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PHOTOSYNTHETIC PATHWAY VARIATION IN LEAFY MEMBERS OF TWO SUBFAMILIES OF THE CACTACEAE

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Patterns of 24-h CO_2 exchange and diel fluctuations in tissue acid concentrations were measured in leafy and leafless shoots of 10 species in the Pereskioideae and eight species in the Opuntioideae (Cactaceae). The species were selected to represent a range of phylogenetic histories. Leafy shoots of all species in the Pereskioideae exhibited C_3 patterns of gas exchange, and net CO_2 exchange of leafless stems in all but one species was negative during the day and night. Although nighttime CO_2 uptake was not observed in shoots or stems of any of the pereskioid taxa, tissue acidity increased at night to a small degree in leaves of six species and stems of five species, indicative of low levels of CAM-cycling. In contrast, in leafy shoots of nearly all species in the Opuntioideae, CO_2 uptake occurred during the day and the night. Gas-exchange rates were typically greater during the day. As is typical of CAM, nighttime maximal water use efficiency often greatly exceeded daytime values. Tissue malic acid concentrations increased overnight in leaves and stems of all eight opuntioid species. Examination of the data from a phylogenetic perspective illustrates evidence of low levels of CAM scattered among the primarily C_3 members of the more ancestral Pereskioideae. Furthermore, such consideration of the taxa in the more derived Opuntioideae (comparing the genera from most ancestral to most derived, that is, *Austrocylindropuntia* → *Quiabentia* → *Pereskiopsis* → *Cylindropuntia*) revealed that CAM became increasingly less important in the leaves of the various taxa, whereas this water-conservative pathway of photosynthesis became increasingly more important in the stems. The results of this study indicate that members of the Pereskioideae should be restricted to moister habitats or must restrict the timing of growth to wet seasons, whereas the observed combinations of the C_3 and CAM pathways in the opuntioid taxa should prove beneficial in conserving water in the sporadically arid tropical and subtropical habitats of these plants.

Keywords: C_3 photosynthesis, Cactaceae, CAM, evolution, gas exchange, leaves, stems.

Introduction

Until recently, plants could be easily categorized into three groups according to their photosynthetic pathway: C_3, C_4, and Crassulacean acid metabolism (CAM; Black 1973; Osmond et al. 1982; Edwards and Walker 1983). Now, however, plants with characteristics of more than one metabolic pathway are known (Koch and Kennedy 1980, 1982; Ting 1985; Griffiths 1988; Monson and Moore 1989; Raghavendra and Das 1993; Kraybill and Martin 1996; Martin 1996). Such plants vary considerably in the degree to which they engage in a particular photosynthetic pathway. Furthermore, different organs of the same plant may exhibit different photosynthetic pathways (Lange and Zuber 1977; Martin et al. 1990). The physiological, ecological, and evolutionary ramifications of such photosynthetic pathway diversity are frequently subjects of speculation (Ting 1985; Griffiths 1988; Monson 1989; Ehleringer and Monson 1993; Martin 1996); however, a complete understanding of such diversity remains elusive.

The Cactaceae is a large derived family, composed of three widely recognized subfamilies: the Pereskioideae, the Opuntioideae, and the Cactoideae (Barthlott and Hunt 1993; Wallace 1995). Although many familiar cacti occur as stem succulents in arid regions, e.g., the deserts of the southwestern United States and northern Mexico, numerous taxa occur as epiphytes in the tropics, as thorny shrubs in subtropical areas, or as succulents at mesic sites at high elevations (Gibson and Nobel 1986). Ancestral taxa, especially those in the Pereskioideae, have large long-lasting leaves that are less succulent than the small ephemeral leaves of the more derived taxa in the Cactaceae (Gibson and Nobel 1986; Barthlott and Hunt 1993; Wallace 1995; also see table 1). Although physiological investigations of cacti are numerous, the great majority of studies include only a single species, typically a leafless stem succulent growing in or collected from the aforementioned arid regions (see references in Nobel 1988). The photosynthetic pathway of these cacti is exclusively CAM, often referred to as "obligate" or "constitutive" CAM.

