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THE OCCURRENCE OF CRASSULACEAN ACID METABOLISM IN EPIPHYTIC FERNS, WITH AN EMPHASIS ON THE VITTARIACEAE

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The potential for nighttime CO_2 uptake and/or increases in tissue acidity characteristic of crassulacean acid metabolism (CAM) was investigated, to varying degrees, in 12 species of Vittariaceae as well as in seven species in four other families of tropical epiphytic ferns. Evidence of CAM (actually CAM cycling, i.e., diel changes in tissue acidity without nighttime atmospheric CO_2 uptake), though highly variable, was found in two species of Vittariaceae. The ecophysiological significance of this finding is puzzling, because the occurrence of these plants in deeply shaded, extremely moist habitats is rare, if not unique, among plants expressing any degree of CAM. The results of this study confirm that CAM among the ferns is not limited to the Polypodiaceae, and they emphasize the polyphyletic nature of the evolution of CAM among higher plants.

Keywords: crassulacean acid metabolism (CAM), CAM cycling, epiphytes, evolution, ferns, photosynthesis, Vittariaceae.

Introduction

Plants with the crassulacean acid metabolism (CAM) photosynthetic pathway are characterized by stomatal opening at night, nighttime uptake of atmospheric CO_2, and diel tissue acid fluctuations (Kluge and Ting 1978). An examination of the phylogenetically widespread occurrence of CAM reveals a clearly polyphyletic origin (Evans 1971; Ting 1976). In most taxa, CAM appears to be a derived character state. Nonetheless, this type of carbon metabolism does appear, albeit infrequently, in more ancestral groups, e.g., among the ferns, isoetids, and gymnosperms (Smith and Winter 1996). Only one gymnosperm, *Welwitschia mirabilis*, has the ability to use CAM photosynthesis, characterized by diel acid fluctuations (Winter and Schramm 1986) and nighttime CO_2 uptake from the atmosphere (D. von Willert, personal communication). Unlike most CAM plants, which are aridland xerophytes or tropical/subtropical epiphytes, CAM plants in the Isoetaceae are aquatic (Keeley 1996). In the latter, CAM is apparently an adaptation that facilitates the absorption of CO_2 at night, when levels of CO_2 dissolved in the water greatly exceed concentrations found during the day.

In most CAM plants, this unique form of photosynthesis constitutes an adaptation that results in an impressive conservation of water (Kluge and Ting 1978; Osmond 1978; Winter 1985; Lüttge 1987; Winter and Smith 1996). Thus, the majority of CAM plants, excluding the isoetids, are found in arid environments or microenvironments. This is reflected in the epiphytic habitat and growth form of epiphytes, because many of these plants lack a continuous source of water, and drought stress between rainfall events can be severe (Winter 1985; Lüttge 1989; Benzing 1990; Martin 1994). The CAM pathway conserves water by maintaining stomatal closure throughout most of the day and limiting stomatal opening to the cooler and more humid nighttime. The acid that accumulates throughout the nighttime, primarily malic acid, results from CO_2 fixation by phospho*enol*pyruvate (PEP) carboxylase. This acid is released from the vacuoles in which it was stored at night and decarboxylated in the light. This decarboxylation has two important consequences: the resultant high concentrations of CO_2 inside the photosynthetic tissue effect stomatal closure, and the released CO_2 is eventually, but slowly, reduced to form carbohydrate. As a result of these biochemical and physiological events, the diagnostic criteria for CAM plants include nighttime CO_2 uptake, diel acid fluctuations, and, although not unique to CAM, high (less negative) stable carbon isotope ratios relative to those of C_3 plants (Kluge and Ting 1978; Osmond 1978; Winter 1985; Lüttge 1987; Winter and Smith 1996).

The CAM photosynthetic pathway is common among tropical and subtropical epiphytes, especially in the families Orchidaceae and Bromeliaceae (Avadhani et al. 1982; Winter et al. 1983; Goh and Kluge 1989; Smith 1989; Martin 1994). Furthermore, all known taxa of ferns exhibiting CAM are tropical epiphytes or, in one species, lithophytes (Kluge et al. 1989; Holtum and Winter 1999). Unlike the situation found in orchids and bromeliads, in which CAM taxa are common and phylogenetically diverse, CAM appears to have arisen in only one taxonomic lineage in the ferns. Specifically, all CAM ferns are found in the Polypodiaceae (Kluge et al.

