



# Crassulacean acid metabolism photosynthesis in Bromeliaceae: an evolutionary key innovation

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Crassulacean acid metabolism (CAM) is a photosynthetic pathway that significantly increases water use efficiency in plants. It has been proposed that CAM photosynthesis, which evolved from the ancestral C3 pathway, has played a role in the diversification of some prominent plant groups because it may have allowed them to colonize and successfully spread into arid or semi-arid environments. However, the hypothesis that CAM photosynthesis constitutes an evolutionary key innovation, thereby enhancing diversification rates of the clades possessing it, has not been evaluated quantitatively. We tested whether CAM photosynthesis is a key innovation in the Bromeliaceae, a large and highly diversified plant family that has successfully colonized arid environments. We identified five pairs of sister groups with and without the CAM feature, including 31 genera and over 2000 species. In all five cases, the clades with CAM photosynthesis were more diverse than their C3 counterparts. We provide quantitative evidence that the evolution of CAM photosynthesis is significantly associated with increased diversification in Bromeliaceae and thus constitutes an evolutionary key innovation. We also found preliminary evidence of an association between the CAM pathway and growth habit in bromeliads, with terrestrial species being more likely to show CAM photosynthesis than epiphytic species. To our knowledge, this is the first case of a physiological attribute shown to be a key innovation in plants. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, 104, 480–486.

**ADDITIONAL KEYWORDS:** adaptation – arid environments – bromeliads – diversification – epiphytism – niche – physiological traits – terrestrial habitat – water economy.

## INTRODUCTION

Crassulacean acid metabolism (CAM) is a photosynthetic pathway widely recognized as a physiological adaptation of plants to aridity, and it is present in approximately 20 000 species from at least 35 plant families (Cushman, 2001; Silvera *et al.*, 2010). This mechanism involves nocturnal uptake of CO<sub>2</sub> and its fixation into malic acid, which is stored in the vacuole and then decarboxylated during daytime for CO<sub>2</sub> to be integrated into the Calvin cycle (Ting, 1985; Taiz & Zeiger, 2002). CAM photosynthesis allows plants to avoid stomatal opening during daytime, when the air

temperature is higher and the probability of losing water by transpiration increases, thereby enhancing water use efficiency (Ehleringer & Monson, 1993; Taiz & Zeiger, 2002). The CAM photosynthetic pathway is a distinctive attribute of large plant families such as Orchidaceae (Silvera *et al.*, 2009) and Bromeliaceae (Givnish *et al.*, 2007), which are very successful in the epiphytic arid microenvironments of tropical forests, and Cactaceae, which are characteristic of arid ecosystems (Ogburn & Edwards, 2009). The involvement of CAM photosynthesis in lineage diversification in arid ecosystems has often been suggested but rarely tested (Cushman, 2001; Silvera *et al.*, 2010). An exception to this is a recent study that showed evidence of correlated divergence in the photosynthetic pathway (with CAM evolving from C3) and

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epiphytism in Orchidaceae, which relates to orchid species diversification (Silvera *et al.*, 2009).

The Bromeliaceae are a mostly Neotropical family (Smith & Till, 1998) composed of eight subfamilies, 58 genera, and 3200 species (Luther, 2008), with almost half of them being epiphytes (Gentry & Dodson, 1987) and approximately two-thirds showing CAM photosynthesis (Martin, 1994; Crayn *et al.*, 2004). Adaptations to aridity in Bromeliaceae include the formation of tanks, a rosette-type growth that impounds water from rainfall among compactly overlapping leaf bases, water-absorbing leaf trichomes that are able to capture atmospheric water vapour, and the CAM photosynthetic pathway (Smith & Downs, 1974; Benzing, 2000; Givnish *et al.*, 2007). The CAM pathway evolved from the C3 ancestral state in Bromeliaceae several times, although reversals have also occurred (Givnish *et al.*, 2007). CAM bromeliads are mainly distributed in arid or semi-arid environments such as the high Andean ranges or deserts of Mexico and western South America, where they are primarily terrestrial (Smith & Downs, 1974; Crayn *et al.*, 2004), or show the epiphytic habit in tropical forests, thus being exposed to low water availability (Benzing, 2000). It has been suggested that the evolution of CAM photosynthesis in Bromeliaceae, and the associated niche broadening to include drier habitats, should have stimulated the diversification of clades (Crayn *et al.*, 2004; Givnish *et al.*, 2007). Although several lines of evidence point to this hypothesis (Givnish *et al.*, 2007), a quantitative verification is yet to be made.

