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ANGIOSPERM RESPONSES TO A LOW-CO_2 WORLD: CAM AND C_4 PHOTOSYNTHESIS AS PARALLEL EVOLUTIONARY TRAJECTORIES

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Crassulacean acid metabolism (CAM) and C_4 photosynthetic syndromes have much in common: they employ a shared biochemical pathway that enables the concentration of CO_2 inside plant cells, they are both considered to be adaptations to stressful environments, and they are both arguably among the most convergent of complex traits, having each evolved multiple times in various plant lineages. They are also both signature elements of stress-adapted floras the world over and play fundamental roles in the ecological success of flowering plants. In spite of these similarities, the obvious phenotypic and ecological differences between certain groups of fully optimized C_4 and CAM plants have led us to generally view these syndromes as very distinct ecological adaptations. A broad look at the distribution of CAM and C_4 plants across a very large phylogeny of angiosperms highlights that while CAM photosynthesis seems to have evolved more often, both CAM and C_4 origins show tight and overlapping clustering in many regions of the tree, suggesting that certain plant lineages are prone to evolve either pathway. Additionally, recent phylogenetic analyses revealed that the origins and diversification of many CAM and C_4 lineages were recent and contemporaneous in time. We postulate that the evolutionary "starting points" for CAM and C_4 pathways could be much more similar than typically acknowledged. Using species with C_3-C_4 and CAM-like intermediate phenotypes as models of CAM and C_4 evolution has been productive, but the distinct advantages that each affords may have promoted rapid ecological divergence that subsequently masked any shared ancestral characteristics between the two pathways. Focusing on newly discovered phylogenetic "hotbeds" of CAM and C_4 evolution will allow for inclusion of relevant C_3 taxa and a finer evaluation of the possible environmental and organismal traits that would strongly favor the evolution of one syndrome over the other.

Keywords: angiosperm evolution, CO_2, CAM, C_4, photosynthesis.

Introduction

Photosynthetic efficiency is inexorably linked to the concentration of CO_2 in the environment. The enzyme that catalyzes carbon fixation in all of Earth's photosynthetic organisms, RuBisCO, possesses a significant flaw: in low-CO_2 conditions and/or high temperatures, it reacts with O_2 as well as CO_2, which results in the energetically wasteful process of photorespiration (Miziorko and Lorimer 1983). Land plants themselves have played a significant role in altering atmospheric CO_2 concentration through time, with roots promoting the silicate weathering of rocks that causes drastic drawdown and long-term storage of carbon (Berner 1994; Royer 2006). The rapid growth rates and high productivity of angiosperms in particular, perhaps realized only by the mid-Cretaceous (Feild et al. 2011), is thought to have played a major role in the strong declining trend in atmospheric CO_2 over the past 100 million years (Ma). The most dramatic changes in CO_2 during this time occurred throughout the Oligocene; over roughly 10 million years, CO_2 declined from roughly 1500 parts per million (ppm) to nearly current-day concentration (<500 ppm), where it has fluctuated at moderate levels ever since (Zachos et al. 2001; Beerling and Royer 2011). Declining atmospheric CO_2 presents multiple problems for terrestrial vegetation; not only is photosynthesis compromised by the oxygenase activity of RuBisCO, but every unit of carbon gained will come at an increasing transpirational cost, thus necessitating both a greater need for water acquisition and a more efficient internal hydraulic network to maintain hydrated tissue (Brodribb et al. 2007). Recent simulations have suggested that a negative feedback cycle between plant productivity and CO_2 has helped to maintain a semistable low-CO_2 world from 24 Ma to the present because extremely low CO_2 (<200 ppm) would result in plant carbon starvation, which in turn will slow weathering and promote a CO_2 buildup in the atmosphere (Pagani et al. 2009).

While this proposed buffering mechanism may prevent a complete "bottoming out" of CO_2 and concomitant collapse of the terrestrial biosphere, it would also contribute to the maintenance of a low CO_2 "ceiling," perpetuating photorespiration and CO_2/H_2O trade-offs as persistent challenges to plant growth from the Oligocene to the present day. A great number of plant lineages have actively responded to this low-CO_2 world via repeated evolution of an internal CO_2-concentrating mechanism (CCM), a sublime solution that drastically increases the $CO_2:O_2$ ratio inside photosynthetic cells. CCMs in land plants fall into two main categories: C_4 photosynthesis and crassulacean acid metabolism

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(CAM). These types employ identical biochemical pathways but differ in their implementation (fig. 1).

The general approach of both CCMs is to first fix atmospheric CO_2 into the four-carbon acid oxaloacetate via a reaction catalyzed by the enzyme phosphoenol-pyruvate-carboxylase (PEPC). Oxaloacetate is typically quickly reduced to malate or aspartate. Malate or aspartate is transported to the site of active RuBisCO, where it is then decarboxylated via one of three enzymes. The release of CO_2 during the decarboxylation step results in an elevated CO_2 concentration around RuBisCO, with estimates of CO_2 inside the bundle sheath of some C_4 plants upward of 10× atmospheric conditions (Furbank and Hatch 1987). The saturation of RuBisCO suppresses photorespiration and allows the Calvin cycle to proceed efficiently. All of the enzymes involved in this biochemical pathway are already found in all plants and were co-opted for this new purpose (Monson 1999; Aubry et al. 2011).