Investigations of the potential modes of photosynthesis of other cacti, especially the ancestral leafy forms found in mesic tropical and subtropical habitats, are much less common. In a survey similar to the current one, although covering fewer species, Nobel and Hartsock (1986) investigated the photosynthetic pathways of both leaves and stems of taxa representing the three subfamilies of the Cactaceae. Three species in the Pereskioideae were included: *Pereskia aculeata*, *Pereskia*

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Table 1

Pereskioid Species, Geographic Ranges, and General Morphology of the Individual Plants

Species	Geographic range	Leaf morphology	Stem morphology
Maihuenia poeppigii (Pfeiffer) K. Schumann	Southern South America	Persistent, tiny, terete, very fleshy; WC_{dm} = 2.6	Small, very thick, very fleshy; WC_{dm} = 1.9
Pereskia aculeata Miller	Throughout Central and South America	Persistent, large, flat, somewhat fleshy; WC_{dm} = 9.9	Medium size, thin, hard; WC_{dm} = 2.0
Pereskia horrida (Kunth) DeCandolle	Northern South America	Persistent, large, flat, fleshy; WC_{dm} = 9.6	Medium size, thin, hard; WC_{dm} = 3.3
Pereskia zinniiflora DeCandolle	Caribbean	Persistent, large, flat, fleshy; WC_{dm} = 5.6	Medium size, thin, hard; WC_{dm} = 4.2
Pereskia quisqueyana Liogier	Caribbean	Persistent, large, flat, fleshy; WC_{dm} = 5.0	Medium size, thin, hard; WC_{dm} = 2.7
Pereskia bahiensis Gürke	Central South America	Persistent, large, flat, fleshy; WC_{dm}= 7.3	Medium size, thin, hard; WC_{dm}= 3.3
Pereskia sacharosa Grisebach	Central South America	Persistent, very large, flat, fleshy; WC_{dm} = 6.6	Medium size, thin, hard; WC_{dm} = 4.2
Pereskia bleo (Kunth) DeCandolle	Northern South America	Persistent, very large, flat, somewhat fleshy; WC_{dm} = 6.9	Medium size, thin, hard; WC_{dm} = 3.0
Pereskia aureiflora Ritter	Central South America	Persistent, large, flat, fleshy; WC_{dm} = 6.3	Medium size, thin, hard; WC_{dm} = 2.4
Pereskia lychnidiflora DeCandolle	Central America	Persistent, large, flat, very fleshy; WC_{dm} = 8.2	Medium size, thin, hard; WC_{dm} = 3.4

Note. Species are arranged in assumed phylogenetic order from more ancestral to more derived. All leaves and stems were green. WC_{dm} is the tissue water content on a dry mass basis. Values shown are for one individual of each species; measurements for a second individual yielded similar results.

grandifolia, and *Maihuenia poeppigii*. In all three taxa, net CO_2 uptake occurred only by the leaves and only during the day. Drought stress served only to reduce daytime CO_2 uptake rates; no nighttime uptake was induced, and net CO_2 exchange of the stems remained zero or negative (Nobel and Hartsock 1987). Although changes in acidity were not measured in either study by Nobel and Hartsock (1986, 1987), Rayder and Ting (1981) reported substantial diel changes in acidity of the leaves of well-watered and drought-stressed plants of both of the above species of *Pereskia* (but see results of Altesor et al. 1992 for *P. aculeata*). Thus, these two species of *Pereskia* are apparently capable of CAM-cycling, during which diel CO_2 exchange patterns are typical of C_3 plants, yet tissue malic acid concentrations increase at night as in CAM plants (Ting 1985; Martin 1996). Similar findings have been reported in field studies of *Pereskia guamacho*, which exhibits only C_3 photosynthesis during the wet season, then undergoes CAM-cycling or CAM-idling (diel acid fluctuations in the absence of stomatal opening [Ting 1985; Martin 1996]) during the dry season (Diaz and Medina 1984; Lüttge et al. 1989; Herrera and Cuberos 1990). The potential for CAM-cycling or CAM-idling in the stems of any of the above taxa has not been investigated.

