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Altitudinal changes in the incidence of crassulacean acid metabolism in vascular epiphytes and related life forms in Papua New Guinea

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Summary. The occurrence of Crassulacean acid metabolism (CAM), as judged from $\delta^{13}C$ values, was investigated in epiphytes and some related plant species at a series of sites covering the approximate altitudinal range of epiphytes in Papua New Guinea. Comprehensive collections were made at each site and the occurrence of water storage tissue and blade thickness was also determined. Some 26% of epiphytic orchids from a lowland rainforest (2–300 m.a.s.l) showed $\delta^{13}C$ values typical of obligate CAM and possessed leaves thicker than 1 mm. A second group of orchids, mostly with succulent leaves, possessed intermediate $\delta^{13}C$ values between -23 and $-26‰$ and accounted for 25% of the total species number. Some species of this group may exhibit weak CAM or be facultative CAM plants. The remainder of the lowland rainforest species appeared to be C_3 plants with $\delta^{13}C$ values between -28 and $-35‰$ and generally possessed thin leaves. Obligate CAM species of orchids from a lower montane rainforest (1175 m.a.s.l) comprised 26% of the species total and mostly possessed thick leaves. The remainder of the species were generally thin-leaved with $\delta^{13}C$ values between -26 and $-35‰$ largely indicative of C_3 photosynthesis. Orchids with intermediate $\delta^{13}C$ values were not found in the lower montane rainforest. Obligate CAM appeared to be lacking in highland epiphytes from an upper montane rainforest and subalpine rainforest (2600–3600 m.a.s.l). However the fern, *Microsorium cromwellii* had a $\delta^{13}C$ value of $-21.28‰$ suggesting some measure of CAM activity. Other highland ferns and orchids showed more negative $\delta^{13}C$ values, up to $-33‰$, typical of C_3 photosynthesis. The highland epiphytic orchids possessed a greater mean leaf thickness than their lowland C_3 counterparts due to the frequent occurrence of water storage tissue located on the adaxial side of the leaf. It is suggested that low daytime temperatures in the highland microhabitats is a major factor in explaining the absence of CAM. The increased frequency of water storage tissue in highland epiphytes may be an adaptation to periodic water stress events in the dry season and/or an adaptation to increased levels of UV light in the tropical-pine environment.

Key words: $\delta^{13}C$ – CAM – Epiphytes – Tropical habitat – Altitude

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Crassulacean acid metabolism (CAM) appears to be an effective way of acquiring carbon whilst limiting water loss by the plant and has been frequently studied in terrestrial succulents growing in arid zones (Kluge and Ting 1978; Osmond 1978; Winter 1985). However, the epiphyte habitat in the humid tropics can also be arid microclimatically and the importance of CAM in this habitat has only recently been appreciated. Tree bark possesses a relatively low capacity to retain water and, in general, epiphytes in the forest canopy are subjected to more marked day-night temperature differences, higher irradiance and stronger winds than their counterparts in the forest understorey (Richards 1952; Smith et al. 1985; Winter 1985).

In the New World, both CAM and C_3 photosynthesis are found in the Bromeliaceae, many of which are epiphytic (Coutinho 1969; McWilliams 1970; Medina 1974; Medina and Troughton 1974; Medina et al. 1977). Recent extensive ecophysiological investigations of the family in Trinidad have shown that the CAM species are characteristic of the drier sites with the C_3 species occupying the wetter areas, although substantial overlap does occur (Griffiths and Smith 1983; Smith et al. 1985, 1986). Less detailed information is available concerning the ecophysiology of CAM in Old World epiphytes although the pathway is known to occur in some genera of the Polypodiaceae (Wong and Hew 1976; Hew 1984; Winter et al. 1983; Sinclair 1984; Winter et al. 1986), Asclepiadaceae (Winter et al. 1983) and Rubiaceae (Winter et al. 1983). However, the Orchidaceae has been the most extensively studied old world family with numerous CAM species and varieties recorded (Milburn et al. 1968; Coutinho 1969; McWilliams 1970; Neales and Hew 1975; Goh et al. 1977; Avadhani et al. 1982; Sinclair 1984; Winter et al. 1986). Indeed, a survey of epiphytic Australian orchids growing at altitudes <1200 m.a.s.l. showed that two thirds of the species examined utilised CAM (Winter et al. 1983). The CAM species generally possessed leaves thicker than about 1 mm and tended to occupy the more exposed sites.

For reasons of accessibility, the above published work on CAM in tropical epiphytes has been largely confined to lowland areas. However, mountains exceeding 3000 m.a.s.l. and lying within 10 degrees latitude of the equator occur in Africa, South America and Malesia (Hnatiuk et al. 1976). A survey of grass species along an altitudinal transect

Table 1. Climatic conditions and habitat descriptions of the study sites. Temperature records represent the mean monthly minima and maxima screen temperatures at each altitude and the habitat descriptions are based on Hope (1980). Data are presented for the upper and lower altitudes of sites D and E. NA represents not available

Site	Lat. S	Long. E	Altitude m.a.s.l.	$T°$ C, min	$T°$ C, max	Precipitation mm year^{-1}	Habitat
A) Lae	6° 44′	146° 59′	30[a]	22.1	29.6	4419	Botanical Garden
B) Gabensis	6° 40′	146° 48′	2–300	NA	NA	NA	Lowland rainforest
C Baiyer River	5° 32′	144° 09′	1175[a]	15.6	28.3	2614	Lower montane rainforest
D) Keglsugl	5° 50′	145° 06′	2600[a]	9.4	18.9	2283	Upper montane rainforest
	5° 48′	145° 04′	3150[b]	6.3	14.9	NA	
E) Mt. Wilhelm	5° 48′	145° 04′	3400[b]	4.9	13.0	NA	Sub-alpine rainforest and
	5° 47′	145° 03′	3580[c]	4.0	11.6	ca. 3450	tropicalpine grassland

