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Distribution of crassulacean acid metabolism in orchids of Panama: evidence of selection for weak and strong modes

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Abstract. Crassulacean acid metabolism (CAM) is one of three metabolic pathways found in vascular plants for the assimilation of carbon dioxide. In this study, we investigate the occurrence of CAM photosynthesis in 200 native orchid species from Panama and 14 non-native species by carbon isotopic composition ($\delta^{13}C$) and compare these values with nocturnal acid accumulation measured by titration in 173 species. Foliar $\delta^{13}C$ showed a bimodal distribution with the majority of species exhibiting values of approximately −28‰ (typically associated with the C_3 pathway), or −15‰ (strong CAM). Although thick leaves were related to $\delta^{13}C$ values in the CAM range, some thin-leaved orchids were capable of CAM photosynthesis, as demonstrated by acid titration. We also found species with C_3 isotopic values and significant acid accumulation at night. Of 128 species with $\delta^{13}C$ more negative than −22‰, 42 species showed nocturnal acid accumulation per unit fresh mass characteristic of weakly expressed CAM. These data suggest that among CAM orchids, there may be preferential selection for species to exhibit strong CAM or weak CAM, rather than intermediate metabolism.

Keywords: carbon stable isotope, crassulacean acid metabolism, evolution, Orchidaceae, photosynthesis.

Introduction

Crassulacean acid metabolism is one of three metabolic pathways found in vascular plants for the assimilation of atmospheric CO_2. In contrast to C_3 and C_4 photosynthesis, CAM is characterised by CO_2 uptake at night, improving the ability of plants to acquire carbon in water-limited and CO_2-limited environments (Winter *et al*. 2005). The CAM pathway is taxonomically widespread among vascular land plants and is found in many succulent species in semi-arid regions, as well as in tropical epiphytes. Uncertainty exists regarding the total number of CAM species among the more than 260 000 species of vascular plants. Excluding the Orchidaceae, recent estimates suggest that there are approximately 9000 species of CAM plants (Winter and Smith 1996). The Orchidaceae represent one of the largest families of vascular plants and contain approximately 20 000 species, of which about three-quarters are estimated to be tropical epiphytes (Atwood 1986; Dressler 1993*b*). The Orchidaceae alone may contribute an additional 7000 species that engage in CAM activity, thus raising the total number of species in which the CAM cycle is present to around 16 000 (Winter and Smith 1996). The purpose of this study is to determine the occurrence of CAM and the extent of CAM activity in a group of orchids from the Republic of Panama, to better assess the functional diversity of Orchidaceae and to better estimate the number of CAM species worldwide.

Because of differential enzyme-mediated discrimination against $^{13}CO_2$ during photosynthetic carbon assimilation between CAM and C_3 photosynthetic pathways (Bender *et al*. 1973; Osmond *et al*. 1973), CAM and C_3 plants exhibit different, but overlapping whole-tissue carbon isotope ratios ($\delta^{13}C$). For CAM species, $\delta^{13}C$ values ranging from −22 to −10‰ have been reported, whereas for C_3 plants, $\delta^{13}C$ values may range from −35 to −20‰ (Ehleringer and Osmond 1989). Thus, $\delta^{13}C$ has been employed as a rapid

Abbreviations used: $\delta^{13}C$, carbon isotopic composition; CAM, crassulacean acid metabolism; SLA, specific leaf area.

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screening method for the presence of CAM activity (Rundel *et al*. 1979; Winter 1979; Winter *et al*. 1983; Kluge *et al*. 1991; Zotz and Ziegler 1997; Crayn *et al*. 2001; Zotz 2004). Despite the fact that whole-tissue $\delta^{13}C$ is also affected by diffusional limitations, plant biochemistry and the $\delta^{13}C$ of source air (O'Leary 1981; Farquhar *et al*. 1989; Griffiths 1992), broad surveys of potential CAM activity utilising plant $\delta^{13}C$ have often produced bimodal distributions of $\delta^{13}C$ values with peaks around −13‰ (signifying strong CAM) and −27‰ (signifying C_3 photosynthesis) (Pierce *et al*. 2002; Crayn *et al*. 2004; Holtum *et al*. 2004). Intermediate values are often interpreted to signify the relative contributions of CAM and C_3 photosynthetic activity (Osmond *et al*. 1973). In fact, O'Leary (1988) predicted a linear relationship between whole-tissue $\delta^{13}C$ values of CAM plants and the fraction of CO_2 fixation occurring during the night and day. This prediction is supported by recent evidence based on quantification of the proportion of CO_2 fixed during the light and dark, and isotopic analysis of the biomass accumulated (Winter and Holtum 2002), in a study that also demonstrated that plants with $\delta^{13}C$ values characteristic of C_3 plants may obtain up to one-third of their carbon through CAM activity. This finding highlights a limitation to surveys that solely employ isotopic composition to estimate the occurrence of CAM and calls for analysis of the extent to which low-level CAM activity is occurring within the C_3 isotopic range, which has important implications regarding estimates of the total number of species in which CAM is expressed. Therefore, this study utilises analysis of nocturnal acidification in conjunction with isotopic composition to determine whether the isotopic distribution of species with CAM in Panamanian orchids is unimodal, with a peak around −15‰ and a skewed margin tailing out towards C_3-type values, or bimodal, with the C_3 isotopic cluster obscuring a second peak of abundance indicative of species with low capacities for dark CO_2 fixation.

Materials and methods

Plant material and cultivation

Plant material was obtained from the commercial greenhouses of Orquideas Tropicales, Inc. (http://www.orquideastropicales.com; validated 14 February 2005), in central lowland Panama, near the town of Chilibre (approximately 35 m above sea level). A total of 214 orchid species were used for the study, including 200 native Panamanian species and 14 non-native species that are commercially grown in Panama (Table 1). Plants were collected from the field over approximately 10 years and grown under semi-natural conditions in an open-sided shadehouse. We sampled 1–4 individuals in the adult vegetative stage for each species. Daily temperature within the shadehouse ranged from approximately 20.3–32.2°C, and light availability at different locations within the greenhouse varied from 7–99% of full sun, corresponding roughly to the natural growing conditions of these plants. Plants were watered daily and nutrients were supplied twice a week with a combination of slow-release fertiliser (Nutricote, Chisso-Asahi Fertiliser Co. Pty Ltd, Tokyo, Japan) and commercial 20–20–20 and 16–32–16 (N–P–K) fertiliser solutions.

Orchid species and nomenclature

We based our nomenclature on a combination of the Field Guide to the Orchids of Costa Rica and Panama (Dressler 1993*a*), recent publications on nomenclatural changes and the Missouri Botanical Garden's VAST (VAScular Tropicos) nomenclatural database and associated authority files (http://mobot.mobot.org/W3T/Search/vast.html; validated 14 February 2005). Genera belonging to the Subtribe Oncidiinae followed nomenclatural changes published since Dressler (1993*a*) (Williams *et al*. 2001*a*, *b*; Dressler and Williams 2003). Similarly, genera belonging to the Subtribe Laeliinae followed recent nomenclatural changes (Higgins 1997; Dressler 2002; Dressler and Higgins 2003) and genera belonging to the Subtribe Pleurothallidinae were based on updated information (Pridgeon and Chase 2001; Pridgeon *et al*. 2001; Luer 2004). The genus *Heterotaxis* has been included in this publication (Ojeda *et al*. 2005). Asian species that are naturalised in Panama, such as *Arundina graminifolia* (Don) Hochr. and *Spathoglottis plicata* Blume, were included as native species (Table 1). Plants identified to genus, but not to species (due to lack of keys, e.g. *Stelis* sp., or uncertainty in delimitation of species names, e.g. *Pleurothallis* sp.) were clearly differentiated from remaining members of the genus present in the greenhouse based on floral and vegetative morphology and were included as separate species. All species used in this study are clearly identified and are maintained in a live collection at Orquideas Tropicales, Inc. for further studies. Vouchers of all species are to be deposited in the herbarium of the Smithsonian Tropical Research Institute in Panama as plants bloom, to ensure comparison of datasets for future research.