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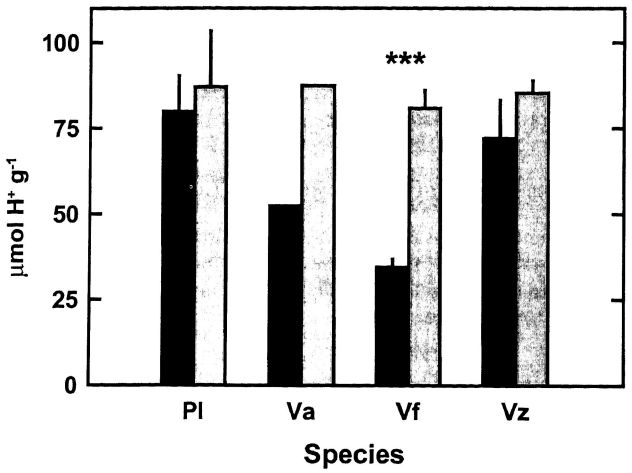

Fig. 1 Evening (black bars) and morning (gray bars) titratable acidity (dry-mass basis) of leaves of well-watered individuals of *Pyrrosia lingua* (*Pl*), *Vittaria auguste-elongata* (*Va*), *Vittaria flexuosa* (*Vf*), and *Vittaria zosterifolia* (*Vz*). Values are means (error bars represent standard errors) of three plants (only one plant of *V. auguste-elongata* was available) that were used in day/night CO_2 exchange measurements. Plants were collected in northeastern Taiwan and then grown in growth chambers in Kansas. Environmental conditions in the gas exchange chambers are provided in "Material and Methods." Asterisks indicate that the evening and morning means are significantly different at $P < 0.001$.

1989; Holtum and Winter 1999). To date, seven species in four genera have been reported as CAM taxa on the basis of measurements of diel acid fluctuations, nocturnal CO_2 uptake, or stable carbon isotope ratios (Hew and Wong 1974; Wong and Hew 1976; Winter et al. 1983; Hew 1984; Sinclair 1984; Ong et al. 1986, 1997; Griffiths 1989; Kluge et al. 1989; Martin et al. 1995; Holtum and Winter 1999). Thus, CAM appears to have evolved only once in the ferns, unlike the situation in the angiosperms. Several years ago, this statement was challenged by a preliminary report of nocturnal acid accumulations characteristic of CAM in a Costa Rican species of Vittariaceae (Carter and Martin 1994). It was the purpose of this study to investigate, in a more thorough manner, the photosynthetic pathway of this species of fern and to determine whether other taxa in the Vittariaceae, as well as selected species in several other families of epiphytic ferns, exhibit the CAM pathway. The findings of this study are important in consideration of the evolution of the CAM photosynthetic pathway among plants.

Material and Methods

Study Sites and Plants

All plants in this study were epiphytic and grew on a wide range of host species. Although most of the taxa were in the Vittariaceae, some plants of four other families of ferns were included. Plants were collected and/or investigated *in situ* in Taiwan and Costa Rica. Further investigations in the lab were conducted in Taiwan and Kansas.

In Taiwan, plants were collected in a subtropical rain forest (Fushan Experimental Forest) at 600 m elevation located 33 km southeast of Taipei. Plants were collected from both disturbed and undisturbed portions of the forest. Climatic conditions at Fushan are subtropical, with daily average air temperatures ranging from 5.7° to 26.9°C (annual mean of 18.6°C) and annual rainfall ranging from 2.8 to 6.8 m (1992–2003), with monthly maxima occurring in the summer. Plants were removed from host trees, transported to Taichung (115 km southwest of Fushan), and affixed to wooden planks under shadecloth on a rooftop for less than a week. Plants were watered daily before they were measured. Environmental conditions for these plants were as follows: maximum photosynthetic photon flux density (PPFD) of ca. 500 μmol m^{-2} s^{-1} (13.5-h daylength), day maximum air temperature of 34°C, night minimum air temperature of 24°C, daytime average humidity ca. 60%, and nighttime average humidity ca. 90%. These plants were used for acid titrations. In addition, some plants were removed from host trees at Fushan and shipped to Kansas, where they were placed in aquariums in a growth chamber under the following environmental conditions: ca. 100 μmol m^{-2} s^{-1} PPFD (12-h photoperiod), 25°/20°C day/night air temperatures, and ca. 50%/70% day/night relative humidities. Rhizomes were placed in 1–2 cm of soil. These plants were used for acid and gas exchange measurements after 3 mo of growth.