[a] McAlpine et al. 1975
[b] Temperature lapse rates from Humphreys 1984
[c] Pindaunde Research Station records, Hnatiuk et al. 1976

in Kenya revealed that C_4 species were not found where the mean annual minimum temperature was below 8° C (Tieszen et al. 1979) but a comparable study does not exist for CAM plants. This paper, accordingly, examines the occurrence of CAM in epiphytes at several sites in Papua New Guinea which cover the approximate altitudinal range over which epiphytes occur. As orchids do not appear to possess C_4 photosynthesis and associated Kranz anatomy (Winter et al. 1983), the carbon isotope ratio has been solely used to discriminate between CAM and C_3 species as successfully used in previous ecophysiological studies (Medina and Troughton 1974; Medina et al. 1977; Griffiths and Smith 1983; Winter et al. 1983). In addition, data are presented for blade thickness (Winter et al. 1983) and the occurrence of water storage tissue which lacks or contains few chloroplasts and which does not carry out appreciable CAM (Kluge and Ting 1978).

Materials and methods

The plant collections in this study were made in Papua New Guinea during July–August 1984. Details of the collection sites and the best available climatic records are shown in Table 1. Collections were made from natural habitats with the exception of lowland orchids from the Lae Botanic Garden and locally-collected species in the orchid display area at the Baiyer River Sanctuary. Thorough vegetation descriptions for Gabensis and Baiyer River do not appear to exist but the highland collections (2600–3600 m.a.s.l) were made from Mt. Wilhelm for which both soil descriptions (Humphreys 1984) and vegetation records (Wade and McVean 1969; Johns and Stevens 1971) are available. Keglsugl (2600 m.a.s.l) lies at the base of Mt. Wilhelm and marks the start of the largely undisturbed upper montane rainforest which terminates at ca. 3350 m.a.s.l. The Mt. Wilhelm site (3400–3600 m.a.s.l) was located in the Pindaunde Valley where the abundance of succulent epiphytes and lithophytes markedly declines at altitudes >3600 m.a.s.l. Vegetation communities between this altitude and the Mt. Wilhelm summit (4509 m.a.s.l) largely consist of (sub-) alpine grassland culminating in alpine heath and tundra (Wade and McVean 1969).

The orchid species in the Lae Botanic Garden were growing in the open on non-living tree fern trunks. Collections made in the orchid display area at the Baiyer River Sanctuary were from solitary individuals growing on exposed tree trunks and remain unidentified. Collections of the naturally-occurring orchids at Baiyer River and those at the other sites were made as comprehensively as possible within the time available. Epiphytes were collected at Gabensis from an area of recent logging activity and at other sites by means of a combination of tree climbing and felling. The majority of orchids were sterile which often precluded identification to the species level. An additional problem is that much work remains to be carried out on the taxonomy of the New Guinea highland orchids. The collection numbers in this study, with the exception of those from Lae Botanic Garden and the Baiyer River orchid display, have been deposited in the Herbarium, Royal Botanic Gardens, Kew, England.

Leaf or frond thickness was determined using a micrometer positioned mid-way along the blade and between the midrib and edge. Reference is made in the text to thick-leaved species where leaf thickness is >approx. 1 mm with thin-leaved species being <approx. 1 mm (see Winter et al. 1983). Blade sections were cut with a razor blade and used to determine the presence or absence of water storage tissue in the early part of the work. Later determinations involved an approximate assessment of the percentage of blade volume occupied by the water storage tissue. Samples for carbon-isotope analysis were air-dried following collection and analysed in Munich. The samples were then dried at 100° C, combusted under oxygen, and $\delta^{13}C$ values of CO_2 determined by ratio mass spectrometry as described previously (Osmond et al. 1975). The carbon-isotope ratio ($\delta^{13}C$) is defined as:

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

Differences in the $\delta^{13}C$ value arise to a great extent from the larger isotope discrimination shown by ribulose bisphosphate carboxylase in C_3 plants as opposed to PEP carboxylase in C_4 and CAM plants. Plants which possess C_3 photosynthesis exhibit a mean $\delta^{13}C$ value of about $-28‰$ in comparison to atmospheric CO_2 which has a $\delta^{13}C$ value of about $-8‰$. On the other hand, C_4 and obligate CAM plants possess a mean $\delta^{13}C$ ratio of about $-14‰$ generally covering the range of -9 to $-19‰$ (Lerman 1975, Troughton 1979). Problems do, however, arise in the case of intermediate $\delta^{13}C$ values which may indicate facultative

CAM. For example, epiphyte species of bromeliads and ferns with $\delta^{13}C$ values in the range -21 to $-27‰$ have been observed to carry out either nocturnal CO_2 uptake or a diurnal rhythm in titratable acidity (Griffiths and Smith 1983; Winter et al. 1983; Smith et al. 1985).

Results

Table 2 provides lists of the species examined in altitudinal groups with details of life form, blade thickness, incidence of water storage tissue and the $\delta^{13}C$ value. The majority of the lowland orchids collected at the Lae Botanic Garden possessed thick leaves and had $\delta^{13}C$ values indicative of pronounced CAM. The two thin-leaved species, *Dendrobium bracteosum* and *D. smilliae*, appeared to possess C_3 photosynthesis.