Orchids used in this study are epiphytic except for five species that are terrestrial (*Arundina graminifolia* (D. Don) Hochr., *Peristeria elata* Hook., *Phragmipedium longifolium* (Rchb. f. & Warsz.) Rolfe, *Sobralia bletiae* Rchb. f. and *Spathoglottis plicata* Blume) and four species that can have epiphytic and terrestrial life forms (*Sobralia chrysostoma* Dressler, *Sobralia decora* Batem., *Sobralia macrophylla* Rchb. f. and *Sobralia wilsoniana* Rolfe).

Leaf thickness and carbon isotope ratio

The thickness of the leaf lamina was measured on fully expanded mature leaves with a micrometer (Mitutoyo, Kawasaki, Japan) during the dry season (March) of 2003. $^{13}C/^{12}C$ ratios were determined for CO_2 derived from 2–4-mg samples of dried tissue of one fully expanded mature leaf per species. Leaf material was analysed at the University of Georgia, Institute of Ecology, with an isotope ratio mass spectrometer. Isotope ratios were calculated relative to the Pee Dee belemnite standard according to the relationship:

$$\delta^{13}C(‰) = [(^{13}C/^{12}C \text{ in sample})/(^{13}C/^{12}C \text{ in standard}) - 1] \times 1000. \quad (1)$$

Leaf characteristics and titratable acidity

Leaf samples were collected from plants during the wet seasons of 2003 and 2004 (August–December). To measure leaf titratable acidity, 3–6 samples per species were taken at the end of the light period (evening, 1745–1830 h) and at the end of the dark period (morning, between 0500–0620 h). In *Trichocentrum caloceras* Endres & Rchb. f., sample size was two at each time point because of limited availability of plant material. Each sample consisted of 3–10 leaf discs of 0.8 cm^2 collected from the central part of the leaf while avoiding major veins when leaves were large enough. For species with very small leaves, or leaves that were too fibrous for the collection of discs, whole leaves or leaf cuts made with scissors or razor blades were collected and their areas were drawn manually on paper. A total of 173 species and >1400 leaf sample titrations were analysed. All leaf samples were weighed before freezing in liquid nitrogen as soon as they were collected so that

Table 1. $\delta^{13}C$ values, leaf traits, and nocturnal fluctuations in titratable acidity for 200 Panamanian native orchid species and 14 non-native species

Titratable acidity represents the mean ± SD of 3–6 replicates at morning and evening, except for *Trichocentrum caloceras* ($n = 2$). * Denotes significance between means of morning and evening at $P < 0.05$ as determined by a Student's *t*-test. NS, not significant

Species name	Leaf $\delta^{13}C$	Leaf thickness (mm)	FM / DM	SLA ($cm^2\ g^{-1}$)	H^+ (evening) ($\mu mol\ H^+\ g^{-1}$ FW)	H^+ (morning) ($\mu mol\ H^+\ g^{-1}$ FW)	ΔH^+
Native to Panama							
Acineta sp.	−28.1	0.55	7.1 ± 0.3	197 ± 20	20.0 ± 0.7	25.1 ± 4.8	5.1 NS
Ada allenii (L. O. Williams ex C. Schweinf.) N. H. Williams	−28.2	0.61	8.1 ± 0.5	191 ± 13	18.4 ± 3.1	19.6 ± 0.5	1.2 NS
Ancipitia crocodiliceps (Rchb. f.) Luer	−28.3	1.14	8.7 ± 0.9	80 ± 11	9.5 ± 1.8	10.4 ± 1.0	0.9 NS
Arundina graminifolia (D. Don) Hochr.	−26.5	0.32	3.5 ± 0.2	119 ± 10	11.5 ± 0.4	11.7 ± 0.8	0.2 NS
Aspasia epidendroides Lindl.	−27.9	0.57	5.3 ± 0.2	147 ± 6	3.5 ± 0.3	8.5 ± 0.2	5.0 *
Aspasia principissa Rchb. f.	−27.9	0.25	4.5 ± 0.2	149 ± 10	35.6 ± 4.2	38.9 ± 2.4	3.2 NS
Brassavola acaulis Lindl.	−15.1	5.01	16.9 ± 0.2	35 ± 3	7.7 ± 1.6	31.6 ± 3.8	23.9 *
Brassavola nodosa (L.) Lindl.	−13.8	3.81	12.5 ± 0.5	37 ± 1	39.5 ± 1.4	96.2 ± 9.4	56.7 *
Brassia arcuigera Rchb. f.	−25.2	0.47	5.3 ± 0.3	86 ± 3	27.2 ± 1.9	32.9 ± 1.5	5.7 *
Brassia caudata (L.) Lindl.	−27.0	0.45	5.5 ± 0.3	133 ± 5	5.5 ± 0.4	27.6 ± 1.3	22.1 *
Brassia verrucosa subsp. *gireoudiana* (Rchb. f. and Warsz.) Dressler and N. H. Williams	−21.3	0.48	6.5 ± 0.3	157 ± 15	9.6 ± 0.8	20.3 ± 0.7	10.7 *
Brenesia johnsonii (Ames) Luer	−26.4	1.23	8.4 ± 0.2	80 ± 2	5.0 ± 0.9	5.5 ± 0.4	0.5 NS
Brenesia lappiformis (A. Heller and L. O. Williams) Luer	−27.0	1.70	8.8 ± 0.8	77 ± 2	6.8 ± 0.7	9.1 ± 1.1	2.4 *
Catasetum bicolor Klotzsch	−25.3	0.18	5.9 ± 0.2	321 ± 43	8.7 ± 1.3	11.2 ± 2.0	2.5 NS
Catasetum maculatum Kunth	−26.8	0.24	5.0 ± 0.3	294 ± 22	40.3 ± 7.3	43.9 ± 6.3	3.6 NS
Catasetum sp.	−24.4	0.26					
Catasetum viridiflavum Hook.	−23.8	0.22	6.0 ± 0.2	299 ± 20	30.8 ± 4.1	30.5 ± 3.0	−0.3 NS
Cattleya dowiana Bateman	−16.2	2.15	9.9 ± 0.2	55 ± 1	16.9 ± 1.2	123.3 ± 10.6	106.4 *
Chelyorchis ampliatum (Lindl.) Dressler and N. H. Williams	−15.3	1.59	7.6 ± 0.1	74 ± 3	5.5 ± 1.3	153.5 ± 3.6	148.0 *
Cischweinfia dasyandra (Rchb. f.) Dressler and N. H. Williams	−30.3	0.43	6.1 ± 0.8	203 ± 29	19.7 ± 5.0	22.9 ± 2.8	3.2 NS
Cischweinfia pusilla (C. Schweinf.) Dressler and N. H. Williams	−27.3	0.47	7.1 ± 0.1	158 ± 9	29.8 ± 1.2	36.6 ± 3.2	6.8 *
Clowesia warscewiczii (Lindl. and Paxton) Dodson	−24.8	0.29	5.8 ± 0.4	278 ± 16	8.8 ± 2.5	9.6 ± 0.4	0.8 NS
Cochleanthes aromatica (Rchb. f.) R. E. Schult. and Garay	−24.3	0.49	5.9 ± 0.6	172 ± 19	37.4 ± 2.7	37.2 ± 0.9	−0.2 NS
Coeliopsis hyacinthosma Rchb. f.	−28.2	0.19	3.7 ± 0.3	186 ± 23	64.3 ± 10.0	79.3 ± 13.2	15.0 NS
Coryanthes hunteriana Schltr.	−26.3	0.25	5.8 ± 0.2	224 ± 7	4.4 ± 0.4	6.1 ± 0.3	1.7 *
Coryanthes sp.	−24.2	0.26					
Cycnoches aureum Lindl. and Paxton	−24.6	0.47	5.4 ± 0.2	266 ± 22	34.9 ± 4.4	35.1 ± 2.8	0.2 NS
Cycnoches guttulatum Schltr.	−24.1	0.19					
Cycnoches warscewiczii Rchb. f.	−28.2	0.31	4.8 ± 0.2	277 ± 21	40.9 ± 1.9	41.4 ± 9.1	0.6 NS
Cyrtochiloides ochmatochila (Rchb. f.) N. H. Williams and M. W. Chase	−26.7	0.62	5.9 ± 1.9	131 ± 28	14.8 ± 2.8	15.2 ± 2.1	0.4 NS
Dichaea dammeriana Kraenzl.	−30.6	0.23	5.5 ± 0.5	294 ± 37	60.4 ± 15.9	69.1 ± 7.8	8.7 NS
Dichaea fragrantissima Folsom	−25.8	0.29	6.2 ± 0.4	224 ± 12	25.0 ± 2.4	25.4 ± 1.9	0.4 NS
Dichaea sp.	−28.8	0.29					
Dimerandra emarginata (G. Mey) Hoehne	−26.3	0.39	5.3 ± 0.5	168 ± 21	11.4 ± 1.5	26.1 ± 6.8	14.7 *
Draconia tuerckheimii (Schltr.) Luer	−27.4	0.54	9.6 ± 1.7	114 ± 12	20.7 ± 1.9	19.1 ± 0.6	−1.6 NS
Dressleria sp.	−24.8	0.36					
Empusella endotrachys (Rchb. f.) Luer	−28.1	1.25	8.6 ± 0.5	93 ± 5	18.1 ± 2.2	20.5 ± 1.9	2.4 NS
Encyclia amanda (Ames) Dressler and Pollard	−14.9	1.15	5.2 ± 0.1	61 ± 7	24.8 ± 2.7	152.4 ± 10.1	127.6 *
Encyclia cordigera (Kunth) Dressler	−16.7	1.42	8.1 ± 0.7	55 ± 4	22.3 ± 0.3	219.2 ± 3.3	196.9 *
Encyclia mooreana (Rolfe) Schltr.	−16.2	0.67	5.5 ± 0.5	44 ± 4	20.3 ± 7.5	50.1 ± 17.0	29.8 *
Encyclia ramonensis (Rchb. f.) Schltr.	−19.0	0.77	4.9 ± 0.3	51 ± 4	15.9 ± 2.3	48.6 ± 9.7	32.7 *
Encyclia stellata (Lindl.) Schltr.	−18.0	0.90	5.2 ± 0.1	59 ± 3	31.2 ± 1.6	115.0 ± 7.0	83.8 *