In Costa Rica, plants were examined *in situ* or collected for transport to Kansas. The study site was within and in the vicinity of Golfito at an elevation of 10–50 m. The annual

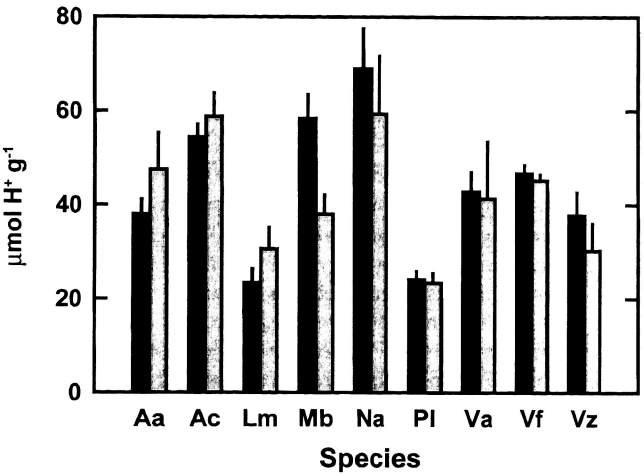

Fig. 2 Evening (black bars) and morning (gray bars) titratable acidity (fresh-mass basis) of leaves of well-watered individuals of *Asplenium antiquum* Makino (*Aa*), *Asplenium cuneatiforme* H. Christ (*Ac*), *Lemmaphyllum microphyllum* C. Presl (*Lm*), *Microsorium buergerianum* (Miq.) Ching (*Mb*), *Nephrolepis auriculata* (L.) Trimen (*Na*), *Pyrrosia lingua* (*Pl*), *Vittaria auguste-elongata* (*Va*), *Vittaria flexuosa* (*Vf*), and *Vittaria zosterifolia* (*Vz*). Values are means (error bars represent standard errors) of measurements made on three consecutive days for the same plants. Plants were collected in northeastern Taiwan and then transported to southwestern Taiwan for sampling (see "Material and Methods"). In no cases were evening and morning means significantly different ($P > 0.05$).

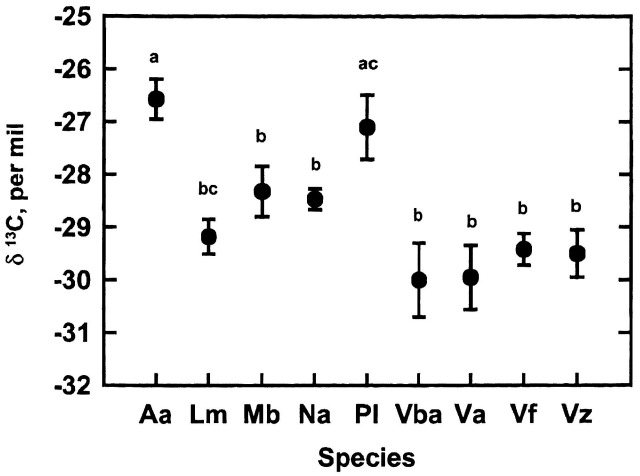

Fig. 3 Stable carbon isotope ratios of leaves of *Asplenium antiquum* (*Aa*), *Lemmaphyllum microphyllum* (*Lm*), *Microsorium buergerianum* (*Mb*), *Nephrolepis auriculata* (*Na*), *Pyrrosia lingua* (*Pl*), *Vandenboschia auriculata* (Blume) Copel. (*Vba*), *Vittaria auguste-elongata* (*Va*), *Vittaria flexuosa* (*Vf*), and *Vittaria zosterifolia* (*Vz*). Values are means (error bars represent standard errors) of three or four plants. Plants were collected in northeastern Taiwan. Means sharing the same letter are not significantly different ($P > 0.05$).

daily temperatures in this area range from 22.4° to 32.3°C, with a mean of 27.4°C, and the average annual rainfall ranges from 4 to 5 m (Coen 1983). At the times of field sampling, rain fell intermittently but seldom for longer than several hours, mornings were sunny, and afternoons were cloudy or partly cloudy. Maximum daytime temperatures were ca. 30°C. Once in Kansas, plants were placed in aquariums as previously described and then placed in a growth chamber under the following environmental conditions: 50–75 μmol m^{-2} s^{-1} PPFD (12-h photoperiod), 25°/20°C day/night air temperatures, and ca. 100% relative humidity day and night. These plants were used for acid and gas exchange measurements after 3 mo of growth.