Four species in the Opuntioideae were also included in the two studies of the Cactaceae by Nobel and Hartsock (1986, 1987), *Austrocylindropuntia subulata*, *Pereskiopsis porteri*, *Quiabentia chacoensis*, and *Opuntia ficus-indica*. Only stems of the latter species, the most derived of the group, were examined; they were strictly CAM, as has been found in numerous past studies (see Nobel 1988). Net CO_2 uptake by the leaves and stems of *P. porteri* and *Q. chacoensis* occurred primarily during the day, although limited stomatal opening and/or CO_2 uptake was observed at night. Diel acid fluctuations were not measured by Nobel and Hartsock (1986). In this regard, although the data are limited, Lehrum et al. (1987) reported lower pH values in the morning relative to values in the afternoon for leaf extracts of the leaves of *Pereskiopsis kellermanii*. Net CO_2 exchange patterns of the leaves of *A. subulata*, the most ancestral taxon studied by Nobel and Hartsock (1986), were similar to those of the two species of *Quiabentia* and *Pereskiopsis*, although, in contrast, their stems were predominately CAM. Desiccation of individuals of *A. subulata*, *P. porteri*, and *Q. chacoensis* effectively eliminated daytime (C_3) uptake and stimulated rates of nighttime CO_2 uptake by the leaves (Nobel and Hartsock 1987). Similar results were

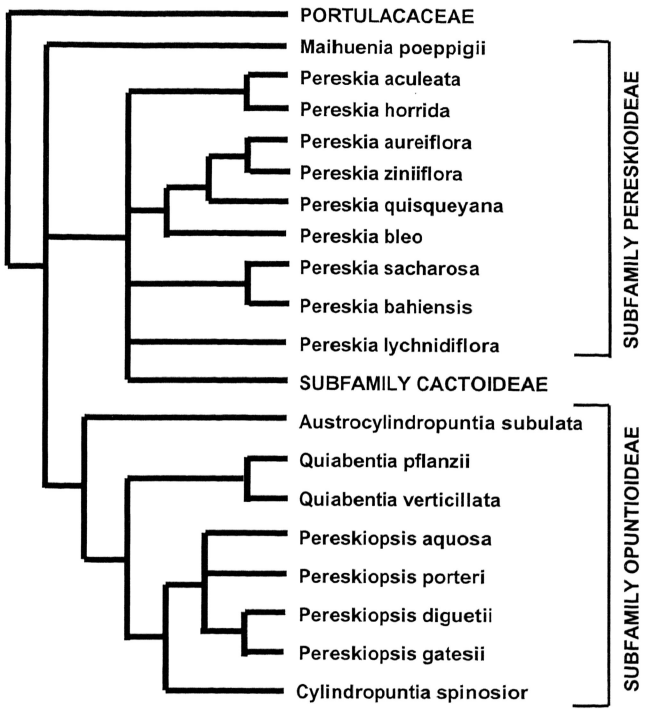

Fig. 1 Inferred phylogeny of the Cactaceae, including species of the subfamilies Pereskioideae and Opuntioideae used in this study. The majority-rule consensus is shown based on data from restriction-site analyses (Wallace 1995) and comparative sequencing using the plastid *rp*l16 intron (R. Wallace, unpublished data).

observed in the stems. Thus, photosynthesis in these species shifted from predominately C_3 (or possibly CAM-cycling?) to CAM with the imposition of drought stress.

Based on the above results, Nobel and Hartsock (1986, 1987) discussed an evolutionary trend of increasing importance of CAM when comparing ancestral with increasingly derived taxa in the Cactaceae. They emphasized the photosynthetic pathway diversity of the Opuntioideae, calling for more work on this subfamily.