(*Continued next page*)

Table 1. *continued*

Species name	Leaf $\delta^{13}C$	Leaf thickness (mm)	FM / DM	SLA ($cm^2\ g^{-1}$)	H^+ (evening) ($\mu mol\ H^+\ g^{-1}$ FW)	H^+ (morning) ($\mu mol\ H^+\ g^{-1}$ FW)	ΔH^+
Epidendrum bilobatum Ames	−27.3	0.35	7.0 ± 0.3	229 ± 15	27.1 ± 1.1	30.7 ± 3.4	3.6 NS
Epidendrum ciliare L.	−18.9	0.45	8.6 ± 0.2	54 ± 3	23.3 ± 2.5	129.6 ± 12.0	106.3 *
Epidendrum coronatum Ruiz and Pav.	−20.4	1.97	10.3 ± 0.2	58 ± 1	10.6 ± 0.3	166.2 ± 6.5	155.5 *
Epidendrum dentilobum Ames, F.T. Hubb. and C. Schweinf.	−21.2	0.50					
Epidendrum difforme Jacq.	−14.4	1.24	17.9 ± 0.9	81 ± 2	12.3 ± 3.7	46.7 ± 6.7	34.4 *
Epidendrum flexicaule Schltr.	−17.0	0.47	8.8 ± 1.0	113 ± 7	13.2 ± 1.1	79.2 ± 2.7	66.0 *
Epidendrum isthmi Schltr.	−28.2	0.48	8.8 ± 0.3	164 ± 17	14.6 ± 0.7	62.7 ± 0.8	48.1 *
Epidendrum lockhartioides Schltr.	−15.7	2.47	9.7 ± 2.6	62 ± 16	41.8 ± 5.4	88.2 ± 18.1	46.4 *
Epidendrum nocturnum Jacq.	−21.7	0.73	9.4 ± 0.6	149 ± 48	18.1 ± 3.6	93.4 ± 5.2	75.3 *
Epidendrum oerstedii Rchb. f.	−18.1	1.97	8.5 ± 0.5	52 ± 3	13.7 ± 0.5	134.2 ± 18.5	120.5 *
Epidendrum porpax Rchb. f.	−20.3	2.20	13.4 ± 4	103 ± 36	29.8 ± 8.3	83.1 ± 7.3	53.3 *
Epidendrum pseudepidendrum Rchb. f.	−26.9	0.32	5.6 ± 0.3	204 ± 26	19.0 ± 3.3	40.2 ± 7.4	21.2 *
Epidendrum radicans Pav. ex Lindl.	−16.2	1.57	9.7 ± 0.2	90 ± 2	12.4 ± 0.9	238.9 ± 6.4	226.5 *
Epidendrum rousseauae Schltr.	−20.4	1.52	15.0 ± 1.2	133 ± 6	13.1 ± 0.1	57.1 ± 1.4	44.0 *
Epidendrum schlechterianum Ames	−15.0	2.34	12.6 ± 0.6	85 ± 14	30.3 ± 7.3	102.1 ± 16.0	71.8 *
Epidendrum stamfordianum Bateman	−17.4	1.55	9.7 ± 0.2	84 ± 4	15.7 ± 2.1	86.7 ± 15.8	71.0 *
Eriopsis rutidobulbon Hook.	−24.8	0.61	4.9 ± 0.8	88 ± 29	22.9 ± 0.7	32.3 ± 0.4	9.4 *
Erycina crista-galli (Rchb. f.) N.H. Williams and M.W. Chase	−24.1	0.15					
Galeandra batemanii Rolfe	−27.5	0.60	4.9 ± 0.1	265 ± 37	30.4 ± 4.6	31.5 ± 8.5	1.0 NS
Galeottia grandiflora A. Rich. and Galeotti	−25.2	0.65	7.5 ± 0.5	187 ± 24	41.6 ± 1.9	44.5 ± 2.5	2.9 NS
Gongora armeniaca (Lindl. and Paxton) Rchb. f.	−29.0	0.25	4.5 ± 0.2	144 ± 10	22.0 ± 2.7	24.2 ± 1.5	2.3 NS
Gongora atropurpurea Hook.	−28.0	0.30	6.8 ± 0.8	236 ± 24	13.3 ± 0.0	13.6 ± 0.2	0.3 NS
Gongora claviodora Dressler	−29.8	0.23	8.1 ± 0.5	407 ± 62	4.6 ± 1.2	4.8 ± 0.2	0.2 NS
Gongora powellii Schltr.	−27.5	0.25	11.1 ± 0.7	432 ± 26	9.1 ± 1.6	7.5 ± 0.7	−1.6 NS
Gongora tricolor (Lindl.) Rchb. f.	−27.0	0.38	8.6 ± 0.5	322 ± 26	18.9 ± 2.5	19.5 ± 2.0	0.6 NS
Gongora unicolor Schltr.	−26.4	0.30					
Guarianthe patinii (Cogn.) Dressler and W. E. Higgins	−16.1	2.45	8.2 ± 1.2	38 ± 1	17.2 ± 5.1	126.8 ± 13.3	109.6 *
Heterotaxis sessilis (Swartz) F. Barros	−13.4	1.74	12.3 ± 0.9	60 ± 4	11.3 ± 1.0	71.2 ± 3.4	59.9 *
Heterotaxis valenzuelana (Nash) I. Ojeda and Carnevali	−28.4	2.65	8.3 ± 0.5	42 ± 5	21.4 ± 2.4	21.8 ± 1.3	0.4 NS
Huntleya burtii (Endres and Rchb. f.) Pfitzer	−27.6	0.30	5.3 ± 0.3	139 ± 13	10.2 ± 1.1	9.1 ± 0.6	−1.1 NS
Huntleya fasciata Fowlie	−27.1	0.66					
Ionopsis utricularioides (Sw.) Lindl.	−12.7	1.63	10.5 ± 0.7	77 ± 7	8.7 ± 0.7	284.4 ± 9.4	275.7 *
Kegeliella atropilosa L. O. Williams and A. H. Heller	−27.4	0.19					
Lockhartia acuta (Lindl.) Rchb. f.	−20.3	1.84	6.9 ± 0.5	76 ± 7	9.2 ± 0.5	21.8 ± 1.2	12.6 *
Lockhartia amoena Endres and Rchb. f.	−26.5	0.65	8.2 ± 0.5	176 ± 17	5.4 ± 0.2	8.5 ± 0.5	3.1 *
Lockhartia hercodonta Rchb. f. ex Kraenzl.	−29.0	0.41	5.7 ± 0.2	174 ± 21	6.9 ± 1.1	19.3 ± 5.1	12.4 *
Lockhartia micrantha Rchb. f.	−24.0	0.71	7.1 ± 0.3	191 ± 29	4.3 ± 1.0	17.9 ± 0.6	13.6 *
Lockhartia pittieri Schltr.	−28.3	0.43	5.5 ± 0.1	155 ± 20	19.2 ± 3.6	21.8 ± 2.3	2.6 NS
Lycaste macrophylla (Poepp. and Endl.) Lindl.	−27.6	0.27	4.7 ± 0.3	207 ± 19	34.2 ± 3.0	32.1 ± 2.0	−0.4 NS
Lycaste powellii Schltr.	−26.7	0.19					
Lycaste tricolor (Klotzsch) Rchb. f.	−27.2	0.18	5.1 ± 0.3	266 ± 43	68.9 ± 12.3	71.2 ± 10.7	2.3 NS
Macroclinium sp.	−14.5	1.00	9.2 ± 0.7	91 ± 22	25.2 ± 4.1	104.6 ± 30.7	79.4 *
Masdevallia lata Rchb. f.	−28.2	1.12	14.3 ± 0.5	141 ± 21	4.0 ± 2.0	6.3 ± 2.5	2.3 NS
Masdevallia tonduzii Woolward	−26.8	0.94	10.7 ± 0.5	88 ± 6	18.2 ± 3.0	18.9 ± 3.2	0.7 NS
Masdevallia zahlbruckneri Kraenzl.	−30.4	0.82					
Maxillaria attenuata Ames and C. Schweinf.	−31.6	0.71					
Maxillaria bicallosa (Rchb. f.) Garay	−29.6	0.52	5.7 ± 0.2	113 ± 2	19.0 ± 1.1	20.0 ± 1.4	1.0 NS