The collection from the lowland rainforest at Gabensis (Site B, 2–300 m.a.s.l) resulted in a $\delta^{13}C$ value for *Dischidia imbricata* suggesting considerable nocturnal CO_2 fixation as found previously for other members of this genus in Australia (Winter et al. 1983). The remainder of the species collected were orchids and their characteristics are summarised in a frequency diagram (Fig. 1). *Bulbophyllum* cf. *sessile, Bulbophyllum* sp. 1, sp. 2 and sp. 3, *Dendrobium pseudocalceolum* and *Robiquetia gracilistipes* formed a group of thick-leaved species with $\delta^{13}C$ values indicative of obligate CAM and accounted for 26% of the total number of species examined at Gabensis. Figure 1 also reveals the existence of another group of orchids with somewhat intermediate $\delta^{13}C$ values between -23 and $-26‰$ accounting for 25% of the total species number. The group consisted of *Bulbophyllum* cf. *macregorii, Ceratostylis* sp. 1 and sp. 2 and *Den-*

Table 2. $\delta^{13}C$ values and some characteristics of the plants surveyed at each site. Species occurring at more than one site are denoted using superscripts. Plants were epiphytes unless the life form is given after the taxon: **Li** Lithophyte, **Te** Terrestrial. Collection numbers can be obtained from the first author (M.J.E.)

Site taxon	Thickness (mm)	Water storage tissue (± or %)	$\delta^{13}C$ (‰)
A LAE (30 m.a.s.l.)			
Orchidaceae			
Dendrobium bracteosum Rchb.f.	0.54	–	−27.29
Dendrobium capituliflorum Rolfe	2.20	–	−20.67
Dendrobium gouldii Rchb.f.	2.33	–	−14.37
Dendrobium helix Cribb	2.03	–	−14.88
Dendrobium insigne Lindl.	1.88	–	−15.28
Dendrobium lineale Rolfe	1.41	–	−13.73
Dendrobium mirbelianum Gaud.	1.84	–	−15.10
[1] *Dendrobium smilliae* F. Muell.	0.51	–	−25.66
Vanda hindsii Lindl.	1.53	–	−14.65
B GABENSIS (2–300 m.a.s.l.)			
Asclepiadaceae			
Dischidia imbricata Steud.	1.80	–	−15.59
Orchidaceae			
Agrostophyllum majus Hook.f.	0.28	–	−31.11
Bulbophyllum cf. *blumei* (Lindl.) J.J. Smith	0.70	–	−24.30
Bulbophyllum cf. *macgregorii* Schltr.	1.12	–	−23.26
Epicranthes cf. *macrorhopalon* (Schltr.) Garay & Kittr.	0.60	–	−25.20
Bulbophyllum cf. *sessile* J.J. Smith	1.38	–	−12.25
Bulbophyllum sp. 1	2.18	–	−13.90
Bulbophyllum sp. 2	1.32	–	−13.05
Bulbophyllum sp. 3	1.28	–	−12.21
Ceratostylis sp. 1	2.75	–	−24.45
Ceratostylis sp. 2	2.35	–	−24.94
Cryptostylis arachnites (Bl.) Hassk., **Te**	0.41	–	−34.03
Dendrobium johnsoniae F. Muell.	1.43	–	−30.25
Dendrobium pseudocalceolum J.J. Smith	1.65	–	−12.49
Dendrobium pseudocalceolum J.J. Smith	1.06		−13.00
[1] *Dendrobium smilliae* F. Muell.	0.27	–	−32.03
Dendrobium smilliae F. Muell.	0.34	–	−28.60
Dendrobium tumoriferum J.J. Smith	1.04	80	−24.44
Dendrobium sect. *Conostalix* Kraenzl.	0.23	–	−30.49
Dendrobium sp. 1	0.23	–	−28.08
Dendrobium sp. 2	0.74	20	−29.36
Dipodium pandanum F.M. Bail.	0.27	–	−29.97
Eria eriaeoides (F.M. Bail.) Rolfe	0.48	–	−31.17
Eria eriaeoides (F.M. Bail.) Rolfe	0.45	–	−31.09
Eria truncicola Schltr.	0.27	–	−30.22
Grammatophyllum papuanum J.J. Smith	0.33	–	−28.00
Robiquetia gracilistipes (Schltr.) J.J. Smith	1.68	–	−11.65
Trichotosia cf. *ferox* Bl.	0.98	–	−28.42
C BAIYER RIVER (1175 m.a.s.l.)			
Orchidaceae			
Agrostophyllum sp. 1	0.25	–	−31.76
Bulbophyllum sp. 4	0.45	–	−34.01
Bulbophyllum sp. 5	0.35	–	−31.20
Bulbophyllum sp. 6	0.57	–	−32.60
Coelogyne cf. *asperata* Lindl.	0.39	–	−33.11
Coelogyne ?fragrans Schltr.	0.50	–	−29.31
Dendrobium sp. 3	0.17	–	−31.49
Dendrobium sp. 4	0.40	–	−28.06
Dendrochilum cf. *longifolium* Rchb.f.	0.39	–	−28.05
Diplocaulobium sp. 1	0.33	–	−28.05
Flickingeria sp. 1	1.00	+	−28.97
Glomera sp. 1	0.50	–	−26.60
Glossorhyncha sp. 1	1.55	+	−27.61
Liparis condylobulbon Rchb.f.	0.50	–	−30.30
Liparis condylobulbon Rchb.f.	0.65	–	a) −35.84 b) −34.55
Luisia teretifolia Gaud.	2.90	–	a) −15.20 b) −15.21
Pedilochilus sp. 1	0.90	+	−29.48
Phreatia sp. 1	0.36	–	−28.31
Phreatia sp. 2	0.90	+	−32.11
Saccolabium sp. 1	0.33	–	−15.24
Unidentified sp. 1	1.75	–	−14.59
Unidentified sp. 2	0.60	–	−29.95