The great and celebrated differences between C_4 and CAM pathways lay not in these added biochemical precursors to the Calvin cycle, which are identical, but rather in how they have isolated RuBisCO and Calvin cycle reactions from a low-CO_2 world. In C_4 plants, both PEPC and RuBisCO operate during the day, when stomata are open: PEPC is active in the mesophyll tissue, whereas RuBisCO, most chloroplasts, and thus the entire C_3 photosynthetic cycle are typically restricted to the bundle sheath cells. CAM plants have instead engaged in a temporal isolation of RuBisCO: they typically exhibit an inverted diurnal stomatal pattern, with stomata mostly open in early evening to early morning and closed for most of the day. PEPC is most active when stomata are open, and malate accumulates in the vacuole overnight. As stomata close, malate is transferred out of the vacuole and decarboxylated to release CO_2; PEPC is deactivated, and RuBisCO and the Calvin cycle become engaged.

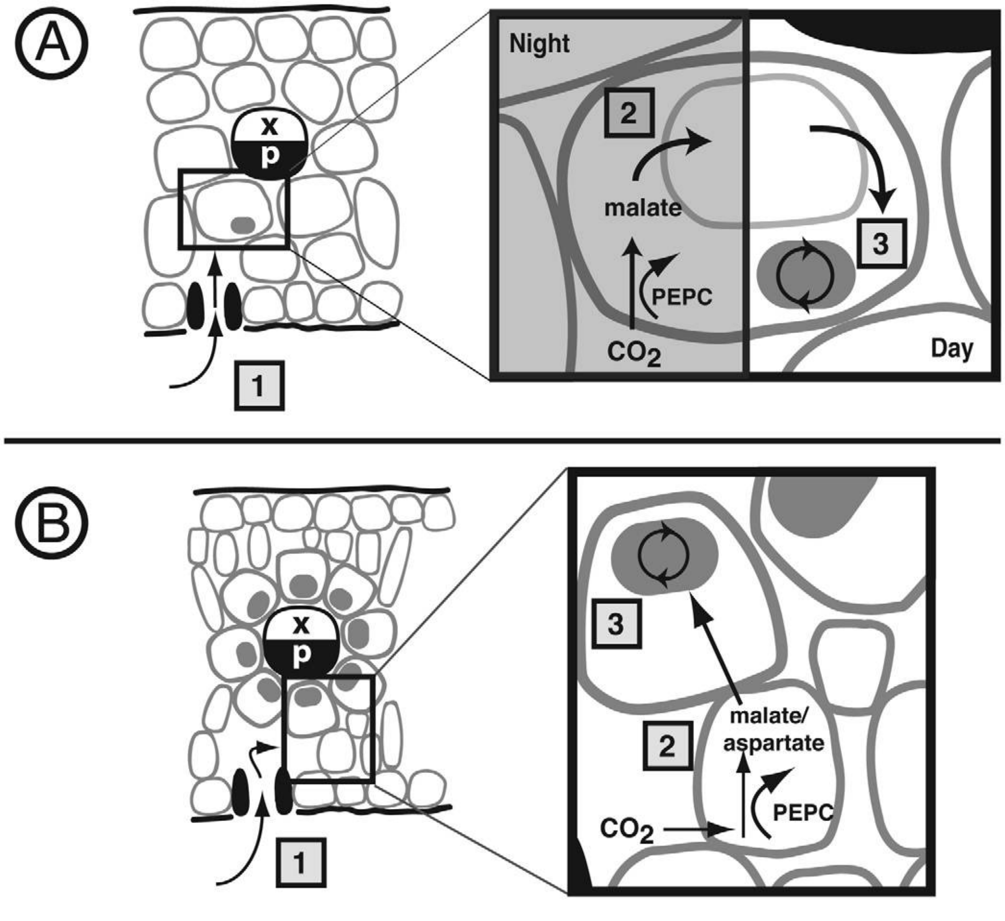

Fig. 1 Basic features of CAM and C_4 photosynthesis. *A*, Full CAM. (1) Stomata are open at night, allowing CO_2 to diffuse into the leaf intercellular air spaces and then into mesophyll cells. CO_2 is converted into HCO_3^- in the cytosol. (2) Within the cytosol, PEP carboxylase (PEPC) catalyzes the reaction between HCO_3^- and the three-carbon PEP to form the four-carbon compound oxaloacetate, which is then reduced to malate. Malate is transported into the vacuole of the same cell and stored for the remainder of the dark period. (3) During daytime, malate is transported out of the vacuole and decarboxylated, releasing CO_2 that is fixed by the Calvin cycle. Stomata are closed. *B*, Full C_4. (1) Stomata are open during the day, and CO_2 diffuses into the leaf intercellular air spaces and mesophyll cells as in CAM. CO_2 is also converted into HCO_3^- in the cytosol of the mesophyll cells. (2) Within the cytosol, PEPC catalyzes the reaction between HCO_3^- and the three-carbon PEP to form the four-carbon compound oxaloacetate, which is then reduced to malate. Depending on the C_4 subtype, either malate or aspartate is transported to the bundle sheath cell (BSC). (3) In the BSC, the four-carbon intermediate is decarboxylated, releasing CO_2 that is fixed by the Calvin cycle.

Thus, PEPC and RuBisCO are active in the very same cells but at different times of day. Having stomata closed during the day also results in a dramatic increase in water use efficiency because lowered nighttime temperature and higher relative humidity will reduce transpiration without affecting rates of CO_2 diffusion into the plant.