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Table 1. *continued*

Species name	Leaf $\delta^{13}C$	Leaf thickness (mm)	FM / DM	SLA ($cm^2\ g^{-1}$)	H^+ (evening) ($\mu mol\ H^+\ g^{-1}$ FW)	H^+ (morning) ($\mu mol\ H^+\ g^{-1}$ FW)	ΔH^+
Maxillaria camaridii Rchb. f.	−27.2	0.29	3.7 ± 0.2	131 ± 8	45.5 ± 2.7	45.0 ± 10	−0.5 NS
Maxillaria cryptobulbon Carnevali and J. T. Atwood	−31.7	0.92	7.4 ± 0.4	89 ± 5	11.1 ± 1.9	12.9 ± 2.5	1.8 NS
Maxillaria discolor (G. Lodd. ex Lindl.) Rchb. f.	−27.5	0.66	5.9 ± 0.9	94 ± 14	21.6 ± 0.1	28.8 ± 0.8	7.2 *
Maxillaria diuturna Ames and C. Schweinf.	−28.8	0.36	4.3 ± 0.4	120 ± 21	29.9 ± 6.3	32.8 ± 4.6	2.8 NS
Maxillaria endresii Rchb. f.	−29.8	0.67	6.1 ± 0.7	109 ± 2	19.8 ± 0.5	27.0 ± 1.2	7.2 *
Maxillaria fulgens (Rchb. f.) L. O. Williams	−28.5	0.68	4.6 ± 0.2	86 ± 4	39.9 ± 2.4	54.4 ± 2.9	14.5 *
Maxillaria ringens Rchb. f.	−28.7	0.96	6.1 ± 0.4	80 ± 3	6.1 ± 0.9	10.0 ± 1.1	3.9 *
Maxillaria sanguinea Rolfe	−31.0	0.59	4.0 ± 0.1	86 ± 18	18.9 ± 2.7	23.0 ± 7.7	4.1 NS
Maxillaria sp.	−27.1	1.72					
Maxillaria tenuifolia Lindl.	−29.0	0.52	4.8 ± 0.3	105 ± 19	12.8 ± 7.4	25.4 ± 6.1	12.6 NS
Maxillaria variabilis Bateman ex Lindl.	−29.8	0.28					
Miltoniopsis roezlii (Rchb. f.) Godefroy-Lebeuf	−27.6	0.37	6.1 ± 0.5	206 ± 20	16.4 ± 2.1	18.9 ± 14.3	2.5 NS
Mormodes fractiflexum Rchb. f.	−22.2	0.27	6.3 ± 0.6	236 ± 22	12.8 ± 1.1	18.9 ± 0.2	6.1 *
Mormodes lancilabris Pabst	−24.2	0.23	5.7 ± 0.3	278 ± 47	14.2 ± 1.7	19.8 ± 0.2	5.6 *
Mormodes powelli Schltr.	−23.5	0.36	6.1 ± 0.3	238 ± 31	15.6 ± 1.7	17.2 ± 0.9	1.6 NS
Mormodes punctatum Rolfe	−24.4	0.30	5.2 ± 0.3	229 ± 25	25.2 ± 3.1	28.8 ± 1.0	3.6 NS
Mormodes skinneri Rchb. f.	−23.0	0.25					
Notylia albida Klotzsch	−13.5	1.52	8.0 ± 0.5	65 ± 4	8.2 ± 0.9	135.4 ± 7.8	127.2 *
Notylia barkeri Lindl.	−11.8	1.31	5.3 ± 0.4	33 ± 3	4.8 ± 0.3	26.3 ± 1.2	21.5 *
Notylia pentachne Rchb. f.	−14.1	1.07	3.8 ± 0.1	30 ± 2	13.7 ± 4.3	48.8 ± 21.0	35.1 *
Oerstedella caligaria (Rchb. f.) Hágsater	−30.2	0.27	5.9 ± 0.2	255 ± 28	14.0 ± 3.7	14.0 ± 3.7	0.0 NS
Oerstedella pseudoschumanniana (Fowlie) Hágsater	−23.6	0.39	4.4 ± 0.3	106 ± 4	13.8 ± 0.9	32.3 ± 5.0	18.5 *
Oerstedella wallisii (Rchb. f.) Hágsater	−27.7	0.34					
Oncidium bracteatum Warsz. and Rchb. f.	−24.0	0.37	4.9 ± 0.3	127 ± 4	13.7 ± 1.5	26.3 ± 3.9	12.6 *
Oncidium carthagenense (Jacq.) Sw.	−12.2	2.32	11.2 ± 0.4	51 ± 5	12.5 ± 0.4	77.3 ± 3.4	64.8 *
Oncidium cheirophorum Rchb. f.	−27.4	0.36	4.7 ± 0.3	163 ± 25	29.9 ± 3.3	30.2 ± 2.7	0.3 NS
Oncidium dichromaticum Rchb. f.	−25.9	0.25	4.0 ± 0.3	156 ± 24	3.7 ± 0.2	7.1 ± 0.1	3.4 *
Oncidium fuscatum Rchb. f.	−24.6	0.50	4.9 ± 0.7	112 ± 24	16.8 ± 1.3	30.2 ± 7.1	13.4 *
Oncidium isthmi Schltr.	−26.9	0.50	6.3 ± 1.0	114 ± 16	11.7 ± 1.1	31.7 ± 1.2	20.0 *
Oncidium klotzscheanum Rchb. f.	−25.0	0.44					
Oncidium maduroi Dressler	−24.7	0.24	4.6 ± 0.1	179 ± 16	17.3 ± 1.9	19.5 ± 0.8	2.2 NS
Oncidium ornithorhynchum Kunth.	−25.2	0.25					
Oncidium panamense Schltr.	−26.2	0.54	6.3 ± 0.1	111 ± 3	11.5 ± 0.7	33.2 ± 0.3	21.7 *
Oncidium parviflorum L. O. Williams	−29.0	0.50	5.3 ± 0.1	114 ± 11	15.7 ± 2.3	18.0 ± 0.8	2.3 NS
Oncidium polycladium Rchb. f. ex Lindl.	−23.2	0.46					
Oncidium powellii Schltr.	−28.9	0.41					
Oncidium schroederianum (O'Brien) Garay and Stacy	−22.2	0.37	5.2 ± 0.5	144 ± 8	5.2 ± 0.4	6.9 ± 0.4	1.7 *
Oncidium stenotis Rchb. f.	−24.5	0.46	6.4 ± 3.1	153 ± 71	20.1 ± 0.8	20.5 ± 1.8	0.4 NS
Ornithocephalus bicornis Lindl.	−13.8	1.55	13.2 ± 1.4	106 ± 17	6.0 ± 1.3	41.5 ± 18.7	35.5 *
Ornithocephalus cochleariformis C. Schweinf.	−14.9	2.20	10.5 ± 1.5	90 ± 16	8.3 ± 2.2	28.8 ± 7.0	20.5 *
Peristeria elata Hook.	−27.0	0.34	5.2 ± 0.3	234 ± 4	28.6 ± 2.6	35.9 ± 0.4	7.3 *
Peristeria guttata Knowles and Westc.	−30.3	0.17	5.2 ± 0.2	309 ± 41	26.3 ± 1.9	34.8 ± 1.8	8.5 *
Peristeria sp.	−28.9	0.41	3.8 ± 0.3	155 ± 8	41.8 ± 5.8	62.9 ± 3.1	21.1 *
Pescatorea cerina Rchb. f.	−27.9	0.70	8.7 ± 0.2	138 ± 6	24.9 ± 0.7	27.2 ± 2.2	2.3 NS
Phloeophila pelecaniceps (Luer) Pridgeon and M. W. Chase	−28.7	0.82	9.5 ± 2.1	93 ± 10	10.8 ± 0.6	10.3 ± 1.0	−0.5 NS
Phragmipedium longifolium (Rchb. f. and Warsz.) Rolfe	−29.7	0.60	4.8 ± 0.2	64 ± 6	25.3 ± 1.1	24.6 ± 0.6	−0.7 NS