Table 2 (continued)

Site taxon	Thickness (mm)	Water storage tissue (± or %)	$\delta^{13}C$ (‰)
Unidentified sp. 2	0.58	–	−29.90
Unidentified sp. 3	1.02	–	−15.27
Unidentified sp. 4	2.55	–	−16.75
Unidentified sp. 5	2.40	–	−26.07
Unidentified sp. 6	0.55	–	−29.78
Unidentified sp. 7	0.90	–	−14.70
Unidentified sp. 8	1.25	–	−16.33
Unidentified sp. 9	0.39	–	−31.61
Unidentified sp. 10	0.39	–	−29.69
Unidentified sp. 11	1.10	–	−14.07
Unidentified sp. 12	0.25	–	−29.33
Unidentified sp. 13	1.50	–	−12.70
Unidentified sp. 14	0.85	–	−28.43
Unidentified sp. 15	0.90	–	−27.96
D KEGLSUGL (2600–3150 m.a.s.l.)			
Grammitidaceae			
[2] *Loxogramme subselliguea* (Bak.) Alston	0.47	–	−29.55
Polypodiaceae			
Belvisia longissima Holtt.	0.35	–	−28.90
[3] *Belvisia revoluta* (Bl.) Copel.	1.60	–	−26.90
Belvisia revoluta (Bl.) Copel., **Te**	1.25	–	−27.45
Belvisia revoluta (Bl.) Copel.	1.55	–	−28.02
Microsorium cromwellii (Ros.) Copel.	0.60	–	−21.28
[4] *Selliguea werneri* (Rosenst.) Pic. Ser.	0.72	–	−28.01
Orchidaceae			
Bulbophyllum sp. 7	1.18	–	−28.01
Bulbophyllum sp. 8	0.35	–	−31.82
Bulbophyllum sp. 9	0.35	–	−31.50
Bulbophyllum sp. 10	0.70	–	−31.02
Bulbophyllum sp. 11	1.15	–	−28.84
Bulbophyllum sp. 12	0.50	<10	−32.26
Calanthe cf. *flava* Hassk.	0.35	–	−26.38
Calanthe sp. 1, **Te**	0.35	–	−32.60
Ceratostylis sp. 3	1.75	+	−26.27
Ceratostylis sp. 4	1.45	+	−26.06
Ceratostylis sp. 5	1.65	+	−29.24
Ceratostylis sp. 6	1.85	30	−31.13
Ceratostylis sp. 7	1.85	30	−24.78
Ceratostylis sp. 8	1.35	20	−32.32
Ceratostylis sp. 9	2.25	40	−27.05
Ceratostylis sp. 10	1.10	–	−29.21
Ceratostylis sp. 11	2.09	60	−31.08
[5] *Dendrobium aurantiroseum* P. Royen ex T.M. Reeve	0.39	–	−34.66
Dendrobium aurantiroseum P. Royen ex T.M. Reeve	0.44	–	−30.61
Dendrobium habbemense P. Royen	0.32	–	−30.58
Dendrobium rigidifolium Rolfe	0.83	–	−28.93
Dendrobium vexillarius J.J. Smith	0.55	–	−25.52
Dendrobium sect. *Grastidium* (Bl.) J.J. Smith	1.17	80	−28.47
Dendrobium sp. 5	0.76	–	−29.85
Diplocaulobium aff. *iboense* (Schltr.) A.D. Hawkes	0.63	–	−26.31
[6] *Epiblastus* sp. 1	0.75	50	−28.40
Epiblastus sp. 2	0.71	60	−27.57
Epiblastus sp. 2	0.89	60	−24.49

Table 2 (continued)

Site taxon	Thickness (mm)	Water storage tissue (± or %)	$\delta^{13}C$ (‰)
Epiblastus sp. 2	0.71	30	−31.02
Glomera cf. *aurea* Schltr.	1.05	60	−24.20
Glossorhyncha sp. 2	1.20	+	−27.80
Glossorhyncha sp. 3	1.35	60	−26.47
Glossorhyncha sp. 4	0.95	70	−26.90
Glossorhyncha sp. 5	1.30	60	−28.55
Glossorhyncha sp. 6	1.10	50	−28.95
Liparis sect. *Distichon* Schltr.	0.60	–	−25.48
Mediocalcar sp. 1	0.62	25	−32.37
Mediocalcar sp. 2	1.85	60	−26.49
Oberonia sp. 1	0.80	70	−31.18
Oberonia sp. 2	0.85	60	−28.62
[7] *Octarrhena filiformis* (L.O. Williams) P. Royen	0.45	–	−26.70
Octarrhena filiformis (L.O. Williams) P. Royen	0.50	–	−27.59
Phreatia elongata Schltr.	1.36	80	−30.79
Phreatia sp. 3	1.60	+	−26.11
Phreatia sp. 3	1.60	20	−26.46
Phreatia sp. 4	0.55	50	−32.96
Phreatia sp. 5	1.50	70	−30.78
Phreatia sp. 6	0.75	60	−31.33
Phreatia sp. 7	1.64	40	−26.71
E MT. WILHELM (3400–3600 m.a.s.l.)			
Grammitidaceae			
[2] *Loxogramme subselliguea* (Bak.) Alston	0.25	–	−29.37
Prosaptia davalliacea (Muel. & Bak.) Copel.	0.30	–	−33.19
Polypodiaceae			
[3] *Belvisia revoluta* (Bl.) Copel	1.10	–	−27.67
[4] *Selliguea werneri* (Rosenst.) Pic. Ser.	0.75	–	−30.05
Iridaceae			
Libbertia pulchella R.Br. Sprengel.	0.20	–	−30.32
Orchidaceae			
Bulbophyllum barbatum P. Royen	1.15	–	−26.18
Bulbophyllum cf. *barbatum* P. Royen	1.15	–	−29.72
Bulbophyllum sp. 15, **Li**	0.60	–	−28.23
Ceratostylis sp. 12, **Li**	2.10	+	−24.25
Ceratostylis sp. 13, **Li**	1.20	+	−27.07
[5] *Dendrobium aurantiroseum* P. Royen ex T.M. Reeve	0.55	–	−26.80
Dendrobium brevicaule Rolfe	0.75	–	−31.86
Dendrobium brevicaule Rolfe	0.70	–	−30.52
Dendrobium dekockii J.J. Smith	0.55	–	−28.98
[6] *Epiblastus* sp. 1	0.65	+	−26.04
Epiblastus sp. 3, **Li**	1.00	+	−26.58
Glossorhyncha sp. 7, **Te**	0.45	–	−30.44
Liparis alpina P. Royen, **Li**	0.70	–	−30.09
Mediocalcar sp. 3, **Li**	1.45	+	−27.92
[7] *Octarrhena filiformis* (L.O. Williams) P. Royen	0.65	–	−27.76
Pedilochilus sp. 2, **Li**	0.60	–	−30.04
Pedilochilus sp. 3, **Li**	0.45	–	−32.20
Pedilochilus sp. 4, **Li**	0.60	–	−32.50
Pterostylis acuminata R.Br., **Te**	0.35	–	−31.52
Thelymitra papuana J.J. Smith, **Te**	1.10	–	−26.35