A second significant difference between C_4 and CAM photosynthesis lies in their relative plasticity. Excluding a handful of wonderfully bizarre C_4 aquatic plants (Keeley 1998*a*; Bowes 2011), the C_4 pathway is a fixed entity—a plant does not switch between C_3 and C_4 carbon fixation as a result of environmental variables or age. There are many examples of intermediate forms of C_4 that achieve elevated CO_2 concentration around some of their RuBisCO without the help of PEPC by restricting their photorespiratory cycle to the bundle sheath cells; while C_3-C_4 intermediates have demonstrated limited differentiation in photosynthetic efficiency among populations (Sayre and Kennedy 1977, 1979), photorespiratory localization is similarly not considered to be an easily inducible or plastic trait. CAM, on the other hand, is an exceptionally flexible system that comes in many flavors. The CAM cycle can be inducible, reversible, and also just partially realized via CAM cycling or CAM idling, which refer to CAM variants that scavenge nighttime-respired CO_2 by PEPC without any accompanying nocturnal opening of stomata (Cushman 2001; Dodd et al. 2002).

This is an extremely simple explanation of each of these CCMs, and there have been many in-depth reviews on the biochemical, molecular, physiological, and ecological aspects of these complex traits (Monson 1989; Ehleringer and Monson 1993; Cushman 2001; Dodd et al. 2002; Sage 2002, 2004; Keeley and Rundel 2003; Silvera et al. 2010; Borland et al. 2011); there is no need for further elaboration here. Surveying the extensive literature on both pathways uncovers a peculiar pattern; since the early 1980s, every decade has seen a handful of compelling review articles that consider CAM and C_4 simultaneously, and a great emphasis is placed on how biochemically similar they are (Cockburn 1985; Monson 1989; Ehleringer and Monson 1993; Sage 2002; Keeley and Rundel 2003; West-Eberhard et al. 2011). Yet the active research world has largely “divided and conquered”: there are CAM biologists and C_4 biologists, with only occasional intermingling. Highly productive research programs in both areas over the years have produced a set of general themes that highlight the distinct nature of each pathway. For example:

1. CAM must have evolved earlier in time because of its presence in lineages such as lycophytes, ferns, and acrogymnosperms. C_4 photosynthesis must be young, especially in many species-poor C_4 eudicot groups, with origins occurring as recently as the Pleistocene. There is little taxonomic overlap between C_4 and CAM species, suggesting that either the selection pressures for the two pathways are very divergent or the evolutionary accessibility of each pathway varies greatly among plant lineages.

2. C_4 photosynthesis has evolved primarily as a means to alleviate high levels of photorespiration because it is prevalent in taxa that live in hot climates. It has also evolved in some arid-adapted eudicot lineages and in some halophytes. Under the right conditions, C_4 photosynthesis results in increased photosynthetic capacity and is thus also prevalent in fast-growing, weedy species. It is uncommon in woody plants and virtually absent from trees (with one bizarre exception).

3. CAM photosynthesis has evolved primarily as an adaptation to drought and occasionally as a response to CO_2 limitation in aquatic systems. Unlike the C_4 pathway, CAM is extremely flexible in its degree of expression, can be stress or age induced, and is also reversible. Many plant growth forms and life histories possess variants of CAM photosynthesis, although nearly all CAM plants exhibit pronounced succulence.

We examine this set of hypotheses in light of recent work addressing CAM and C_4 evolution. We begin with hypothesis 1, as some of the underlying assumptions here are especially problematic. The good news is that recent advances in phylogenetics alleviate the need for such assumptions and allow us to address more directly the timing and origins of both pathways. Inferring the organismal and environmental conditions that promoted the repeated evolution of C_4 and CAM is a far more difficult task but one that is ultimately more interesting. We argue that using C_3-C_4 and C_3-CAM intermediate species as models of the first evolutionary “steps” has been exceptionally fruitful, but the distinct advantages of these two intermediate phenotypes may have spurred the evolution of a complementary set of traits that could easily be misinterpreted as ancestral. In light of current knowledge, it seems reasonable to assume that the “starting point” phenotype for both pathways is quite similar. In this case, the relative advantages of rudimentary stages might have immediately redirected subsequent adaptation, resulting in the emergence of two divergent evolutionary trajectories and the accumulation of many classical C_4 and CAM syndromes across the angiosperm tree of life.

The Miocene Uprising of Two Global Photosynthetic Revolutions: C_4 and CAM

Many authors have interpreted CAM as being older than C_4 because it is reported from a greater number of species (ca. 16,000 vs. 7500; Smith and Winter 1996; Sage 2004) as well as families (Raven and Spicer 1996; Keeley and Rundel 2003; Silvera et al. 2010). This approach poses several problems. For example, variability in diversification rates between clades could be misleading, especially if the evolution of CAM confers significantly higher diversification rates. In addition, the taxonomic breadth of a particular characteristic cannot possibly inform on its age of origin, unless it is shared across all taxa and inferred to have also been present in their ancestor. The presence of some form of CAM in “ancient lineages” such as *Isoetes*, the ferns, and in the acrogymnosperm *Welwitschia* has been interpreted as evidence that this pathway is older than the C_4 pathway. However, all extant lineages have been continually evolving throughout their history, and it is entirely possible that CAM is a recent innovation in each of these taxa. This is likely the case for the CAM fern lineages, which have undergone extensive diversification subsequent to the evolution of angiosperms (Schneider et al. 2004). While *Welwitschia*-like fossils of pollen, leaves, and strobili date at least as far back as the lower Cretaceous (Taylor et al. 2009), the single living taxon *Welwitschia mirabilis*, our only record of CAM-like physiology in the group