In spite of the fact that there are ca. 20 species in the subfamily Pereskioideae and at least 250 species in the subfamily Opuntioideae (Gibson and Nobel 1986; Barthlott and Hunt 1993), investigations of photosynthesis in the more ancestral taxa of these subfamilies are limited to the few species mentioned above. Given this dearth of information about the potential diversity of photosynthetic types and about phylogenetic trends in the evolution of photosynthetic pathways in these cactus subfamilies, it was the purpose of the current study to expand the knowledge base regarding photosynthetic pathways in the Pereskioideae and the Opuntioideae. In addition to day/night measurements of CO_2 (and water vapor) exchange of leaves and stems, diel changes in tissue acidities in both tissue types were also included. Thus, several of the species previously investigated were included to address the potential for CAM-cycling in the more ancestral, putatively C_3 taxa of these subfamilies. As a result, it was possible to differentiate the ancestral C_3 form of photosynthesis from the more derived CAM, as well as to differentiate CAM-cycling, a form of C_3-CAM intermediacy.

Material and Methods

Whole plants or cuttings were obtained from botanical gardens (primarily the Botanischer Garten und Botanisches Museum, Freie Universität, Berlin) and propagated in the greenhouses of Iowa State University. After growing for periods ranging from several months to several years, cuttings or whole plants were first rooted in pure sand, then transplanted in a 1 : 2 (v/v) mixture of sand and standard greenhouse soil (7 : 2 : 1 : 1 mixture of clay loam, peat moss, Perlite, and vermiculite) and placed in a growth chamber at the

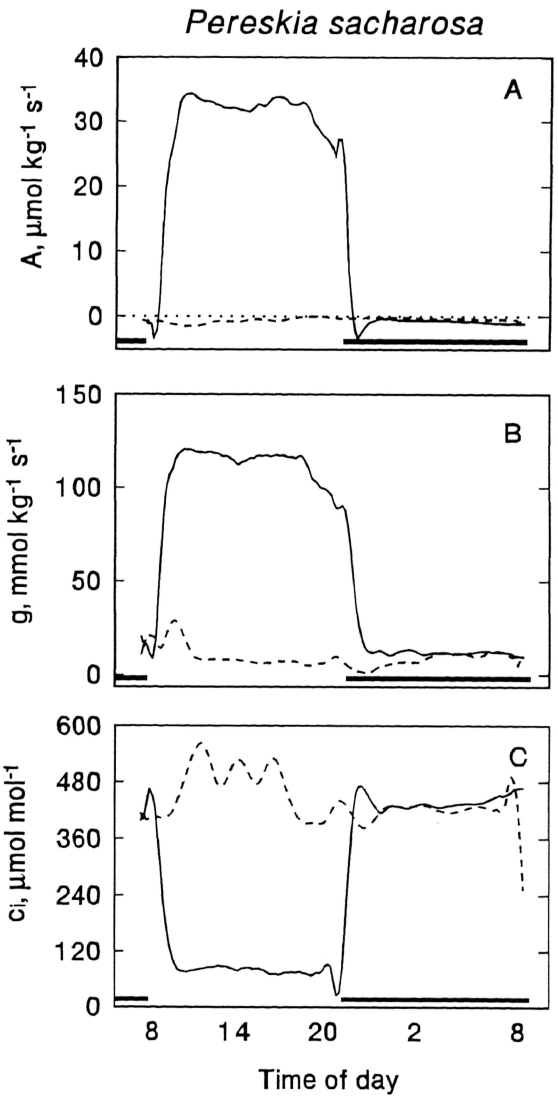

Fig. 2 *A*, Diel patterns of net CO_2 exchange, *A*; *B*, conductance, *g*; and *C*, internal CO_2 concentration, c_i for leafy shoots (solid lines) and leafless stems (dashed lines) of *Pereskia sacharosa*. The thick black bars along the *x*-axes indicate nighttime. Data are expressed on a dry mass basis and represent one individual; results for a second individual were similar. Based on these results, water use efficiency (WUE) values and integrated amounts of CO_2 exchange for the day, night, and 24-h period are presented in table 3. Tissue was removed for determination of acid concentrations at the end of the day and at the end of the second night shown (acid data are presented in table 4).