(*Continued next page*)

Table 1. *continued*

Species name	Leaf $\delta^{13}C$	Leaf thickness (mm)	FM / DM	SLA ($cm^2\ g^{-1}$)	H^+ (evening) ($\mu mol\ H^+\ g^{-1}$ FW)	H^+ (morning) ($\mu mol\ H^+\ g^{-1}$ FW)	ΔH^+
Pleurothallis leucantha Schltr.	−16.1	1.58	18.1 ± 2.2	138 ± 8	9.7 ± 0.4	82.9 ± 17.1	73.2 *
Pleurothallis racemiflora (Sw.) Lindl. ex Hook.	−24.6	1.87	13.1 ± 4.1	81 ± 37	5.9 ± 0.4	10.3 ± 0.5	4.4 *
Pleurothallis sp.	−29.7	1.19	5.4 ± 0.4	45 ± 4	10.7 ± 0.7	11.5 ± 1.2	0.8 NS
Polystachya sp.	−27.8	0.25					
Prosthechea abbreviata (Schltr.) W. E. Higgins	−26.5	0.25	4.6 ± 0.5	169 ± 25	44.5 ± 7.7	61.1 ± 13.2	16.6 NS
Prosthechea aemula (Lindl.) W. E. Higgins	−28.4	0.56	6.4 ± 0.9	112 ± 13	6.5 ± 0.5	15.1 ± 1.5	8.6 *
Prosthechea chacaoensis (Rchb. f.) W. E. Higgins	−28.6	0.47	6.9 ± 0.2	142 ± 9	8.9 ± 0.3	22.6 ± 1.4	13.7 *
Prosthechea chimborazoensis (Schltr.) W. E. Higgins	−30.4	0.60	6.8 ± 0.3	98 ± 3	9.9 ± 1.7	14.5 ± 0.9	4.6 *
Prosthechea prismatocarpa (Rchb. f.) W. E. Higgins	−25.4	0.72	4.4 ± 0.5	77 ± 24	32.3 ± 4.9	39.0 ± 7.0	6.7 NS
Prosthechea vespa (Vell.) W. E. Higgins	−28.2	0.50	7.1 ± 0.3	150 ± 12	36.2 ± 1.1	36.6 ± 2.9	0.4 NS
Psygmorchis pusilla (L.) Dodson and Dressler	−13.8	0.80					
Rhynchostele bictoniensis (Bateman) Soto Arenas and Salazar	−28.0	0.53					
Rodriguezia compacta Schltr.	−13.2	1.15	6.6 ± 0.4	69 ± 4	33.1 ± 1.2	102.3 ± 1.8	69.2 *
Rodriguezia lanceolata Ruiz and Pavon	−13.7	1.43	9.5 ± 0.4	60 ± 6	7.4 ± 0.5	184.8 ± 4.1	177.4 *
Scaphyglottis behrii (Rchb. f.) Benth. and Hook. f. ex Hemsl.	−31.0	0.32	5.2 ± 0.4	197 ± 25	14.4 ± 3.7	16.4 ± 2.5	2.0 NS
Scaphyglottis bidentata Lindl.	−26.8	0.31					
Scaphyglottis imbricata (Lindl.) Dressler	−27.0	0.37	3.0 ± 0.3	98 ± 17	17.7 ± 4.1	33.9 ± 1.7	16.2 *
Scaphyglottis laevilabia Ames	−30.2	0.24	3.5 ± 0.2	186 ± 20	31.5 ± 2.6	37.6 ± 3.2	6.1 NS
Scaphyglottis sp.	−24.9	0.29					
Schomburgkia undulata var. *lueddemannii* (Prill.) H. G. Jones	−15.3	1.35					
Sievekingia butcheri Dressler	−26.1	0.50	5.5 ± 0.3	115 ± 10	15.2 ± 1.7	16.9 ± 2.5	1.7 NS
Sigmatostalix macrobulbon Kraenzl.	−31.9	0.14					
Sigmatostalix picturatissima Kraenzl.	−28.8	0.23	4.6 ± 0.1	137 ± 14	32.6 ± 6.6	36.9 ± 1.1	4.3 NS
Sobralia bletiae Rchb. f.	−27.9	0.32	3.9 ± 0.1	133 ± 17	8.4 ± 0.5	22.8 ± 1.0	14.4 *
Sobralia callosa L. O. Williams	−27.2	0.58	2.7 ± 0.3	84 ± 16	16.3 ± 5.4	16.6 ± 1.4	0.3 NS
Sobralia chrysostoma Dressler	−27.8	0.43	3.5 ± 0.1	132 ± 4	29.3 ± 2.3	30.0 ± 2.4	0.7 NS
Sobralia decora Batem.	−29.0	0.31	3.3 ± 0.3	128 ± 11	24.4 ± 2.8	24.0 ± 4.9	−0.4 NS
Sobralia macrophylla Rchb. f.	−27.0	0.50	4.4 ± 0.3	148 ± 9	6.4 ± 0.2	6.4 ± 1.0	0.0 NS
Sobralia wilsoniana Rolfe	−27.0	0.37	3.2 ± 0.0	100 ± 4	25.4 ± 1.6	26.5 ± 0.4	1.2 NS
Spathoglottis plicata Blume	−28.9	0.22	4.6 ± 0.6	225 ± 95	29.0 ± 4.0	31.7 ± 0.9	2.7 NS
Specklinia barboselloides (Schltr.) Pridgeon and M. W. Chase	−29.3	1.72	6.5 ± 0.6	113 ± 11	8.6 ± 1.6	9.3 ± 1.0	0.7 NS
Specklinia barbulata (Lindl.) Luer	−21.0	0.63	5.8 ± 1.9	79 ± 19	7.3 ± 1.3	13.0 ± 2.1	5.7 *
Specklinia calyptrostele (Schltr.) Pridgeon and M. W. Chase	−27.3	0.10	9.9 ± 3.6	149 ± 44	88.8 ± 15.0	96.3 ± 11.6	7.6 NS
Specklinia fulgens (Rchb. f.) Pridgeon and M. W. Chase	−26.5	1.35	8.9 ± 0.4	95 ± 13	9.4 ± 2.7	16.2 ± 5.9	6.8 NS
Specklinia imraei (Lindl.) Luer	−29.0	0.90	8.9 ± 0.5	94 ± 3	13.1 ± 1.3	15.3 ± 2.0	2.2 NS
Stanhopea ecornuta Lem.	−27.1	0.37	4.1 ± 0.1	120 ± 9	13.8 ± 1.1	13.8 ± 0.6	0.0 NS
Stanhopea oculata (G. Lodd.) Lindl.	−29.6	0.53					
Stanhopea pulla Rchb. f.	−31.5	0.27	4.5 ± 0.4	130 ± 14	9.8 ± 3.0	11.0 ± 3.1	1.2 NS
Stanhopea sp.	−27.7	0.61					
Stanhopea wardii G. Lodd. ex Lindl.	−29.5	0.54	7.0 ± 0.3	180 ± 10	7.3 ± 1.1	8.1 ± 0.7	0.8 NS
Stelis sp. 1 (yellow form)	−25.1	1.00	11.5 ± 1.1	151 ± 8	15.8 ± 1.6	17.9 ± 2.3	2.1 NS
Stelis sp. 2	−26.5	1.23					
Ticoglossum krameri (Rchb. f.) Lucas Rodr. ex Halb.	−31.7	0.35	7.5 ± 0.9	148 ± 15	14.4 ± 2.5	14.8 ± 2.4	0.4 NS