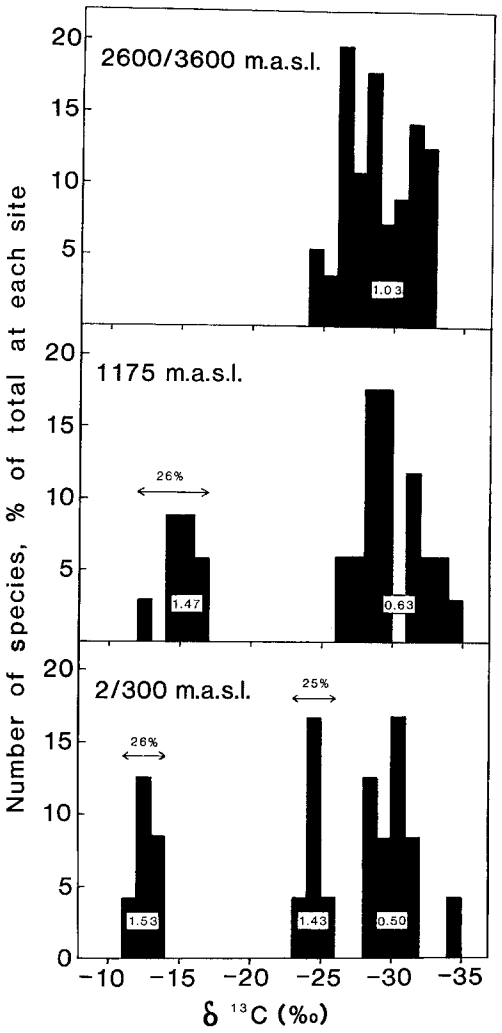

Fig. 1. Frequency diagram of $\delta^{13}C$ values in epiphytic and lithophytic orchids with respect to altitude (data taken from Table 2). The distribution is based on 23 spp. at 2–300 m.a.s.l. (Site B) and 34 spp. at 1175 m.a.s.l. (Site C). Data for the highland orchids at 2600–3600 m.a.s.l. (Sites D, E) have been combined and consisted of a total of 56 spp. Mean values were obtained in cases of more than one determination for a single species within an altitudinal group. Numerical values within a histogram refer to mean leaf thickness and values above a histogram indicate the percentage of the species recorded for the site

drobium tumoriferum all with thick leaves together with *Bulbophyllum* cf. *blumei* and *Epicranthes macrorhopalon* with somewhat thinner leaves. The mean leaf thickness of this group is little different to the obligate CAM species (Fig. 1) and some species of this group with intermediate $\delta^{13}C$ values may well possess weak CAM or be facultative CAM plants. The rest of the species collected from Gabensis with $\delta^{13}C$ values between -28 and $-35‰$ are presumably C_3 plants. They all possessed thin leaves with the exception of *Dendrobium johnsoniae*.

The epiphytes collected from the lower montane rainforest at Baiyer River (Site C, 1175 m.a.s.l) were all orchids. The obligate CAM species accounted for 26% of the total species number (Fig. 1) and consisted of *Luisia teretifolia, Saccolabium* sp. 1 and seven unidentified species (sp. 1, 3, 4, 7, 8, 11, 13). All the obligate CAM orchids at Baiyer River possessed thick leaves with the exception of *Saccolabium* sp. 1. The remainder of the orchid species at Baiyer River showed $\delta^{13}C$ values between -26 and $-35‰$ indicative of largely C_3 photosynthesis. The group consists mostly of thin-leaved species with a mean leaf thickness similar to the C_3-type orchids at Gabensis (Site B, 2–300 m.a.s.l).