(Winter and Schramm 1986; von Willert et al. 2005), could have conceivably evolved CAM at any point between then and now. Of all the nonangiosperm examples of CAM, only *Isoetes* currently appears to check out as a very early derivation of the pathway: fossils assignable to crown group *Isoetes* date to the earliest Triassic (Retallack 1997), and CAM has been reliably reported from the vast majority of *Isoetes* species examined (Keeley 1998*b*). It is important to keep in mind, however, that as an aquatic CAM taxon, the factors driving the evolution of CAM in *Isoetes* are quite distinct from those found in terrestrial CAM taxa (Keeley 1990).

Recent phylogenetic dating and diversification analyses increasingly support the idea that both CAM and C_4 pathways made their global mark relatively recently in time, beginning largely in the Miocene. Among C_4 plants, the common suggestion that C_4 grasses are older than C_4 eudicots does not generally appear to be true, as the subset of origins within each group that can be dated with reasonable confidence completely overlap in time, with most origins appearing within the past 20 Ma (Christin et al. 2011*a*). Dating the timing of transitions to CAM photosynthesis is inherently more challenging because the plasticity of CAM reduces confidence in coding species as CAM-like or not (Winter and Holtum 2002; Edwards and Diaz 2006; Borland et al. 2011), which in turn makes ancestral reconstructions very uncertain. That said, evidence is mounting that major diversification events in many established CAM lineages are quite recent, occurring throughout the mid-late Miocene and into the Pliocene (Klak et al. 2004; Good-Avila et al. 2006; Givnish et al. 2007; Bruyns et al. 2011; Arakaki et al. 2011). A global surge of succulent CAM plant diversification apparently coincided with the global rise of C_4 grasslands ~8–5 Ma (Cerling et al. 1997; Edwards et al. 2010), suggesting a recent and contemporaneous land grab by both C_4 and CAM syndromes. The global nature of these events suggests that they may have been triggered by massive changes in Earth's climate that would have provided new ecological opportunities for both pathways, such as a further decline in atmospheric CO_2 (Tripati et al. 2009).

Phylogeny Can Place a Finer Point on the Relative Relatedness of CAM and C_4 Lineages

In spite of their complexity, both C_4 and CAM syndromes have evolved a remarkable number of times; the latest tally of C_4 origins numbered 62 (Sage et al. 2011), which has already been increased to at least 69 as a result of more extensive phylogenetic work in the grasses (GPWG II 2012). Less is known about the number of CAM origins. While there has been no real attempt at a "count," its scattered taxonomic distribution strongly suggests that the number of CAM origins must be on par with that of C_4 (Smith and Winter 1996; Silvera et al. 2010).

There has been occasional interest in comparing the distribution of CAM and C_4 origins across the land plant tree of life (Sage 2002; Keeley and Rundel 2003). These efforts have been squarely based in taxonomy and have generally concluded that C_4 and CAM pathways are rather evolutionarily distinct simply because C_4 and CAM are rarely both found in the same genus. However, here again taxonomy can deceive us—two genera can be quite closely related, while two members of a single genus could have been evolving independently for many millions of years. Recent advances in phylogenetics allow us to move beyond genus counts and take a first broad look at the patterns of CAM and C_4 evolution simultaneously (fig. 2). We mapped the known occurrence of C_4 and CAM pathways that were sampled in a 9412-taxon phylogeny of angiosperms (Smith et al. 2009). This approach may roughly estimate the real phylogenetic distribution of these traits if we assume that the sampling of C_4 and CAM species is proportional to their real occurrence. At the grossest scale this appears to be the case because ~2% of taxa here are C_4, which corresponds to the hypothesized real occurrence of C_4 taxa across angiosperms (~2%–4%, depending on estimates of angiosperm species richness). Certain patterns are immediately evident. It does appear that CAM has evolved more often and is more broadly distributed across the tree, although each trait independently exhibited highly significant phylogenetic clustering as evaluated with the *D* statistic (Fritz and Purvis 2010), implemented in the R package "caper" ($P = 0.00$ in both cases).

But more to the point, this simple mapping exercise also identifies multiple regions of the phylogeny that are hotbeds of both C_4 and CAM origins. For example, it is now clear that *Chamaesyce*, a mostly C_4 lineage, is nested squarely in *Euphorbia*, a large clade containing many origins of CAM (Steinmann and Porter 2002). Similarly, the disintegration of Portulacaceae has resulted in the C_4 *Portulaca* being nested within a larger clade of C_3 and CAM taxa, including the cacti (Hershkovitz and Zimmer 1997; Applequist and Wallace 2001; Edwards et al. 2005; Nyffeler et al. 2008; Nyffeler and Eggli 2010). Even leaving aside the cases of nonmonophyletic taxa, many distinct C_4 and CAM genera are more closely related than one could infer using only taxonomic rankings. For example, we now know that within the Caryophyllales, Molluginaceae (with multiple C_3-C_4 and C_4 origins) is sister to the Portulacineae, a lineage of ~2200 species containing at least one C_4 origin (in *Portulaca*) and many independent transitions to CAM (Arakaki et al. 2011; Christin et al. 2011*b*). We suspect that focused phylogenetic work and extensive photosynthetic surveys in other lineages will uncover many other clusters of intermingled C_4 and CAM origins. These areas will be especially useful for inferring the ecological, anatomical, and genetic preconditions that have promoted the evolution of one pathway over the other.