Table 2

Opuntioid Species, Geographic Ranges, and General Morphology of the Individual Plants

Species	Geographic range	Leaf morphology	Stem morphology
Austrocylindropuntia subulata (Mühlenpfordt) Engelmann	Western South America	Persistent, large, terete, very fleshy; WC_{dm} = 11.3	Large, very thick, very fleshy; WC_{dm} = 8.3
Quiabentia pflanzii (Vaupel) Backeberg & F. Kunth	Central South America	Persistent, small, flat, fleshy; WC_{dm} = 14.7	Medium size, thick, hard; WC_{dm} = 10.8
Quiabentia verticellata (Vaupel) Backeberg & F. Kunth	Central South America	Persistent, small, flat, fleshy; WC_{dm} = 11.8	Medium size, thick, hard; WC_{dm} = 9.9
Pereskiopsis aquosa (F.A.C. Weber) Britton & Rose	Northern Central America	Persistent, small, flat, fleshy; WC_{dm} = 10.6	Medium size, thick, hard; WC_{dm} = 10.9
Pereskiopsis diguetii (F.A.C. Weber) Britton & Rose	Northern Central America	Persistent, small, flat, fleshy; WC_{dm} = 12.4	Medium size, thick, hard; WC_{dm} = 9.2
Pereskiopsis gatesii Baxter	Northern Central America	Persistent, small, flat, fleshy; WC_{dm} = 12.9	Medium size, thick, hard; WC_{dm} = 8.1
Pereskiopsis porteri Britton & Rose	Northern Central America	Persistent, small, flat, fleshy; WC_{dm} = 10.8	Medium size, thick, hard; WC_{dm} = 5.7
Cylindropuntia spinosior (Engelmann) F. Kunth	Southern North America	Ephemeral, small, terete, very fleshy; WC_{dm} = 15.3	Medium size, thick, very fleshy; WC_{dm} = 15.9

Note. For further information, see table 1 note.

University of Kansas under the following environmental conditions: photosynthetic photon flux density (PPFD) of 500–700 μmol m^{-2} s^{-1} at shoot height during a 14-h photoperiod, 30°/20°C day/night air temperatures, and 40%/55% day/night relative humidities (RH). Plants were watered only after the soil in their pots had thoroughly dried. A commercial greenhouse fertilizer was added to the water weekly. All plants were well established, growing vigorously, and often flowering when used for the measurements described below. Plants were always kept well watered during all measurements.

Attached shoots (with leaves) were sealed in gas-exchange cuvettes for 3 d, during which CO_2 and H_2O vapor exchange was continuously monitored. The open gas-exchange system, differential infrared gas analyzer, dew point meters, cuvettes, and methods of gas-exchange calculations are described in Harris and Martin (1991) and Gravatt and Martin (1992). Environmental conditions during the gas-exchange measurements were similar to those in the growth chamber. At the end of the second day and at the beginning of the third day, leaf tissue was removed from the shoot inside the cuvette, frozen at −65°C, and subsequently analyzed for acidity (see below). At the end of the third day, the plant shoot was carefully removed from the cuvette, all its leaves were detached, and the plant was replaced in the growth chamber. The leaves were weighed, dried at 65°C until no further weight change, and then reweighed. These weights were used to determine the leaf water content (dry mass basis), WC_{dm}, for each species: (fresh mass − dry mass)/dry mass. Total amounts of CO_2 absorbed during the day and night were calculated by integrating the area under the respective portions of the gas-exchange curve using a commercial software program.