The collections for the highland species from upper montane rainforest at Keglsugl (Site D, 2600–3150 m.a.s.l) and from sub-alpine rainforest and tropicalpine grassland on Mt. Wilhelm (Site E, 3400–3600 m.a.s.l) are described separately in Table 2 but the data for the epiphytic and lithophytic orchids have been combined in Fig. 1. The highland ferns possessed $\delta^{13}C$ values typical of C_3 photosynthesis except for *Microsorium cromwellii* with a $\delta^{13}C$ value of $-21.28‰$ (Table 2). In fact, this $\delta^{13}C$ determination was the least negative found for all vascular plants examined from altitudes >2600 m.a.s.l. *Libbertia pulchella* (Iridaceae) and all the orchids possessed $\delta^{13}C$ values within the range -24 to $-33‰$ indicative of largely C_3 photosynthesis (Table 2). The mean leaf thickness in the highland epiphytic and lithophytic orchids of 1.03 mm is substantially greater than their counterparts at lower altitudes (Fig. 1). This can largely be accounted for by the more frequent occurrence of water storage tissue which was present in 59% of the highland species compared to 12% at Baiyer River (Site C, 1175 m.a.s.l) and 9% at Gabensis (Site B, 2–300 m.a.s.l). The water storage tissue in the highland orchids was invariably located underneath the dorsal epidermis. The frequency distribution of $\delta^{13}C$ values of the highland orchids (Fig. 1) shows that a small number of species possessed $\delta^{13}C$ values of -24 to $-26‰$. However, both *Dendrobium vexillarius* and *Liparis* sect. *Distichon* are thin-leaved and the thick-leaved species, *Ceratostylis* sp. 7 and sp. 12 and *Glomera* cf. *aurea*, all possessed water storage tissue (Table 2). It is, therefore, unlikely that any of these species are facultative CAM plants.

The data for the highland orchids demonstrate that CAM is little utilised as an ecological adaptation at altitudes >2600 m.a.s.l. in Papua New Guinea but mean leaf thickness is substantially increased compared with populations from lower altitudes due to the greater incidence of leaf water storage tissue.

Discussion

Obligate CAM species of epiphytic orchids accounted for 26% of the total at both Gabensis (Site B, 2–300 m.a.s.l) and Baiyer River (Site C, 1175 m.a.s.l) which compares closely with a value of 24% for an Australian lowland rainforest. By contrast, 62% of orchids from relatively dry, open Australian forests were found to be CAM species (Winter et al. 1983). The obligate CAM orchids at both Gabensis and Baiyer River, as well as the Lae Botanic Garden, possessed thick leaves with the exception of *Saccolabium* sp. 1 at Baiyer River. A similar correlation between obligate CAM and leaf succulence has been noted previously for members of the Bromeliaceae and Euphorbiaceae (McWilliams 1970), Crassulaceae (Teeri et al. 1981) as well as the Orchidaceae (Neales and Hew 1975; Winter et al. 1983).

At Gabensis, in addition to the obligate CAM orchids, there was an intermediate group comprising 25% of the species examined which possessed $\delta^{13}C$ values of between -23 and $-26‰$ (Fig. 1). As noted previously, it is not possible to identify facultative CAM species using $\delta^{13}C$ values and particular difficulties also occur in the case of epiphytes. The $\delta^{13}C$ values of C_3 plants occurring in the lower levels of a rainforest have been found to be 5‰ more

negative than the leaves collected from the forest canopy (Medina and Minchin 1980). This is attributed to CO_2 near the floor of rainforests being depleted in ^{13}C due to both root respiration and the respiratory decomposition of plant materials. It also appears that conditions of high humidity and low irradiance produce more negative $\delta^{13}C$ values in C_3 species due to a reduction in the proportion by which uptake of CO_2 is limited by diffusion (Farquhar et al. 1982a, b). The above factors may be responsible for the high incidence in the present study of epiphytic and lithophytic orchids with $\delta^{13}C$ values more negative than $-31‰$ (24% of the species total in Table 2) compared with most C_3 plants (Lerman 1975, Troughton 1979). A relatively large number of the C_3 species of bromeliads in Trinidad also possessed $\delta^{13}C$ values more negative than $-31‰$ (Griffiths and Smith 1983). It appears then that epiphytes in the humid tropics possess relatively low $\delta^{13}C$ values which strengthens the likelihood of some of the intermediate group of orchids at Gabensis exhibiting weak CAM or being facultative CAM plants.

The $\delta^{13}C$ values of the highland epiphyte species (Sites D, E; 2600–3600 m.a.s.l) provided no evidence for the occurrence of CAM with the exception of the fern *Microsorium cromwellii* which, with a $\delta^{13}C$ value of $-21.28‰$, may well be a CAM species (Table 2). Previous studies on ferns have produced evidence for CAM in the genera *Drymoglossum* and *Pyrrosia*, also in the family Polypodiaceae (Wong and Hew 1976; Hew 1984; Winter et al. 1983; Sinclair 1984; Winter et al. 1986). The majority of *Microsorium* spp. possess thin fronds (Holttum 1968) and this report appears to be the first suggestion of CAM within the genus. The absence of obligate CAM in the highland orchids is somewhat surprising, particularly in the lower altitudes of the upper montane rainforest. Keglsugl (2600 m.a.s.l), for example, with mean monthly minimum and maximum screen temperatures of 9.4 and 18.9° C (McAlpine et al. 1975) represents the approximate altitudinal limit in the area for the cultivation of maize, *Zea mays* and sweet potato, *Ipomoea batatas* which are both chilling sensitive species. Precipitation at Keglsugl, located in a basin centre, is slightly lower than at Baiyer River (Site C, 1175 m.a.s.l) where obligate CAM orchids occur (Table 2, Fig. 1). Mean annual evaporation estimates for highland New Guinea suggest values between 1600 and 1800 mm over the altitudinal range 1200–2000 m.a.s.l. and, thereafter, a decline with increasing elevation to give a value of approx. 500 mm at Pindaunde Research Station, 3580 m.a.s.l. (Humphreys 1984). It is, therefore, reasonable to suppose that epiphytes at Keglsugl and Baiyer River are subjected to approximately the same degree of annual water stress compared with the Mt. Wilhelm epiphytes (Site E, 3400–3600 m.a.s.l) where water stress is much reduced.