Early Steps toward C_4 or CAM Syndromes May Strongly Direct Subsequent Evolution

Returning to themes 2 and 3, there are clear and irrefutable physiological distinctions between fully optimized C_4 and CAM plants. Obligate CAM plants exhibit much higher water use efficiencies than C_4 plants, and C_4 plants can achieve higher photosynthetic capacities and thus faster growth rates and also perform very well at hot temperatures. These differences establish particular ecological and organismal scenarios in which C_4 or CAM might work especially well. The CAM pathway, for example, is a very common element of the "succulent syndrome," which refers to a combination of traits that

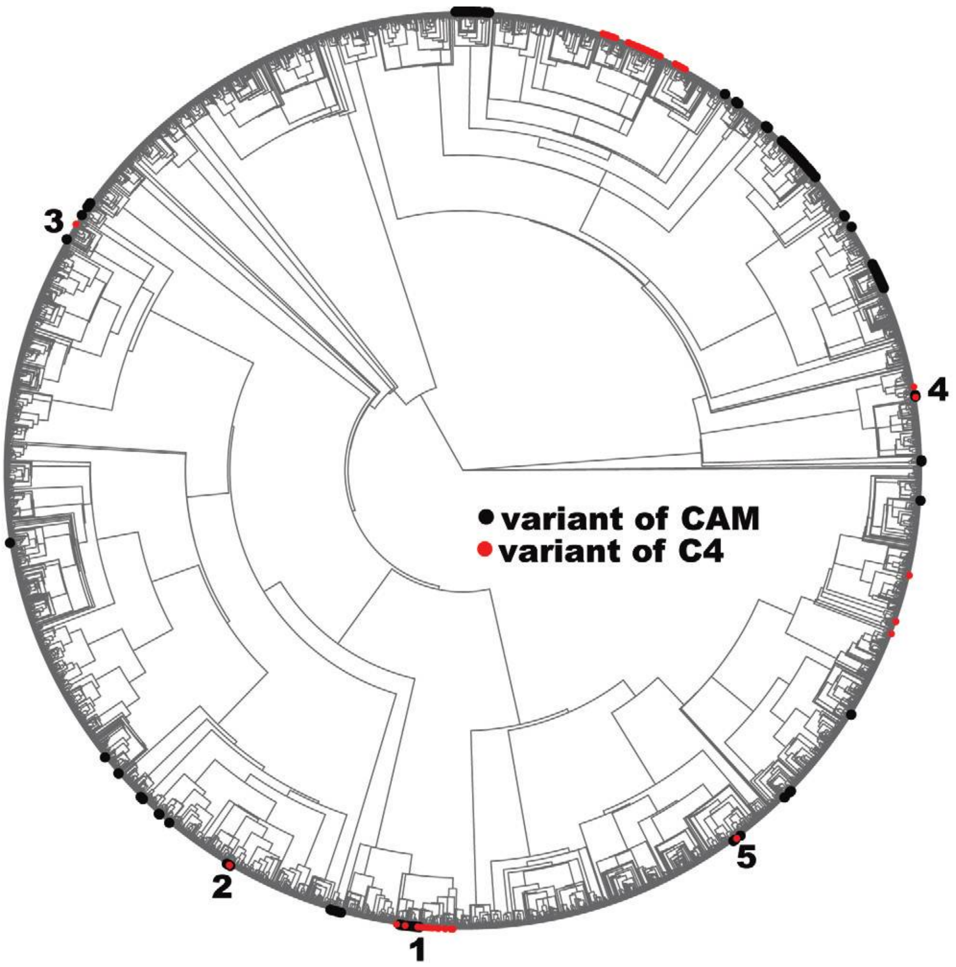

Fig. 2 Phylogenetic distribution of CAM and C_4 photosynthesis. We coded known occurrences of C_4 and CAM pathways that were captured in a recent large-scale phylogenetic analysis of 9414 angiosperms (Smith et al. 2009). Red dots represent taxa with C_3-C_4 or C_4 photosynthesis; black dots represent taxa with some variant of CAM photosynthesis (CAM idling, CAM cycling, inducible CAM, or full, "constitutive" CAM). Numbers refer to five areas of the tree that contain clustered origins of both pathways: (1) Caryophyllales, (2) *Chamaesysce*/*Euphorbia*, (3) Brassicales, (4) Hydrocharitaceae, and (5) Asteroideae (Compositae).

have repeatedly coevolved to optimize a particular water use strategy in arid-adapted plants (Ogburn and Edwards 2010). In contrast, C_4 photosynthesis is often associated with fast-growing and highly productive plant species that live in high-light environments but do not experience exceptional levels of water stress. It is therefore natural to assume that C_4 has been repeatedly selected for in weedy, fast-growing plants that live in hot climates, and CAM has been repeatedly selected for in succulent plants that live in areas with long and frequent droughts. In other words, the C_4 and CAM pathways are simply the finishing touches to a pair of very distinctly preadapted phenotypes.