Determinations of Diel Tissue Acid Fluctuations

Tissue acidity was determined in the laboratory in Taichung and in Kansas and in the field in Costa Rica. In the lab, tissue was ground and titrated after it was removed from a freezer (−10°C), whereas tissue in the field was ground and titrated immediately after it was removed from the plants *in situ*. For all plants, a leaf or portion of a leaf was removed at the end of the light period (evening) and frozen (or titrated). This was then repeated at the end of the dark period or shortly thereafter (morning).

In one case with *Anetium citrifolium*, leaf tissue was removed from plants in the field near Golfito, packed in ice in a thermos bottle for 24 h during transport to San Jose, and then frozen for 2 d. The frozen tissue was then packed in ice in a thermos bottle, transported to Kansas, frozen overnight, and analyzed the next day. Leaf samples of *Tillandsia balbisiana*, an epiphytic bromeliad known to be a CAM plant (Martin 1994), were collected and treated in an identical fashion. The difference between the acidity of the evening and morning leaf samples was typical for this plant (data not shown), indicating that the unusual treatment of the collected tissue did not adversely affect the detection of tissue acid fluctuations indicative of CAM. In all cases, the tissue was weighed and then ground in deionized water with a mortar and pestle; the resultant slurry was titrated to pH 7.0 with 0.01 N NaOH.

Determinations of Plant CO_2 Exchange

For several species, whole plants were sealed into gas exchange cuvettes for determination of CO_2 exchange. The rhizomes were washed of adhering soil and organic matter and kept moist. The latter prevented measurements of leaf transpiration but did not substantially alter rates or patterns of CO_2 exchange, as determined by measurements of the fronds of some plants when the rhizomes were sealed outside the chambers. Details of the cuvettes and the gas exchange system are provided in Harris and Martin (1991) and Gravatt and Martin (1992). The gas exchange data were analyzed using equations and theory from Šesták et al. (1971) and Farquhar and Sharkey (1982). Day/night air temperatures were maintained at 25°/20°C, and day/night air relative humidities were kept at 60%/80%. Light levels inside the cuvettes were 60 μmol m^{-2} s^{-1} PPFD (12-h photoperiod). For several species, some plants were not watered for 5–6 d in the growth chamber before measurements. Rhizomes of these plants were not kept moist during the gas exchange measurements.

Stable Carbon Isotope Analyses

Tissue collected in Taiwan or Costa Rica was oven-dried (70°C), pulverized, and then combusted for determination of

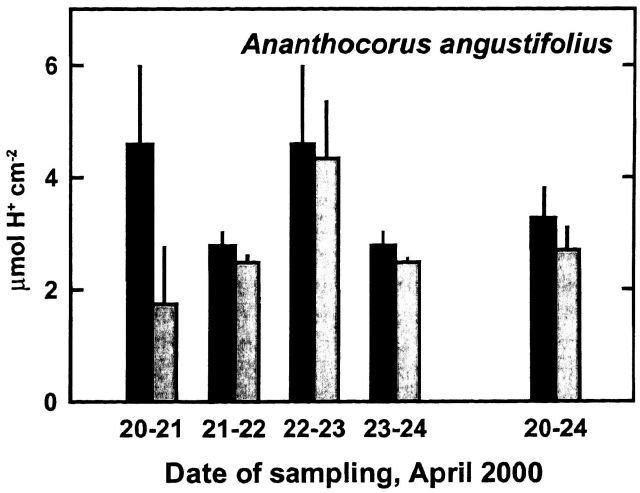

Fig. 4 Evening (black bars) and morning (gray bars) titratable acidity (leaf area basis) of leaves of *Ananthocorus angustifolius* (Sw.) Und. & Maxon. Values are means (error bars represent standard errors) of four (*20–21* and *21–22*) or two (*22–23* and *23–24*) plants (*20–24* represents all dates) sampled *in situ* in Costa Rica. Plants were well hydrated. In no cases were evening and morning means significantly different ($P > 0.05$).