It appears then that the lack of obligate CAM species in the highland epiphytic and lithophytic orchids (Fig. 1) must be due to reduced temperature (Table 1). Temperature optima for nocturnal CO_2 uptake and synthesis of malic acid in CAM plants generally occur within the range 10 to 24° C, often with appreciable activity still present at 0 to 5° C (Medina and Delgado 1976; Gerwick and Williams 1978; Medina and Osmond 1981; Wagner and Larcher 1981; Winter 1985). By contrast, the rate of daytime degradation of malic acid is considerably reduced at low temperatures and, for example, in *Sempervivum montanum* shows an approximately linear decrease from 40° C to a low value at 15° C (Wagner and Larcher 1981). It also appears that low temperatures delay the initiation of malic acid decarboxylation following nocturnal acidification (Winter and Tenhunen 1982). Indeed, low daytime temperatures tend to result in the promotion of net CO_2 fixation during the daytime in a range of CAM species (Kluge and Ting 1978; Winter 1985). It is conceivable that the lack of obligate CAM in the highland orchids reported in the present paper (Table 2, Fig. 1) is due to low daytime leaf temperatures, a feature which will be exacerbated by the partially shaded epiphytic microhabitats.

The highland orchids, whilst lacking obligate CAM, are however distinguished by a high degree of leaf succulence due to the frequent occurrence of water storage tissue, mostly located on the dorsal part of the leaf. Xeromorphic adaptation is commonly found in epiphytes ranging from leaf epidermal trichomes and water storing "tanks" in some of the Bromeliaceae to the pseudobulbs found in many of the Orchidaceae. Aerial roots and either internal or external water storage tissue are also commonly found in members of both families (Medina 1974; Fu and Hew 1982; Griffiths and Smith 1983; Winter et al. 1983; Smith et al. 1986). The role of water storage tissue in succulents in general appears to be to buffer the chlorenchyma during periods of water stress (Barcikowski and Nobel 1984; Schmidt and Kaiser 1987). Whilst the New Guinea highland orchids are not subjected to greater annual water stress compared to those in the lower montane rainforest (Table 1), they are subjected to an appreciable seasonality in precipitation. There is a pronounced wet season at Keglsugl and on Mt. Wilhelm from December to April, with May–July representing a relatively sudden dry season, and the succeeding months marking a gradual return to wetter weather (Hnatiuk et al. 1976; Humphreys 1984). As many as 22 consecutive dry days have been recorded at Pindaunde Research Station although somewhat less than half this number would normally be characteristic of a dry spell. Indeed, there are reports of epiphytic ferns and bryophytes in the subalpine rainforest becoming dry and shrivelled on occasion during the dry season (Hnatiuk et al. 1976). The frequent presence of water storage tissue in the highland orchids (Table 2) could, therefore, be regarded as an adaptation to prolonged periods of water stress within an environment where daytime temperatures are insufficiently high to promote CAM. It is also possible that the dorsal location of the water storage tissue, if containing phenols and flavonoids, represents an adaptation to the high level of UV light in the tropical-pine environment. As UV damage to the photosynthetic machinery of an individual leaf appears to be cumulative with time (Caldwell 1981), the presence of water storage tissue in long-lived leaves of orchids occupying the highland epiphytic microhabit may confer selective advantage.

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References

Avadhani PN, Goh CJ, Rao AN, Arditti J (1982) Carbon fixation in orchids. In: Arditti J (ed) Orchid biology, reviews and perspectives, II. Comstok/ Cornell University Press Ithaca London, pp 173–193

Barcikowski W, Nobel PS (1984) Water relations of cacti during desiccation: distribution of water in tissues. Bot Gaz 145:110–115

Caldwell MM (1981) Plant response to solar ultraviolet radiation: In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Physiological plant ecology. Encycl Plant Physiol, New Series, Vol 12A. Springer, Berlin Heidelberg New York, pp 169–197

Coutinho LM (1969) Novas observacoes a ocortenica do "efeito de de Sassure" e suas relacoes com a suculencia, a temperatura folhear e os movimentos estomaticos. Botanica 24:79–102

Farquhar GD, Ball MC, Caemmerer S von, Roksandic Z (1982a) Effect of salinity and humidity on $\delta^{13}C$ value of halophytes – evidence for diffusional isotope fractionation determined by the ratio of intercellular/atmospheric partial pressure of CO_2 under different environmental conditions. Oecologia (Berlin) 52:121–124

Farquhar GD, O'Leary MH, Berry JA (1982b) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust J Plant Physiol 9:121–137

Fu CF, Hew CS (1982) Crassulacean acid metabolism in orchids under water stress. Bot Gaz 143:294–297

Gerwick BC, Williams GJ (1978) Temperature and water regulation of gas exchange of *Opuntia polyacantha*. Oecologia (Berlin) 35:149–159

Goh CJ, Avadhani PN, Loh CS, Hanegraaf C, Arditti J (1977) Diurnal stomatal and acidity rhythms in orchid leaves. New Phytol 78:365–372

Griffiths H, Smith JAC (1983) Photosynthetic pathways in the Bromeliaceae of Trinidad: relations between life-forms, habitat preference and the occurrence of CAM. Oecologia (Berlin) 60:176–184

Hew CS (1984) *Drymoglossum* under water stress. Amer Fern J 74:37–39

Hnatiuk RJ, Smith JMB, McVean DN (1976) The climate of Mt. Wilhelm. Mt. Wilhelm Studies 2, ANU, BG/4, Canberra

Holttum RE (1968) Flora of Malaya Vol. II Ferns of Malaya. Government Printing Office, Singapore