(*Continued next page*)

Table 1. *continued*

Species name	Leaf $\delta^{13}C$	Leaf thickness (mm)	FM / DM	SLA ($cm^2\ g^{-1}$)	H^+ (evening) ($\mu mol\ H^+\ g^{-1}$ FW)	H^+ (morning) ($\mu mol\ H^+\ g^{-1}$ FW)	ΔH^+
Trichocentrum caloceras Endres and Rchb. f.	−14.4	2.39	15.6 ± 3.8	75 ± 20	16.0 ± 2.1	56.5 ± 0.7	40.5 *
Trichocentrum capistratum Rchb. f.	−13.4	1.74	11.2 ± 1.2	56 ± 8	16.2 ± 0.4	37.8 ± 5.5	21.7 *
Trichocentrum nudum (Bateman ex Lindl.) M. W. Chase and N. H. Williams	−14.5	10.02					
Trichocentrum nudum subsp. *stipitatum* (Lindl.) Dressler and N. H. Williams	−16.1	8.30	17.6 ± 0.4	19 ± 1	14.1 ± 0.6	35.5 ± 0.5	21.4 *
Trichocentrum teres (Ames and C. Schweinf.) M. W. Chase and N. H. Williams	−13.3	10.10	20.8 ± 1.6	36 ± 10	6.6 ± 0.3	48.1 ± 5.3	41.5 *
Trichopilia leucoxantha L. O. Williams	−29.3	0.46	5.2 ± 0.2	143 ± 9	10.5 ± 2.1	10.8 ± 2.2	0.3 NS
Trichopilia maculata Rchb. f.	−25.5	0.75	6.5 ± 0.3	91 ± 14	12.6 ± 3.3	25.8 ± 1.1	13.2 *
Trichopilia marginata Henfr.	−26.9	0.72	3.9 ± 0.2	67 ± 6	10.9 ± 0.6	12.0 ± 0.6	1.1 NS
Trichopilia sp.	−29.3	1.50	6.6 ± 1.0	128 ± 11	14.7 ± 3.1	14.9 ± 1.0	0.2 NS
Trichopilia suavis Lindl. and Paxton	−29.3	0.50	4.0 ± 0.2	86 ± 5	11.8 ± 0.9	13.9 ± 0.3	2.1 *
Trichosalpinx blaisdellii (S. Watson) Luer	−29.9	0.85	8.4 ± 2.5	98 ± 22	7.7 ± 1.5	7.1 ± 0.8	−0.6 NS
Trichosalpinx orbicularis (Lindl.) Luer	−25.1	0.95	5.2 ± 0.9	52 ± 6	15.5 ± 1.9	19.5 ± 2.7	4.0 NS
Trigonidium egertonianum Bateman ex Lindl.	−32.3	0.40	4.0 ± 0.6	121 ± 33	5.3 ± 0.5	9.3 ± 0.8	4.0 *
Trigonidium sp.	−27.3	0.59					
Vanilla pfaviana Rchb. f.	−23.4	0.56	10.5 ± 0.4	141 ± 5	29.4 ± 0.8	52.1 ± 1.8	22.7 *
Vanilla planifolia Andrews	−16.4	1.96	14.2 ± 0.5	63 ± 2	15.7 ± 0.2	151.8 ± 3.1	89.7 *
Vanilla pompona Schiede	−16.5	1.83	17.4 ± 0.4	92 ± 3	14.6 ± 0.2	118.8 ± 3.8	104.2 *
Warszewiczella lipscombiae (Rolfe) Fowlie	−27.9	0.43	6.4 ± 0.1	130 ± 11	16.5 ± 1.4	19.0 ± 2.0	2.5 NS
Xylobium colleyi (Bateman ex Lindl.) Rolfe	−27.9	0.45	6.3 ± 0.6	152 ± 14	40.6 ± 1.4	41.8 ± 6.6	1.2 NS
Xylobium elongatum (Lindl. and Paxton) Hemsl.	−28.3	0.26	4.2 ± 0.5	157 ± 13	12.3 ± 1.4	11.7 ± 1.2	−0.6 NS
Xylobium foveatum (Lindl.) G. Nicholson	−28.9	0.42					
Xylobium sulfurinum (Lem.) Schltr.	−28.2	0.37					
Non-native species							
Aspasia lunata Lindl.	−31.3	0.30					
Bulbophyllum macranthum Lindl.	−15.1	1.96	7.7 ± 0.2	68 ± 11	11.6 ± 1.6	37.3 ± 3.6	25.6 *
Bulbophyllum putidum (Teijsm. and Binn.) J.J. Sm.	−15.2	1.77	11.8 ± 1.5	99 ± 15	10.1 ± 6.3	23.5 ± 7.9	13.5 *
Coelogyne ovalis Lindl.	−25.8	0.32	5.5 ± 0.2	183 ± 25	17.7 ± 4.1	33.9 ± 1.7	11.8 *
Encyclia alata (Bateman) Schltr.	−16.1	1.14					
Epidendrum fulgens Brongn.	−16.9	1.10					
Lycaste aromatica (Graham ex Hook.) Lindl.	−26.6	0.20	4.8 ± 0.3	287 ± 14	59.1 ± 3.4	59.9 ± 2.7	0.8 NS
Miltonia bluntii (natural hybrid between *Miltonia clowesii* Lindl. x *Miltonia spectabilis* Lindl.)	−25.9	0.29					
Mormodes horichii Fowlie	−27.4	0.29	5.1 ± 0.3	224 ± 17	25.6 ± 2.6	25.1 ± 3.3	−0.5 NS
Myrmecophila tibicinis (Bateman) Rolfe	−13.7	1.85					
Oncidium flexuosum Sims	−24.4	0.26	5.8 ± 0.2	288 ± 37	37.9 ± 9.4	74.0 ± 10.4	36.1 *
Oncidium leucochilum Bateman ex Lindl.	−26.7	0.41	6.5 ± 0.2	164 ± 40	12.9 ± 2.2	15.6 ± 3.9	2.7 NS
Oncidium sphacelatum Lindl.	−27.9	0.53	5.0 ± 0.1	114 ± 8	8.3 ± 6.3	31.2 ± 2.1	22.9 *
Trichocentrum luridum (Lindl.) M. W. Chase and N. H. Williams	−13.6	1.11					

titratable acidity could be expressed per unit fresh leaf mass. Leaf area was measured on tracings of leaf-cuts or whole leaves with a LI3100 leaf area meter (Li-Cor, Lincoln, NE). Leaf samples were freeze-dried before titrations and dry mass was determined for calculation of the ratio of fresh mass to dry mass (FM / DM) and of specific leaf area (SLA; area per unit dry mass). FM / DM and SLA allow titratable acidity per unit fresh mass to be converted to a dry mass or leaf area basis for comparative purposes. Leaf samples were boiled sequentially in 20% ethanol and deionised water, and titratable acidity was measured as the amount of 5 or 10 mM NaOH required to neutralise extracts to pH 7.0 with a pH meter.