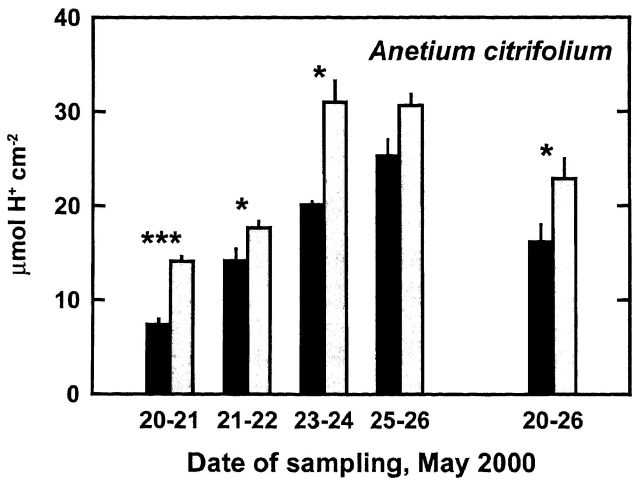

Fig. 5 Evening (black bars) and morning (gray bars) titratable acidity (leaf area basis) of leaves of *Anetium citrifolium* (L.) Splitgb. Values are means (error bars represent standard errors) of four plants (*20–26* represents all dates) sampled *in situ* in Costa Rica. Plants were well hydrated. One asterisk indicates that the evening and morning means were significantly different at $P < 0.05$, and three asterisks indicate a difference at $P < 0.001$.

the stable isotopic composition of its carbon at the University of Arkansas Stable Isotope Facility with a Carlo Erba elemental analyzer (NA1500 CHN Combustion Analyzer, Carlo Erba Strumentazione, Milan, Italy) coupled to a Finnigan Delta+ mass spectrometer (Finnigan MAT, Bremen, Germany) via a Finnigan Conflo II interface. The spectrometer had been calibrated using the Pee Dee Belemnite standard. The instrument error (twice the standard deviation) associated with each measurement was ±0.1‰. Leaf samples were collected from either epiphytes in the field in Fushan and Golfito or dried specimens at the University of San Jose (Costa Rica) herbarium (the fig. 9 legend provides collection sites).

Statistical Analyses

All evening and morning acidity means were compared using the Student's *t*-test; when the data did not meet the assumptions of the parametric *t*-test, the data were analyzed with the nonparametric Mann-Whitney *U*-test (Sokal and Rohlf 1981). The species means for the stable isotope data were compared using an ANOVA, followed by the Tukey multiple comparison-of-means test (Sokal and Rohlf 1981). In all tests, statistically significant differences were inferred only when $P \leq 0.05$.

Results

Day/night patterns of net CO_2 exchange in three species of *Vittaria* (Vittariaceae), as well as *Pyrrosia lingua* (Polypodiaceae), all collected in Taiwan, showed no evidence of CAM; i.e., CO_2 uptake occurred only during the day, and only CO_2 release was observed at night (data not shown). In two of these species, *Vittaria auguste-elongata* and *Vittaria flexuosa*, however, diel acid fluctuations indicative of CAM were measured in the same plants used for the gas exchange experiments (fig. 1), although only one plant of *V. auguste-elongata* was available for measurements, obviating the use of statistics with this species. No acid fluctuations were observed in the individuals of *Vittaria zosterifolia* and *P. lingua* used for the gas exchange measurements (fig. 1).

Although gas exchange was not measured for the other epiphytic ferns examined in Taiwan, measurements of diel acid fluctuations provided no evidence of CAM in nine species, including the four previously discussed, in four families of ferns (fig. 2). Statistics were used to analyze these data, although only one plant of each species was examined, albeit several leaves were sampled over a period of 3 d (hence the error bars in fig. 2).

Stable carbon isotopes were measured for leaves of nine species of epiphytic ferns collected in Taiwan, including the eight previously discussed. The resultant carbon isotope ratios indicated that all species assimilated atmospheric CO_2 through the C_3 photosynthetic pathway (fig. 3). Values for two of the ferns, *Asplenium antiquum* and *P. lingua*, were significantly higher (less negative) than those for the other ferns. Although this might reflect the more exposed (and potentially more stressful) microenvironment characteristic of *P. lingua*, the habitat of *A. antiquum* is not more exposed than those of the other species investigated (W.-L. Chiou, personal communication). Nonetheless, given the above acidity and gas exchange data, it is highly unlikely that the higher carbon isotope ratios reflected nighttime CO_2 uptake in either of these species.