On the other hand, it seems just as reasonable to think that the evolution of CAM-like or C_4-like behavior in any given lineage may itself select for further changes that would allow for the full advantages of each pathway to be realized. In the earliest stages of CAM and C_4 evolution, the ancestral phenotypes could be far more similar to one another than are the end points—or even the midpoints. Once that first step toward either pathway has been made, the initial selective advantage could strongly promote subsequent divergence and specialization that would result in repeated evolution of the classical CAM and C_4 syndromes. The functional attributes of CAM variants and C_3-C_4 intermediate species strongly support this type of model (Monson 1989; Sage 2002). Recycling nocturnally respired CO_2 as in CAM cycling improves plant water use efficiency, with some estimates suggesting that recapturing nocturnal respiration could conserve as much as 44% of water loss simply by allowing for lower stomatal conductance during the day (Martin and Higley 1988). This initial boost to a plant's water status would allow for greater tolerance of longer droughts, but it would not necessarily increase daytime photosynthetic rates. On the other hand, the great advantage of C_3-C_4 intermediate species derives from their utilization of the photorespiratory cycle to saturate RuBisCO with CO_2, which reduces the impact of photorespiration on the carbon budget and elevates instantaneous photosynthetic rates (Hunt et al. 1987; Monson and Moore 1989; Huxman and Monson 2003). The evolution of C_3-C_4 intermediacy and intermediate CAM variants thus carries immediate and significant ecological consequences, and the relative advantages of these partially realized adaptations open up different avenues for subsequent ecological and physiological specialization. The establishment of greater drought tolerance via CAM cycling would be optimized by further increases in tissue succulence, which would simultaneously allow for greater levels of malate storage at night, as well as a larger whole-plant reservoir of water. On the other hand, increases in daytime photosynthetic efficiency afforded by the localization of the photorespiratory cycle would promote a live-hard, die-young phenotype that achieves high growth rates in hot temperatures when other resources are less limiting.

Previous researchers have been right to emphasize the critical role that C_4 and CAM intermediates can play in reconstructing the evolution of these syndromes (Monson 1989; Sage 2002), but in many ways these stages may already be too advanced to inform about the very initial conditions that would decide "which road into the wood" was taken. In a low-CO_2 world, photorespiratory stress and drought stress go hand in hand. In most cases a C_3 plant will be suffering from neither or from both. Reduced stomatal conductance in response to water stress will increase photorespiration as a result of decreasing internal CO_2 concentrations (Farquhar and Sharkey 1982) and increased internal temperatures, whereas photorespiration is minimized when water is plentiful and stomatal conductivity can remain high. Likewise, high temperatures increase photorespiration directly, but they also exacerbate water stress because of stronger evaporative demand. Reducing either photorespiratory stress or water stress is simply a means to maintain a better carbon balance, and both solutions will be advantageous in a hot and/or dry environment. Assuming that C_3-C_4 intermediacy evolved in weedy annual plants simply because it is commonly associated with those traits presents the same logical flaw as assuming that the C_4 pathway evolved in highly productive grasslands: we still do not know which aspects of the intermediate phenotype were present before the development of a rudimentary C_4 or CAM system.

In much the same way that intermediate stages have been successfully used as models of the stepwise evolution of full C_4 and CAM syndromes, we must now identify the C_3 conditions that have given rise to each of these intermediates. A phylogenetic approach can uniquely contribute to this agenda by identifying appropriate C_3 taxa for comparison. Focusing on lineages containing many clustered C_4 and CAM origins will be especially useful for uncovering the factors that promote the evolution of one CCM over the other.

Closely Related C_4 and CAM Species Show Considerable Overlap in Climate Space

We illustrate this line of investigation with a very preliminary look at the environmental context of C_4 versus CAM evolution in the above-mentioned Molluginaceae + Portulacineae (Caryophyllales), referred to here as the "Portullugo clade." Molluginaceae is mostly C_3 but includes a minimum of two independent C_3-C_4 lineages, as well as a third lineage that contains another C_3-C_4 intermediate and two presumably independent subsequent transitions to a fully optimized C_4 syndrome (Christin et al. 2011*b*). Its sister taxon, the Portulacineae, contains many C_3 and CAM intermediate-type lineages, in addition to several transitions to full CAM syndromes, and at least one origin (but possibly multiple) of C_4 in *Portulaca* (Guralnick and Jackson 2001; Guralnick et al. 2008; Voznesenskaya et al. 2010). Portullugo is roughly 50 million years old and has a worldwide distribution, although it is most common in semiarid and arid tropical regions. It also includes the cacti, one of the most ecologically important New World CAM lineages.

A precursory look at species' climate envelopes across Portullugo supports the hypothesis that C_4-evolving lineages (Molluginaceae) and CAM-evolving lineages (Portulacineae) occupy very similar climate space and, furthermore, there is no obvious difference between C_3 Portullugo and species with either CAM or C_4-like adaptations (fig. 3). We performed a principal components analysis on 19 bioclimatic variables (Hijmans et al. 2005) for 883 taxa from Portullugo using lo-