Evolutionary key innovations are attributes whose appearance allows some taxonomic groups to successfully exploit formerly underused ecological niches, resulting in enhanced diversification rates of lineages (Simpson, 1953; Heard & Hauser, 1995). An outstanding example of key innovation in plants is the evolution of the flower, which is largely responsible for the enormous differences in species richness between angiosperms and gymnosperms (Stebbins, 1981). The association between putative key innovations and diversification rates can be evaluated by comparing the species richness in a clade possessing such a trait and a sister group (i.e. the most phylogenetically-related clade) that lacks it (Slowinski & Guyer, 1993; Barraclough, Nee & Harvey, 1998). Attributes shown to be key innovations within flowering plants include: annual growth habit, herbaceous life-form, climbing habit, floral nectar spurs, fleshy fruits, modifications of seeds that facilitate dispersal, and defensive resin canals (Farrell, Dussourd & Mitter, 1991; Eriksson & Bremer, 1992; Ricklefs & Renner, 1994; Hodges, 1997; Dodd, Silvertown & Chase, 1999; Andreasen & Baldwin, 2001; Smith, 2001; Gianoli, 2004). Interestingly, the possible role of physiological traits as key

innovations in plants has not been addressed; studies have primarily focused on morphological attributes.

The Bromeliaceae include several particularly species-rich clades. Approximately one-third of the species of the family are concentrated in eight genera (*Aechmea*, *Dyckia*, *Guzmania*, *Neoregelia*, *Pitcairnia*, *Puya*, *Tillandsia*, and *Vriesea*; Luther, 2008). It is possible to infer that one or more characters present in them have been responsible for their higher diversification rates compared to closely-related groups. CAM photosynthesis, along with other adaptations to aridity, has been mentioned as a possible key innovation for the diversification of Bromeliaceae by allowing the colonization and establishment in formerly nonsuitable arid or semi-arid habitats (Givnish *et al.*, 1997, 2007; Benzing, 2000; Reinert, Russo & Salles, 2003; Crayn *et al.*, 2004). The present study aimed to test the hypothesis that CAM photosynthesis is a key innovation for Bromeliaceae (i.e. to determine whether clades of bromeliads possessing the CAM pathway are more diverse than their sister groups with C3 photosynthesis). In addition, to estimate the independence of this hypothetical evolutionary pattern, we assessed whether the presence of CAM photosynthesis and epiphytism are correlated in Bromeliaceae, as has been shown for Orchidaceae (Silvera *et al.*, 2009).

## MATERIAL AND METHODS

Valid genera of Bromeliaceae and their number of species were considered *sensu* Luther (2008), excluding subspecies, varieties, hybrid genera, and hybrid species. We carried out an extensive search of the available literature to determine the presence or absence of CAM photosynthesis in bromeliad species. Facultative CAM, constitutive CAM, and species reported as 'probably CAM' were classified as CAM species, whereas C3 and species reported as 'probably C3' were classified as C3 species. A genus was considered CAM when the majority of the reported species was so classified. Importantly, we only considered cases where (1) the photosynthetic pathway for a whole genus was indicated in the text or in tables (e.g. 'all *Greigia* species are C3') or (2) a given number of species within a genus were explicitly identified and their corresponding photosynthetic pathways informed in the text or in tables (e.g. *Puya chilensis*: CAM; *Puya alpestris*: C3) (i.e. we did not take into account studies merely asserting that a given number of species within a genus were C3 or CAM).

To determine sister groups within Bromeliaceae, we used available phylogenies for the family (Crayn *et al.*, 2004; Givnish *et al.*, 2004, 2007) and for the main subfamilies (Barfuss *et al.*, 2005; Rex *et al.*, 2009; Schulte, Barfuss & Zizka, 2009; Jabaily &