The potential for diel changes in tissue acidity was measured for several consecutive days in three species of epiphytic

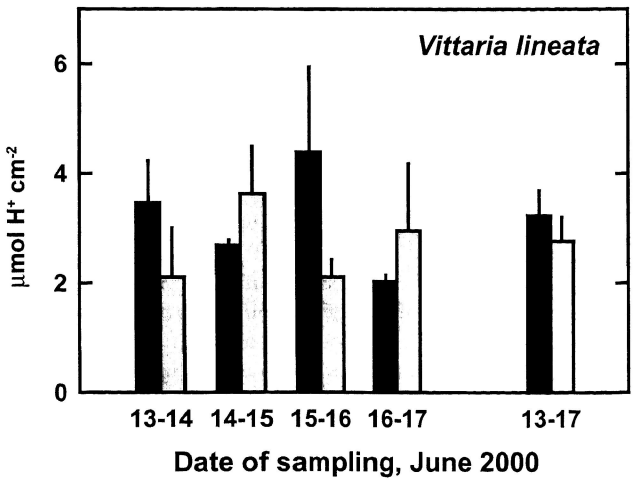

Fig. 6 Evening (black bars) and morning (gray bars) titratable acidity (leaf area basis) of leaves of *Vittaria lineata* (L.) Sw. Values are means (error bars represent standard errors) of four plants (*13–17* represents all dates) sampled *in situ* in Costa Rica. Plants were well hydrated. In no cases were evening and morning means significantly different ($P > 0.05$).

ferns in the Vittariaceae *in situ* in Costa Rica (figs. 4–6). Evidence of CAM acid fluctuations was found only in *Anetium citrifolium* (fig. 5). Morning tissue acidities were greater than evening acidities on three of the four days sampled.

Plants of *Ananthocorus angustifolius*, *A. citrifolium*, and *Vittaria lineata* were transported to Kansas for further analysis. Whether the plants were well watered or droughted for several days, on the basis of acidity (fig. 7) and gas exchange (data not shown) measurements, no evidence of CAM was found in *A. angustifolius* and *V. lineata*. This was generally true of *A. citrifolium* as well, although small but statistically significant CAM acid fluctuations were found on one occasion in well-watered plants (fig. 8). Although it may have been caused by a statistical aberration, given the relatively large number of experiments in which the potential for tissue acid fluctuations was examined, this result matches those found on three of four days in the field with this species. Because all CO_2 uptake by *A. citrifolium* occurred only during the day (data not shown), the results from the field, coupled with the laboratory data, indicate that this epiphytic fern has the capacity to undergo CAM cycling, especially in the field. Despite this, the plants usually engaged only in C_3 photosynthesis, at least in the lab. The lack of nocturnal assimilation of CO_2 from the atmosphere observed in the lab in any of the fern taxa is supported by measurements of stable carbon isotope ratios in *A. citrifolium* and 11 other epiphytic species of Vittariaceae collected in Costa Rica (fig. 9), which indicated that atmospheric CO_2 was assimilated solely by way of C_3 photosynthesis during the day.

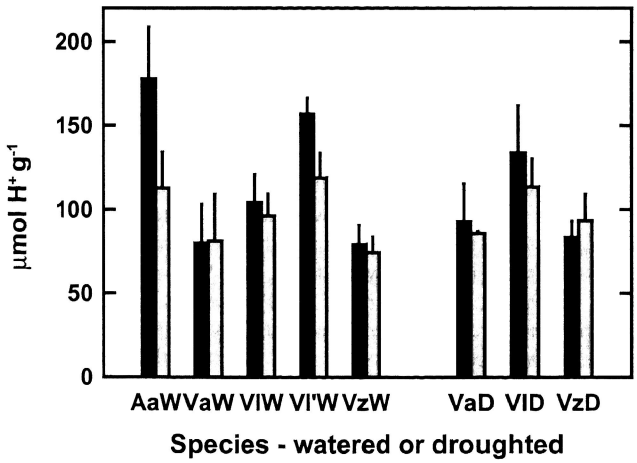

Fig. 7 Evening (black bars) and morning (gray bars) titratable acidity (dry-mass basis) of leaves of well-watered individuals of *Ananthocorus angustifolius* (*AaW*), *Vittaria auguste-elongata* (*VaW*), *Vittaria lineata* (*VlW* and *Vl'W*), and *Vittaria zosterifolia* (*VzW*) and of droughted (6 d without water) individuals of the three *Vittaria* species (*VaD*, *VlD*, and *VzD*). Values are means (error bars represent standard errors) of the following numbers of plants: *AaW* = 5–6; *VaW* and *VaD* = 2; *VlW* and *VlD* = 6–7; *Vl'W* = 5; *VzW* = 4; and *VzD* = 3. Plants were collected in southern Costa Rica and then grown and sampled in growth chambers (all but *AaW* and *Vl'W*) or gas exchange chambers (*AaW* and *Vl'W*) in Kansas. Environmental conditions in the chambers are provided in "Material and Methods." In no cases were evening and morning means significantly different ($P > 0.05$).