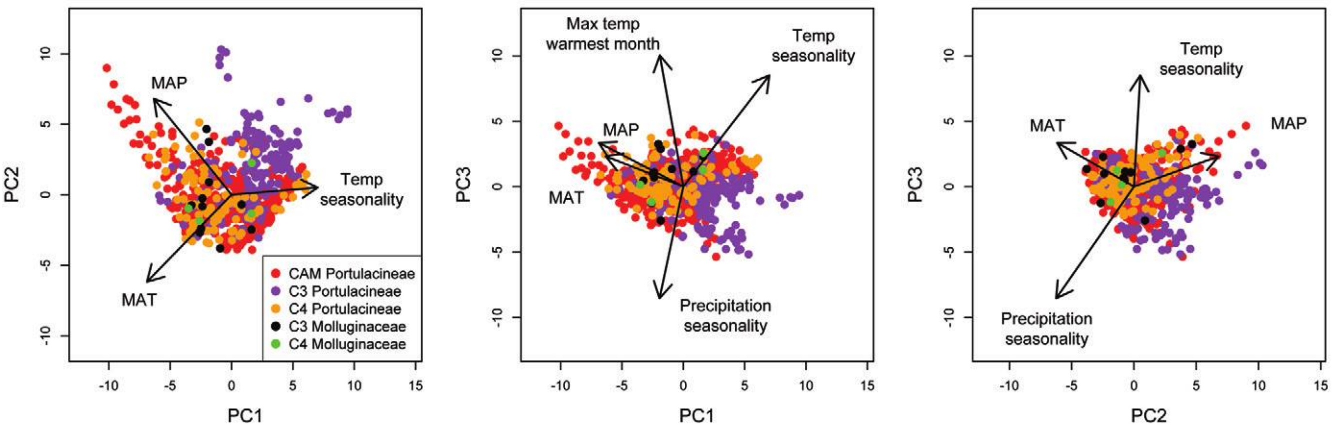

Fig. 3 Relationships among the first three principal components (PCs) for 19 climate variables estimated from species occurrence data of the Portullugo (Caryophyllales). See text for eigenvalues and variable loadings for each PC axis. Points represent species means and are colored by major clade (Portulacineae, Molluginaceae) and photosynthetic pathway (C_3, C_4, CAM).

cality information obtained from the Global Biodiversity Information Facility (http://www.gbif.org/). The first three principal components (PC) axes explained a total of 84% of the variation in the data set (PC1: 43.5%, PC2: 27.2%, and PC3: 13.1%). On the first two PC axes, we recovered two main vector clusters of climate variables: one relating to temperature and one to precipitation. These clusters are roughly orthogonal but do not line up neatly with either axis (fig. 3). The graph of PC1 versus PC2 is therefore best interpreted in terms of its quadrants: the upper left quadrant signifies wet, aseasonal environments, the lower left indicates hot and aseasonal, the upper right indicates cold and seasonal, and the lower right indicates dry and seasonal. PC3 marks an axis along which seasonality in temperature and precipitation are negatively correlated; i.e., low values for PC3 indicate high precipitation seasonality but low annual temperature variation. There was no obvious clustering of species in climate space when separated by major lineage or even by photosynthetic type, suggesting that C_4 and CAM pathways evolved in this group under the same broad set of environmental conditions and did not promote any obvious exploration of new climate space not occupied by their C_3 relatives. The one exception may be the movement of CAM epiphytic cacti into wetter habitats, as evidenced by the long tail in the upper left quadrant of PC1 versus PC2 (fig. 3). If anything, a portion of the C_3 Portulacineae seems to occupy a colder climate space than all the others. These points represent a handful of lineages within the Montiaceae that have specialized in alpine regions in western North America and the high Andes.

We also mapped individual environmental variables onto a phylogeny of a subset of taxa that were included in the most recent phylogenetic analysis of Portullugo (Arakaki et al. 2011). Figure 4 highlights maximum temperature of the warmest month and warm-season precipitation, two variables assumed to be extremely influential in the evolution of the C_4 syndrome but less so in CAM (Sage 2004; Keeley and Rundel 2003). Again, we see no clear differences in these variables, between either major clades or photosynthetic types. A phylo-