Hope GS (1980) New Guinea mountain vegetation descriptions. In: van Royen P, The alpine flora of New Guinea, Vol 1. J Cramer, Vaduz, pp 153–122

Humphreys GS (1984) The environment and soils of Chimbu Province, Papua New Guinea, with particular reference to soil erosion. Research Bull. 35, DPI, Port Moresby

Johns RJ, Stevens PF (1971) Mount Wilhelm flora: a checklist of the species. Div Bot, Dept Forests, Lae, Bulletin 6:1–60

Kluge M, Ting IP (1978) Crassulacean acid metabolism. Ecological studies, vol 16. Springer, Berlin Heidelberg New York

Lerman JC (1975) How to interpret variations in the carbon isotope ratio of plants: biologic and environmental effects. In: Marcelle R (ed) Environmental and biological control of photosynthesis. Dr W Junk, The Hague, pp 323–335

McAlpine JR, Keig G, Short K (1975) Climatic tables for Papua New Guinea. CSIRO, Melbourne

McWilliams EL (1970) Comparative rates of dark CO_2 uptake and acidification in the Bromeliaceae, Orchidaceae and Euphorbiaceae. Bot Gaz 131:285–290

Medina E (1974) Dark CO_2 fixation, habitat preference and evolution within the Bromeliaceae. Evolution 28:677–686

Medina E, Delgado M (1976) Photosynthesis and night CO_2 fixation in *Echeveria columbiana* v. Poellnitz. Photosynthetica 10:155–163

Medina E, Minchin P (1980) Stratification of $\delta^{13}C$ values in Amazonian rain forests. Oecologia (Berlin) 45:377–378

Medina E, Osmond CB (1981) Temperature dependence of dark CO_2 fixation and acid accumulation in *Kalanchoë daigremontiana*. Aust J Plant Physiol 8:641–649

Medina E, Troughton JH (1974) Dark CO_2 fixation and the carbon isotope ratio in Bromeliaceae. Plant Sci Lett 2:357–362

Medina E, Delgado M, Troughton JH, Medina JD (1977) Physiological ecology of CO_2 fixation in Bromeliaceae. Flora 166:137–152

Milburn TR, Pearson DJ, Ndegwe NA (1968) Crassulacean acid metabolism under natural tropical conditions. New Phytol 67:883–879

Neales TF, Hew CS (1975) Two types of carbon fixation in tropical orchids. Planta 123:303–306

Osmond CB (1978) Crassulacean acid metabolism: a curiosity in context. Ann Rev Plant Physiol 29:379–414

Osmond CB, Ziegler H, Stichler W, Trimborn P (1975) Carbon isotope discrimination in alpine succulent plants supposed to be capable of crassulacean acid metabolism. Oecologia (Berlin) 18:209–217

Richards PW (1952) The tropical rainforest. Camb Univ Press, London New York Melbourne

Schmidt JE, Kaiser WM (1987) Response of the succulent leaves of *Peperomia magnoliaefolia* to dehydration. Plant Physiol 83:190–194

Sinclair R (1984) Water relations of tropical epiphytes. III. Evidence for Crassulacean acid metabolism. J Exp Bot 35:1–7

Smith JAC, Griffiths H, Bassett M, Griffiths NM (1985) Day-night changes in the leaf water relations of epiphytic bromeliads in the rain forests of Trinidad. Oecologia (Berlin) 67:475–485

Smith JAC, Griffiths H, Lüttge U (1986) Comparative ecophysiology of CAM and C_3 bromeliads. I. The ecology of the Bromeliaceae in Trinidad. Plant, Cell Environ 9:359–376

Teeri JA, Tonsor SJ, Turner M (1981) Leaf thickness and carbon isotope composition in the Crassulaceae. Oecologia (Berlin) 50:367–369

Tieszen LL, Senyimba MM, Imbamba SK, Troughton JH (1979) The distribution of C_3 and C_4 grasses and carbon isotope discrimination along an altitudinal and moisture gradient in Kenya. Oecologia (Berlin) 37:337–350

Troughton JH (1979) $\delta^{13}C$ as an indicator of carboxylation reactions. In: Gibbs M, Latzko E (eds) Photosynthesis II: Photosynthetic carbon metabolism. Encycl Plant Physiol, New Series, Vol 6. Springer, Berlin Heidelberg New York, pp 140–147

Wade LK, McVean DN (1969) Mt. Wilhelm Studies I. The alpine and subalpine vegetation. ANU, BG/1, Canberra

Wagner J, Larcher W (1981) Dependence of CO_2 gas exchange and acid metabolism of the alpine CAM plant *Sempervivum montanum* on temperature and light. Oecologia (Berlin) 50:88–93

Winter K (1985) Crassulacean acid metabolism. In: Barber J, Baker NR (eds) Photosynthetic mechanisms and the environment. Elsevier, pp 329–387

Winter K, Tenhunen JD (1982) Light-stimulated burst of carbon dioxide uptake following nocturnal acidification in the Crassulacean acid metabolism plant *Kalanchoë daigremontiana*. Plant Physiol 70:1718–1722

Winter K, Wallace BJ, Stocker GC, Roksandic Z (1983) Crassulacean acid metabolism in Australian vascular epiphytes and some related species. Oecologia (Berlin) 57:129–141

Winter K, Osmond CB, Hubick KT (1986) Crassulacean acid metabolism in the shade. Studies on an epiphytic fern, *Pyrrosia longifolia*, and other rainforest species from Australia. Oecologia (Berlin) 68:224–230

Wong SC, Hew CS (1976) Diffusive resistance, titratable acidity, and CO_2 fixation in two tropical epiphyte ferns. Amer Fern J 66:121–124

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