Results

Whole-tissue $\delta^{13}C$ values of orchid leaves ranged from a minimum of −32.3 to a maximum of −11.8‰. The frequency distribution of isotopic values showed bimodal distribution with a large mode at −28‰ and a smaller mode near −15‰ (Fig. 1). Leaf thickness varied from 0.1 mm in *Specklinia calyptrostele* (Schltr.) Pridgeon & M.W. Chase to 10.1 mm in *Trichocentrum teres* (Ames & C. Schweinf.) M.W. Chase & N.H. Williams (Table 1). Within the group of species with $\delta^{13}C$ values commonly observed for C_3 plants (−33 to −22‰), leaf thickness averaged 0.5 ± 0.4 mm (mean ± s.d.), whereas in species with $\delta^{13}C$ values usually associated with the CAM pathway (−20 to −12‰), leaf thickness averaged 2.2 ± 2.1 mm and in species with values in an intermediate range (−22 to −20‰), leaf thickness averaged 1.2 ± 0.7 mm. All leaves thicker than 3 mm had $\delta^{13}C$ values indicative of pronounced CAM (Fig. 2).

A total of 87 of 173 species exhibited significant differences ($P<0.05$) between evening and morning titratable acidity per unit fresh leaf mass (Fig. 3*A*) and $\delta^{13}C$ values of these 87 species spanned the C_3–CAM range (−32.3 to −11.8‰). The remaining 86 species, in which the differences between evening and morning titratable acidity were non-significant, spanned a range that was less enriched in ^{13}C (−31.7 to −23.5‰) (Fig. 3*B*). For species with $\delta^{13}C$ values more negative than −20‰, significant nocturnal acidity increase averaged 17.9 (range: 1.7–155.5) µmol H^+ per unit fresh leaf mass. For species with $\delta^{13}C$ values less

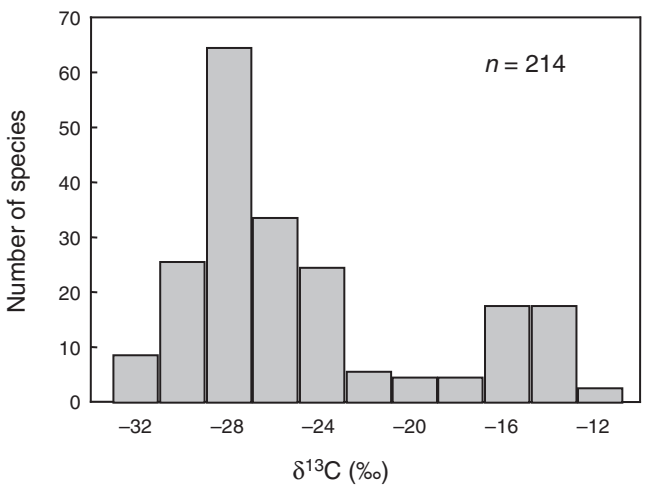

Fig. 1. Frequency of leaf $\delta^{13}C$ values of 214 species of orchids. Each bar represents a 2‰ range of $\delta^{13}C$.

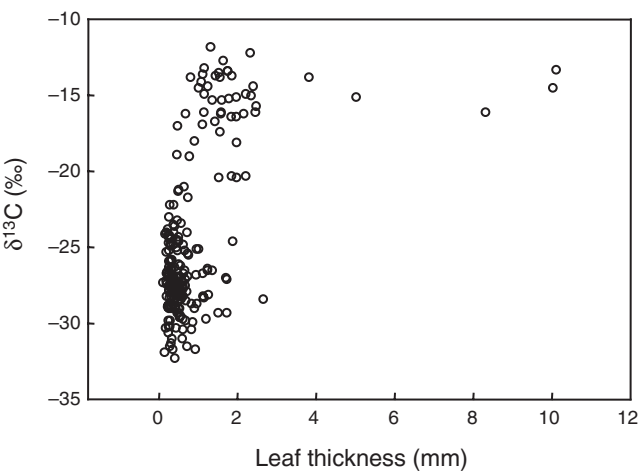

Fig. 2. Leaf $\delta^{13}C$ as a function of leaf thickness for 214 orchid species.

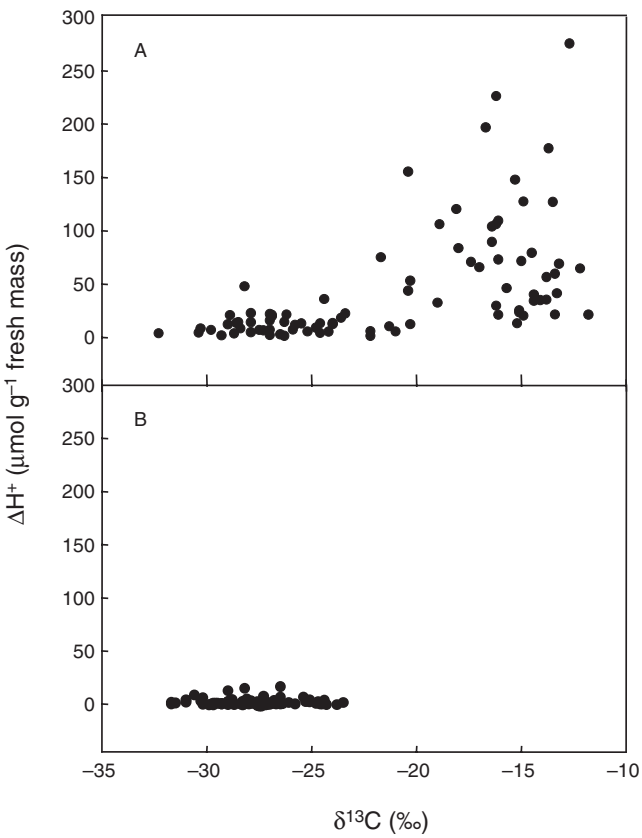

Fig. 3. The relationship between $\delta^{13}C$ and (*A*) significant nocturnal acidification ($P<0.05$) and (*B*) non-significant nocturnal acidification ($P>0.05$) determined by *t*-tests between evening and morning titratable acidity values for 173 orchid species.

negative than −20‰, the nocturnal acidity increase averaged 80.4 (range: 13.5–275.7). Forty-two species of 128 species with C_3-type $\delta^{13}C$ values (more negative than −22‰) showed significant nocturnal acidification per unit fresh leaf mass, whereas significant nocturnal acidification was always associated with species exhibiting intermediate and CAM-type $\delta^{13}C$ values. For the 31 species with $\delta^{13}C$ values more negative than −22‰ and a small but significant acidification (<15 μmol H^+ per unit fresh leaf mass), repeat measurements were performed, and in all cases, significant acidification was confirmed.

In the frequency distribution of isotopic values for the 173 species in which both $\delta^{13}C$ and titratable acidity were measured (Fig. 4), we observed the same bimodal distribution as observed for all 214 species (Fig. 1). The distribution of the subset of species that were shown to exhibit significant nocturnal acidification also formed a bimodal distribution, exhibiting modes at −28‰ and approximately −15‰, thus tracing the pattern of the complete dataset and revealing a cluster of CAM activity within the characteristic isotopic range for C_3 plants (Fig. 4).