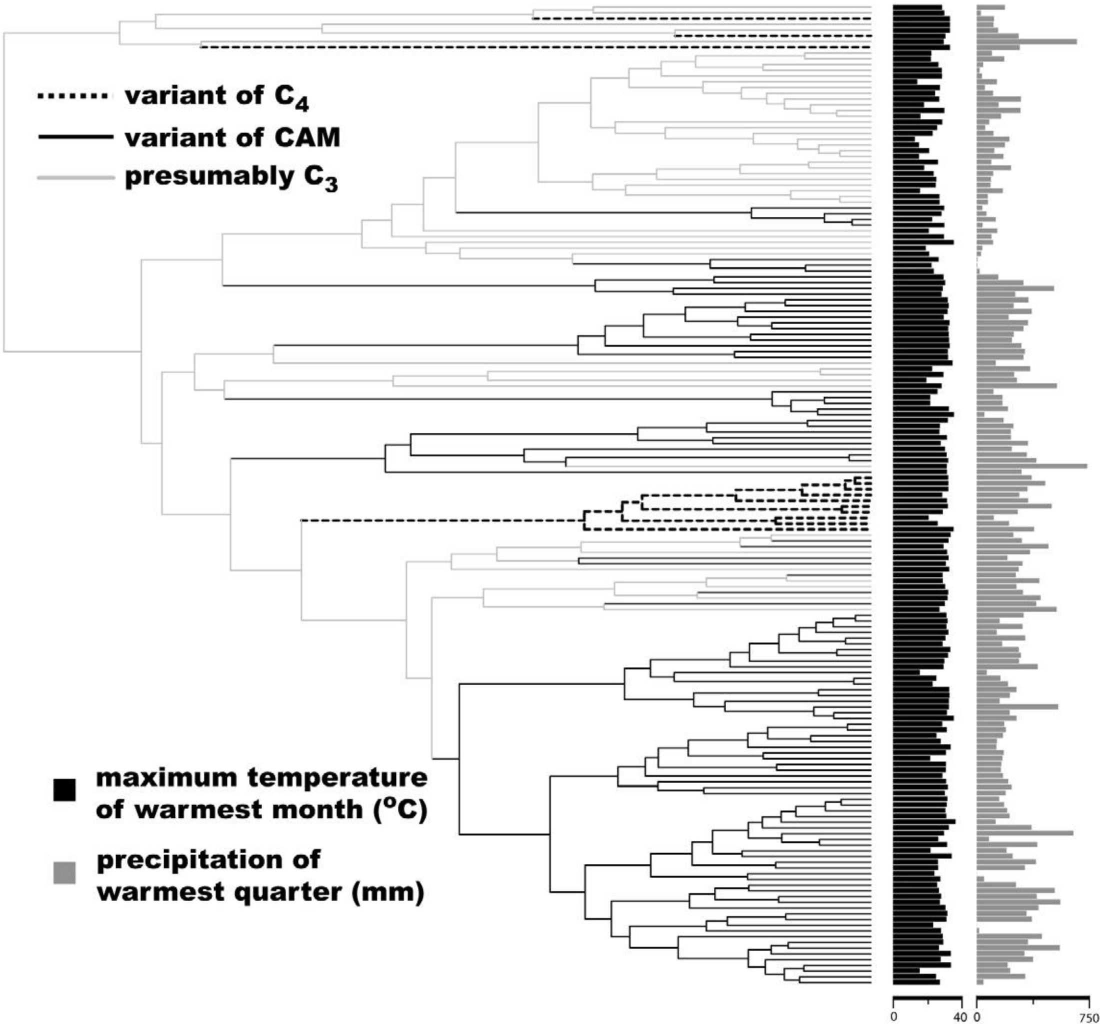

Fig. 4 CAM, C_4, and climate in the Portullugo. Phylogeny of 171 species of Portullugo (Caryophyllales) for which we have available climate data, pruned from a 254-taxon analysis of the group (Arakaki et al. 2011). Species mean values of two environmental variables that have been strongly associated with C_4 ecological success (high temperatures, warm-season precipitation), but less so with CAM, are illustrated along the tips of the phylogeny.

genetic regression confirms this visual pattern, with photosynthetic pathway having no significant effect on warm-season precipitation ($P = 0.87$) but a nearly significant effect on maximum temperature ($P = 0.06$). A second analysis in which we tested for the effect of each photosynthetic pathway separately, however, revealed again that this latter signal was being driven entirely by specialized members of Montiaceae that have adapted to very cold climates (C_3 effect, $P = 0.02$; C_4 effect, $P = 0.26$; CAM effect, $P = 0.23$).

Developmental Enablers of CAM and C_4 Syndromes

The climate analysis presented here admittedly can paint only with the broadest of brush strokes, but we include it simply to illustrate how both C_4 and CAM could likely have evolved as a response to similar environmental pressures. We suspect that a more global analysis of climate envelopes for C_4 and CAM plants from other lineages will largely confirm these initial patterns for the Portullugo. Climate envelopes cannot inform on most aspects of a plant's ecological character, however; more detailed ecological information, which is surely needed in this case, requires actual studies of organismal traits and observations from the field. We do not doubt that microhabitat preference, phenology, and many aspects of plant ecophysiology differ wildly within this shared climate space, and we may discover that some of these variables were key elements in tipping the balance toward the evolution of one pathway over the other.

An alternative (though certainly not mutually exclusive) set of tipping points likely resides within the rich and varied details of organismal anatomy and genomic structure. While contemplating why evolution has never produced a plant that engages in C_4 and CAM cycles simultaneously, Sage (2002) outlined a persuasive model of CAM versus C_4 evolution, where the first step toward each pathway precluded subsequent evolution of the other and this first step was governed entirely by leaf anatomy. In the C_4 model, he proposed (as have others; e.g., Monson and Rawsthorne 2000) that a reduced mesophyll–to–bundle sheath ratio was necessary before the establishment of a C_3-C_4 intermediate physiology. On the CAM side, in order to effectively recapture and store nighttime-respired carbon, a leaf must be at least mildly succulent, with large mesophyll cells and substantial vacuolar space for storing the C_4 acids overnight. What makes this hypothesis especially elegant is that these two aspects of leaf anatomy must be, to a large degree, mutually exclusive: without a concomitant increase in venation density, enlarged mesophyll cells will necessarily lead to an increased mesophyll–to–bundle sheath ratio. Large mesophyll cells could thus provide an elevated accessibility to the evolution of rudimentary CAM cycling while simultaneously reducing the efficacy of a photorespiratory cycle that is localized to the bundle sheath.

Could a factor as simple as the relative cell size of the ancestral C_3 phenotype underlie the complex evolutionary patterns of two of the most critical evolutionary innovations in the history of angiosperms? We doubt it, considering the hundreds of times these pathways have evolved and within such a diversity of organismal contexts. However, in the case of Portullugo, we find this model extremely compelling, and a preliminary data set quantifying leaf succulence and cell size across the group supports it (Ogburn and Edwards 2012). Focusing future research efforts in these phylogenetic "hot spots" of CAM and C_4 evolution will provide the means to further develop these ideas.

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