**Table 1.** Species richness contrasts between crassulacean acid metabolism (CAM) and C3 sister groups in Bromeliaceae

CAM genera	Number of species	C3 genera	Number of species	$P_c$	Sources
<i>Billbergia</i> – <i>Lymania</i> – <i>Wittrockia</i> – <i>Edmundoa</i> – <i>Neoregelia</i> – <i>Nidularium</i> – <i>Canistrum</i> – <i>Araeococcus</i> – <i>Quesnelia</i> – <i>Aechmea</i> – <i>Cryptanthus</i> – <i>Ortophytum</i> – <i>Ananas</i> – <i>Neoglazovia</i> – <i>Hohenbergia</i> – <i>Bromelia</i> – <i>Ursulaea</i>	773	<i>Fernseea</i> (Bromelioideae)	2	0.0026	1/2–6
<i>Encholirium</i> – <i>Dyckia</i> – <i>Deuterocohnia</i>	170	<i>Fosterella</i> (Pitcairnioideae)	30	0.1508	6–8/4–6, 9–10
<i>Puya</i> (Puyoideae)	273	<i>Fascicularia</i> – <i>Ochagavia</i> – <i>Greigia</i> (Bromelioideae)	38	0.1226	1, 8, 11/4, 6, 10
<i>Hechtia</i> (Hectioideae)	52	<i>Glomeropitcairnia</i> – <i>Catopsis</i> (Tillandsioideae)	20	0.2817	6, 12/2–6, 10
<i>Tillandsia</i>	596	<i>Racinaea</i> (Tillandsioideae)	61	0.0929	6, 8, 12/2–6

Subfamilies are indicated in parenthesis; when both groups within a contrast belong to the same subfamily, the latter is indicated after the C3 genus or genera.  $P_c$ , probability that the CAM clade actually has the observed species richness or greater (Slowinski & Guyer, 1993; see Methods). Sources: phylogeny and species richness/photosynthetic pathway. 1, Schulte *et al.* 2009; 2, Medina 1974; 3, Griffiths & Smith 1983; 4, Martin 1994; 5, Pierce, Winter & Griffiths 2002; 6, Crayn *et al.* 2004; 7, Givnish *et al.* 2004; 8, Rex *et al.* 2009; 9, Crayn *et al.* 2000; 10, Reinert *et al.* 2003; 11, Jabaily & Sytsma 2010; 12, Barfuss *et al.* 2005.

Sytsma, 2010). Genera with uncertain phylogenetic position or without enough information about the species' photosynthetic pathway were not considered. The analysis was conducted on five contrasts, including 31 genera and 2015 species (Table 1).

To test the hypothesis that CAM clades are more diverse than their C3 sister groups, we used the method of Slowinski & Guyer (1993), which is based on a model of random speciation and extinction. For each of the five sister-group pairs, the probability that the clade with CAM photosynthesis has a species richness of  $r$  or greater was calculated by the formula:  $P_c = p(r/n) + p(r + 1/n) + \dots + p(n - 1/n) = (n - r)/(n - 1)$ , where  $n$  is the actual number of total species in both clades and  $r$  is the observed number of species in the clade with CAM photosynthesis. The natural logarithm ( $\ln$ ) of these independently calculated probabilities ( $P_c$ ) was summed for the five pairs, and the result multiplied by  $-2$  and tested against a chi-square distribution with  $2k$  degrees of freedom, where  $k$  is the number of pairs evaluated (Fisher's combined probability test; Sokal & Rohlf, 1995).

The independence of the evolution of CAM photosynthesis and the growth habit (epiphytic versus terrestrial) was assessed via tables of contingency for each of four Bromeliaceae clades where phylogenies were available and both the growth habit and the

photosynthetic pathway were clearly identified at the species level (Table 2). Specifically, to test the explicit hypothesis that transitions from C3 to CAM photosynthesis are more probable on epiphytic clades than on terrestrial ones, we used the method of Sillén-Tullberg (1993), which allows testing for the contingency of states in two discrete characters. The resulting probabilities ( $P$ -values) of the four independent chi-square tests were processed as described above (Fisher's combined probability test).

## RESULTS

We found that, in all five cases, the CAM clade had greater species richness than the corresponding sister clade with C3 photosynthesis (Table 1). The pattern of greater taxonomic diversification in CAM groups was statistically significant ( $\chi^2 = 27.18$ , d.f. = 10,  $P = 0.002$ ; Fisher's combined probability test). Therefore, results validated the hypothesis that the CAM pathway is an evolutionary key innovation for Bromeliaceae. The contrasts between sister groups showing different photosynthetic pathways included five of the eight Bromeliaceae subfamilies (Table 1).