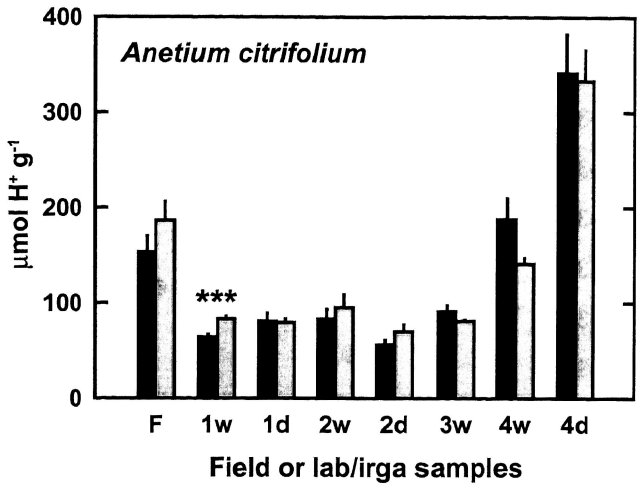

Fig. 8 Evening (black bars) and morning (gray bars) titratable acidity (dry-mass basis) of leaves of individuals of *Anetium citrifolium* in the field (*F*), in growth chambers under well-watered (*1w*, *2w*) or droughted (*5* d without water) conditions (*1d*, *2d*), and in gas exchange chambers under well-watered (*3w*, *4w*) or droughted (*5* d without water) conditions (*4d*). Identical numerals indicate that the same group of plants was used under well-watered and droughted conditions. Values are means (error bars represent standard errors) of the following numbers of plants: $F = 4$; *1w* and $1d = 5$; *2w* and $2d = 4$; *3w*, *4w*, and $4d = 3$–4. Plants were collected in southern Costa Rica and then grown and sampled in Kansas. Environmental conditions in the growth and gas exchange chambers are provided in "Material and Methods." In only one case were evening and morning means significantly different (asterisks indicate that $P < 0.001$).

Discussion

In this study, the photosynthetic pathway of 19 species of tropical/subtropical epiphytes in five families of ferns from Taiwan and Costa Rica was investigated in the field and in the lab. Some evidence of CAM was found in two of those species, and evidence of CAM was not always found when plants of these taxa were examined on different days and under different conditions. Nonetheless, the potential for CAM acid fluctuations was conclusively discovered in *Vittaria flexuosa* and *Anetium citrifolium*. Because CAM acid fluctuations were never accompanied by nighttime CO_2 uptake in the few instances in which the latter was measured and because the stable carbon isotope ratios of the leaf tissue were highly negative (note that Winter et al. 1983 and Zotz and Ziegler 1997 also reported carbon isotope ratios indicative of C_3 gas exchange in five species of Vittariaceae, including *A. citrifolium*), the results provide evidence of CAM cycling in these two species, not CAM *sensu stricto*.

Plants with CAM cycling, by definition, exhibit CAM acid fluctuations in the absence of nighttime CO_2 uptake (Ting 1985; Martin 1996). Although this form of photosynthetic

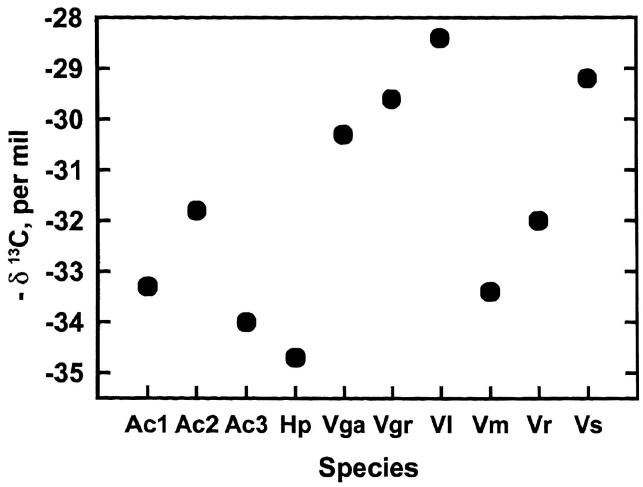

Fig. 9 Stable carbon isotope ratios of leaves of *Anetium citrifolium* (*Ac1* and *Ac2* for two plants from Golfito; *Ac3* for a plant from Sarapiquí), *Hecistopteris pumila* (Spr.) J. Sm. (*Hp*; from Puerto Viejo, Sarapiquí), *Vittaria gardneriana* Fée (*Vga*; collection location unknown), *Vittaria graminifolia* Kaulf. (*Vgr*; from Pérez Zeledón), *Vittaria lineata* (*Vl*; from Golfito), *Vittaria minima* (Bak.) Benedict (*Vm*; from Las Cruces, Cota Brus), *Vittaria remota* Fée (*Vr*; from Cartago), and *Vittaria stipitata* Kunze (*Vs*; from San Vito de Java, Puntarena). Values are for single plants that were collected throughout Costa Rica.