Discussion

Species in which the CAM cycle is present are distributed bi-modally along the entire isotopic range of study species, with one peak in the characteristic CAM region and a second peak embedded within the characteristic C_3 region. Roughly one third of the orchid species that exhibit $\delta^{13}C$ values in the range that is commonly associated with C_3 photosynthesis are capable of nocturnal acidification. Therefore, although $\delta^{13}C$ values reflect the photosynthetic pathway through which carbon is predominately assimilated, many species with C_3-type $\delta^{13}C$ values exhibit low-level CAM activity.

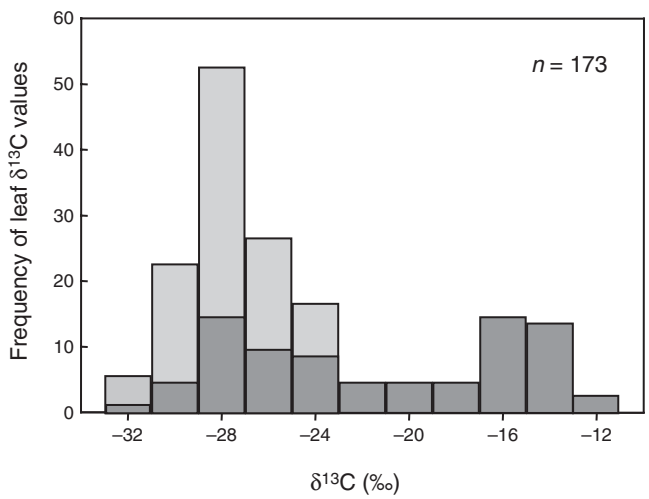

Fig. 4. Frequency of leaf $\delta^{13}C$ values for orchid species with the presence (dark grey) or absence (pale grey) of CAM, based on titratable acidity measurements. Each bar represents a 2‰ range of $\delta^{13}C$.

Based on the nocturnal carbon gain calibration line proposed by Winter and Holtum (2002), we conclude that orchids associated with the frequency mode of −14 to −16‰ acquire approximately 60–73% of their carbon via CAM, whereas species that exhibit low-level CAM activity with $\delta^{13}C$ values near −28‰, may obtain 5% or less of their carbon via CAM photosynthesis. Interestingly, intermediate species, which assimilate roughly 40% of their carbon via CAM, are not common and correspond to the frequency minimum at approximately −20‰, between the two abundance modes (Figs 1, 4). Although the calibration line proposed by Winter and Holtum (2002) does not specifically consider the effect of recycling of respiratory CO_2 via CAM on $\delta^{13}C$ values, CO_2-cycling is not a carbon-acquiring feature, and its effect on the overall carbon isotope composition of non-stressed, non-senescing CAM-tissues is probably small. Our results illustrate the need to consider species with weakly expressed CAM in estimates of the frequency of the CAM pathway among vascular plant species, and raise interesting questions regarding the role of low-level CAM activity in tropical epiphytes.

Recent studies at two lowland sites in Panama, based their results solely on $\delta^{13}C$ values, and found that 19% and 25%, respectively, of the epiphytic flora, and 33% and 40%, respectively, of the Orchidaceae are composed of CAM species, most of which are prevalent in exposed sites (Zotz and Ziegler 1997; Zotz 2004). In contrast, only 21% of the orchid species in this study exhibit strong enough CAM to be identified solely on the basis of $\delta^{13}C$ values. Including species in which the presence of the CAM cycle was verified through acid titration, increases the percentage of species with CAM to 50%, and includes species in which CAM is only weakly expressed. $\delta^{13}C$ values of cultivated orchids in our study are similar to those reported *in situ* (Table 1; Zotz and Ziegler 1997), and there is particularly close agreement for $\delta^{13}C$ values among species with strongly expressed CAM. Species in the C_3 isotopic range tend to be more depleted in ^{13}C in the field than in cultivation, possibly because cultivated plants grow in more open sites. Nonetheless, these differences are small and our data are consistent with studies in other taxonomic groups where some species with C_3-type $\delta^{13}C$ values have been shown to exhibit a small degree of CAM (Holtum and Winter 1999; Pierce *et al*. 2002). The presence of CAM in 50% of Panamanian orchid species studied by us is in line with previous predictions that 50% of tropical epiphytes in the Orchidaceae could show CAM activity (Winter and Smith 1996). In addition, this study suggests CAM activity in 15 genera previously not known to exhibit CAM (Smith and Winter 1996): *Aspasia*, *Brassia*, *Brenesia*, *Cischweinfia*, *Coryanthes*, *Eriopsis*, *Macroclinium*, *Mormodes*, *Oerstedella*, *Peristeria*, *Prosthechea*, *Scaphyglottis*, *Sobralia*, *Trichopilia* and *Trigonidium*. Because we only cover approximately 1% of all known orchid species, further studies are

required to corroborate our findings. It is apparent that more investigation of the distribution of C_3 and CAM photosynthetic options in this large family will have a strong bearing on our understanding of the evolution of CAM, the role of CAM in adaptive radiations and the overall number of CAM-equipped species.

The isotopic bimodal distribution of species with CAM observed in this study suggests that strongly or weakly expressed CAM is favoured over intermediate metabolism. In this regard, strong CAM is likely to be favoured in species that inhabit more severely water-limited environments and that have evolved a greater degree of anatomical features, such as leaf succulence, which facilitate operation of the CAM cycle and storage of nocturnally produced malic acid (Ting 1985). However, we found that leaves as thick as 2.65 mm can show C_3-type $\delta^{13}C$ values. Leaf thickness may be mainly due to hydrenchyma that does not participate in CAM activity (Winter *et al*. 1983). In this regard, plots of $\delta^{13}C$ value *v.* chlorenchyma thickness instead of leaf thickness may yield better relationships (Zotz and Ziegler 1997). The expression of weak CAM, on the other hand, allows species to recycle respiratory CO_2 and to take up atmospheric CO_2 at low rates during the night (Ting 1985; Winter and Smith 1996; Wanek *et al*. 2002). Low-level CAM activity can aid survival during drought when C_3 photosynthetic CO_2 uptake is strongly reduced due to stomatal closure and scavenging of respired CO_2 and nocturnal CO_2 uptake, however low, become an increasingly large proportion of the 24-h CO_2 exchange balance (Lüttge 1987; Holtum and Winter 1999; Pierce *et al*. 2002). Plants that exhibit periodic low to medium-level CAM activity, such as stressed *Clusia* sp., would also exhibit C_3-type $\delta^{13}C$ values (Holtum *et al*. 2004). However, since our study species were regularly watered, it is likely that the CAM orchid species within the C_3 isotopic range exhibit weak CAM permanently rather than occasionally. The relatively low frequency of intermediate metabolism suggests that species relying predominately on one pathway or the other are favoured based on the available ecological niches. It is also possible that anatomical or physiological limitations exist for the assimilation of equal amounts of carbon through both pathways.

In conclusion, our data indicate that CAM capacity is widespread among a group of Panamanian orchids. We demonstrate that surveys of CAM occurrence based on leaf thickness and $\delta^{13}C$ value can underestimate the number of CAM-equipped species and that the number of species with CAM concealed within the C_3 peak of the isotopic frequency distribution is larger than previously thought. Several studies have highlighted the role of weak CAM (or periodic CAM) during severe drought (Franco *et al*. 1992; Borland *et al*. 1993; Holtum and Winter 1999). We do not know whether the presence of low-level CAM in plant lineages may serve as a selective advantage for adaptive radiations through changing climatic conditions during evolutionary time scales. Further studies on the occurrence, function and expression of CAM in species with C_3-type $\delta^{13}C$ values are necessary to fully explore the relationship between CAM and microhabitat preferences of orchid species in the context of their phylogeny, thereby improving our understanding of the functional significance and the evolutionary origins of the CAM pathway.

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