Assessment of the independence of the evolution of the CAM pathway and the growth habit included four clades (Table 2), with a total of 1627 species. In three cases, the transitions to CAM photosynthesis

**Table 2.** Contingency tables testing the association between the evolution of crassulacean acid metabolism (CAM) photosynthesis and growth habit in four Bromeliaceae clades

	C3→C3	C3→CAM	<i>P</i>	Phylogeny sources
<i>Encholirium</i> – <i>Dyckia</i> – <i>Deuterocohnia</i> – <i>Fosterella</i> – <i>Pitcairnia</i> – <i>Pepinia</i> (587 species)				
Epiphytic	4	0	0.0463	2, 4, 5
Terrestrial	40	67		
<i>Puya</i> – <i>Fascicularia</i> – <i>Ochagavia</i> – <i>Greigia</i> (311 species)				
Epiphytic	1	0	0.7189	1, 5, 6
Terrestrial	102	51		
<i>Hechtia</i> – <i>Glomeropitcairnia</i> – <i>Catopsis</i> (72 species)				
Epiphytic	20	0	0.0001	4, 7
Terrestrial	2	33		
<i>Tillandsia</i> – <i>Racinaea</i> (657 species)				
Epiphytic	49	98	0.9362	4, 5, 7
Terrestrial	3	8		

Total species richness in the clade is indicated in parenthesis. The number of branches in the phylogenies with C3→C3 and C3→CAM transitions for epiphytic and terrestrial taxa are shown (Sillén-Tullberg, 1993). *P*-values are calculated from Yates' corrected chi-square distribution. Phylogeny sources are as indicated in Table 1.

were much more common for the terrestrial taxa than for the epiphytic taxa (Table 2). Overall, there was a significant association between photosynthetic pathway and growth habit ( $\chi^2 = 25.36$ , d.f. = 8,  $P = 0.0013$ ; Fisher's combined probability test). Thus, evolution of the CAM pathway in Bromeliaceae is apparently not linked to the development of an epiphytic habit, instead being rather favoured by a terrestrial habitat.

## DISCUSSION

We quantitatively verified the hypothesis that the CAM photosynthetic pathway constitutes an evolutionary key innovation for the Bromeliaceae. This finding supports earlier claims that the evolution of CAM photosynthesis, and the ensuing colonization of arid environments, has promoted taxonomic diversification in Bromeliaceae (Cushman, 2001; Crayn *et al.*, 2004; Givnish *et al.*, 2007). The colonization of novel habitats, comprising potential 'adaptive zones' where lineages are released from competitors, is one of the mechanisms by which a given attribute may promote diversification of clades (Heard & Hauser, 1995). Several traits have proven to be evolutionary key innovations in plants, most of them being morphological or life-history traits (Eriksson & Bremer, 1992; Ricklefs & Renner, 1994; Hodges, 1997; Dodd *et al.*, 1999; Andreasen & Baldwin, 2001; Smith, 2001; Gianoli, 2004). However, to our knowledge, no physiological character had been shown to be a key innovation, with the closest case being the appearance of

latex and resin canals that confer resistance against herbivores (Farrell *et al.*, 1991). The paucity of such plant physiological attributes in the key innovation literature is more likely the result of a lack of sufficient information rather than to a minor role of physiological traits in plant adaptation to novel environments.

The number of contrasts included in the present study is close to the number of times CAM photosynthesis supposedly arose within Bromeliaceae. According to Crayn *et al.* (2004), this photosynthetic pathway appeared independently at least four times within the family. This statement is supported by other studies where four approximately defined monophyletic CAM clades were shown after mapping the presence or absence of CAM photosynthesis onto the Bromeliaceae phylogeny (Crayn *et al.*, 2000; Reinert *et al.*, 2003; Givnish *et al.*, 2007). We considered five CAM clades in the analysis, instead of four, because we chose to 'split' the Bromelioideae subfamily into two clades, in accordance with evidence that places *Greigia*, *Ochagavia*, and *Fascicularia* as a sister group to *Puya* (Rex *et al.*, 2009; Jabaily & Sytsma, 2010) and *Fernseea* as a sister group of the CAM Bromelioideae (Schulte *et al.*, 2009). Although our number of contrasts may seem small compared to other similar studies (Gianoli, 2004), it reflects the actual number of appearances of CAM photosynthesis within the family.