intermediacy has been reported for a large number and wide variety of terrestrial angiosperms (Martin et al. 1988; Martin 1996), it is unreported in ferns and rare in epiphytes in general (e.g., for bromeliads, Martin 1994 and Zotz 1997; for a species of orchid, Zotz and Tyree 1996). Thus, the finding of CAM cycling in *V. flexuosa* and *A. citrifolium* comprises the first report of this poorly understood combination of photosynthetic pathways in epiphytic ferns. The ecophysiological significance of CAM cycling is currently poorly understood, although hypothetical benefits include conservation of carbon, conservation of water, and daytime reduction in potential photoinhibition (Ting 1985; Martin et al. 1988; Harris and Martin 1991; Herppich 1997; Herppich et al. 1998). It has also been suggested that CAM cycling itself confers little benefit to a plant but serves as a precursor to CAM-idling, which may benefit plants with CAM biochemistry when they are under severe drought stress (Rayder and Ting 1981; Sipes and Ting 1985; Martin 1996).

Given the extremely high rainfall characteristic of the environments in Fushan (Taiwan) and Golfito (Costa Rica) in which the two species exhibiting evidence of CAM cycling were found, coupled with the high humidity and deep shade of their microenvironment (all species were found in the rain forest understory, and *A. citrifolium* was growing on the lower parts of tree trunks overhanging a stream), it is difficult to envision drought stress as a problem for these epiphytes. Furthermore, photoinhibition in these habitats would presumably be an infrequent occurrence. Thus, the hypothetical benefits of CAM cycling commonly suggested do not appear to be viable for these tropical epiphytic ferns. Although the evolution of CAM in response to elevated nocturnal CO_2 concentrations in the dense canopy of a rain forest is an interesting proposition (Knauft and Arditti 1969; Benzing 1990; Carter and Martin 1994), it would not appear to apply to CAM cycling because the stomata of such plants are closed at night, preventing capitalization on the increased nighttime availability of CO_2. Thus, the presence of CAM cycling, albeit sporadic, in these tropical epiphytic ferns is puzzling and worthy of further investigation.

Although the results of this study do not support those of Carter and Martin (1994), in that no evidence for CAM (or CAM cycling) was found in *Vittaria lineata*, CAM cycling was found in two other species in the Vittariaceae, including a species of *Vittaria*. Given the sporadic nature of CAM cycling in *V. flexuosa* and *A. citrifolium* uncovered in this study, it seems likely that CAM (or CAM cycling) may also be a sporadic phenomenon in *V. lineata*, which could explain the disparity between the findings of this study and those of Carter and Martin (1994). This is not the first indication that CAM might be a highly variable feature of some epiphytic ferns. Winter et al. (1983) presented stable carbon isotope ratios for *Pyrrosia confluens* (Polypodiaceae) that ranged from −19.2‰ to −25.3‰, possibly reflecting sampling of different plants with varying mixtures of C_3 and CAM photosynthesis (Griffiths 1993; Pierce et al. 2002; Winter and Holtum 2002). Their data for *Pyrrosia rupestris* could be interpreted similarly. In addition, Holtum and Winter (1999) reported "weak CAM" and CAM cycling in three epiphytic species in the Polypodiaceae. Findings of C_3-CAM flexibility are by no means limited to epiphytic ferns; some members of the Clusiaceae and the Crassulaceae, for example, are particularly plastic in this regard (Gravatt and Martin 1992; Pilon-Smits et al. 1996; Smirnoff 1996; Hietz et al. 1999; Lüttge 1999; Wanek et al. 2002).

The findings of this study provide more evidence that CAM, *sensu lato*, does indeed occur in the fern family Vittariaceae. This is an important finding because all previous evidence for CAM in the ferns was limited to taxa in one fern family, the Polypodiaceae. Thus, this confirmation of CAM in another distinct family of ferns, the Vittariaceae (Hasebe et al. 1995; Schneider et al. 2004), further emphasizes the polyphyletic nature of the evolution of the CAM photosynthetic pathway.

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