The CAM pathway evolved from an ancestral C3 state during the evolution of Bromeliaceae, probably being selected during the advance of different

groups in the family into arid regions (Varadarajan & Gilmartin, 1988; Givnish *et al.*, 2007). This pattern of origin and diversification of the CAM pathway in Bromeliaceae would be similar to that reported for Cactaceae (Ocampo & Columbus, 2010), as well as for Orchidaceae (Silvera *et al.*, 2009), a family that shares the dominance of the rather dry epiphytic habitat in tropical forests with Bromeliaceae (Gentry & Dodson, 1987). The CAM pathway is considered to be a vital adaptation for plants living in arid or semi-arid environments (Cushman, 2001). For those Bromeliaceae species that rely on foliar structures to obtain fog water and inhabit extremely dry environments, such as the rootless *Tillandsia landbeckii* that grows unattached on the Atacama Desert sands, CAM photosynthesis is essential for survival (Rundel *et al.*, 1997; Rundel & Dillon, 1998). For terrestrial genera such as *Puya*, CAM photosynthesis appears to be a key feature for those species that live in the drier regions of its distribution range (Benzing, 2000).

The basal phylogenetic position of the C3 genera *Brocchinia* and *Lindmania*, both endemic to the Guayana Shield, implies that the probable origin of Bromeliaceae was in this warm and rainy region (Givnish *et al.*, 2004, 2007). In the subsequent geographical expansion of the family, morphological and physiological adaptations to aridity were probably the main mechanisms behind its ecological and taxonomic diversification (Givnish *et al.*, 1997, 2007). Thus, the successful colonization of drier environments, such as the epiphytic habitat in tropical forests and semi-arid highlands in the Andes, was mediated by the appearance of tanks, absorbing trichomes and CAM photosynthesis (Givnish *et al.*, 1997; Benzing, 2000). This photosynthetic pathway probably allowed bromeliads to advance into extremely arid zones, such as deserts, by minimizing water loss during carbon assimilation (Ehleringer & Monson, 1993). On the other hand, the CAM photosynthetic pathway also entails disadvantages. Thus, cold climate limits the distribution of CAM plants (Ehleringer & Monson, 1993) mainly because of the sensitivity of PEPC, the carbon fixation enzyme, to low temperatures (Lambers, Chapin & Pons, 1998). This could explain the scarcity of CAM plants at higher latitudes in southern South America. In the case of Orchidaceae species, it has been established that the prevalence of CAM photosynthesis decreases with altitude (Silvera *et al.*, 2009).

A comprehensive study in the Orchidaceae revealed the involvement of CAM photosynthesis in the adaptive radiations within the family, and demonstrated correlated evolutionary divergence between photosynthetic pathways and plant growth habit, with CAM epiphytic species being more prevalent at lower altitudes (Silvera *et al.*, 2009). Our study found prelimi-

nary evidence suggesting that CAM photosynthesis is more likely to occur in terrestrial than in epiphytic clades within Bromeliaceae. This result is somewhat unexpected given that some of the most important and abundant genera of Bromeliaceae are mainly epiphytic and CAM (Smith & Downs, 1974; Martin, 1994). Although we cannot rule out the possibility that our noncomprehensive dataset is not representative of the whole family, there is also an explanation for such a pattern based on biogeographical and ecological issues. In the case of orchids and tropical bromeliads, such as the clade dominated by *Tillandsia*, the epiphytic habitat represents a low water environment where the water-conserving CAM strategy is advantageous, and the terrestrial habitat is associated with high soil moisture. By contrast, in the case of temperate bromeliads, which are mainly distributed in Andean highlands and deserts, the CAM pathway is associated with the terrestrial habitat, which is semi-arid or arid and much more challenging in terms of water economy than the epiphytic habitat. This is the case for *Puya*, *Dyckia*, *Encholirium*, and *Deuterocohnia*, the CAM representatives of their clades, which live in arid and semi-arid environments of South America (Smith & Downs, 1974), and it is also the case for *Hechtia*, which is distributed in arid regions of North America (Smith & Downs, 1974; Crayn *et al.*, 2004). Further research, including a more comprehensive dataset, containing a greater number of tropical groups, will shed light into the actual relationship between the evolution of CAM photosynthesis and growth habit in Bromeliaceae.

In the present study, we provide quantitative evidence indicating that the evolution of CAM photosynthesis is significantly associated with increased diversification in Bromeliaceae and thus constitutes an evolutionary key innovation for this family, as has been often stated. To our knowledge, this is the first case of a physiological attribute being shown to represent a key innovation in plants. We also found preliminary evidence of an evolutionary association between CAM photosynthesis and growth habit in the Bromeliaceae, which deserves further investigation. Future studies that aim to explain the diversification of bromeliads should include, in addition to CAM photosynthesis, other characteristic adaptations to aridity in the family, such as tanks, absorbing trichomes, and succulence.

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