One week from the start of the whole-shoot gas-exchange measurements, the same shoot, now lacking leaves, was resealed in the gas-exchange cuvette. The intervening time allowed petiole scars to heal yet was usually too short to allow regrowth of leaves (when this occasionally occurred, the new immature leaves were removed and their dry weight was added to that of the leaves previously removed). Gas exchange of the leafless stem, sampling for stem acid concentrations (small portions near the stem apex were removed at the times noted above), and final determination of the stem dry weight (the latter was also included with that of the leaves in gas-exchange calculations for the whole shoot) were measured as described above. These weights were also used to calculate values of stem WC_{dm}.

In order to include measurements of both whole shoots (including leaves) and leafless stems and to incorporate as many species as possible, only two individuals of each species were measured. An attempt was made to ensure that these individ-

Table 3

Gains, Losses, and Net Exchange of CO_2 Integrated over a Day, Night, and 24-h Period (mmol CO_2 kg^{-1}) for Leafy Shoots and Leafless Stems of 10 Species of Pereskioideae

	Day			Night			24 h			
Species and tissue	Uptake	Loss	Net	Uptake	Loss	Net	Uptake	Loss	Net	WUE
Maihuenia poeppigii:										
Shoot	33	0	33	0	20	−20	33	20	13	6
Stem	0	12	−12	0	21	−21	0	33	−33	
Pereskia aculeata:										
Shoot	3496	0	3496	0	103	−103	3496	103	3393	4
Stem	0	305	−305	0	49	−49	0	354	−354	
Pereskia horrida:										
Shoot	2701	0	2701	0	110	110	2701	110	2591	8
Stem	423	0	423	10	33	−23	433	33	400	3
Pereskia ziniiflora:										
Shoot	1863	0	1863	0	555	−555	1863	555	1308	4
Stem	0	74	−74	0	182	−182	0	256	−256	
Pereskia quisqueyana:										
Shoot	774	0	774	0	66	−66	774	66	708	4
Stem	0	70	−70	0	119	−119	0	189	−189	
Pereskia bahiensis:										
Shoot	2597	0	2597	0	436	−436	2597	436	2161	4
Stem	0	334	−334	0	440	−440	0	774	−774	
Pereskia sacharosa:										
Shoot	1358	0	1358	0	34	−34	1358	34	1324	7
Stem	0	29	−29	0	14	−14	0	43	−43	
Pereskia bleo:										
Shoot	1578	0	1578	0	112	−112	1578	112	1466	4
Stem	0	32	−32	0	49	−49	0	81	−81	
Pereskia aureiflora:										
Shoot	905	0	905	0	177	−177	905	177	728	4
Stem	0	197	−197	0	80	−80	0	277	−277	
Pereskia lychnidiflora:										
Shoot	1488	0	1488	0	102	−102	1488	102	1386	5
Stem	0	38	−38	0	59	−59	0	97	−97	

Note. Water use efficiencies (WUE; μmol CO_2 $mmol^{-1}$ H_2O) are instantaneous maxima during the day (data for stems given for *P. horrida* only, because no other species exhibited stem CO_2 uptake). Corresponding diel CO_2 exchange data for *P. sacharosa* and *P. horrida* are given in figs. 2 and 3. Lengths of day and night were 14 and 10 h each, respectively. Data are expressed on a dry mass basis. Species are arranged in assumed phylogenetic order from more ancestral to more derived. Values shown are for one individual of each species; measurements for a second individual yielded similar results.

uals were genetically distinct, but this was not always possible. As a result, and because the data obtained for the two plants were usually quite similar, results of only one are presented below.

After storage (typically several weeks) in the ultracold freezer, the leaf or shoot tissue was either ground in a mortar and pestle (pereskioid species) or thawed and sliced (opuntioid species). The liquid in the latter slices was removed by centrifugation as described by Smith and Lüttge (1985), and the malic acid concentration of the tissue liquid was determined using standards of known malic acid concentrations and the enzymatic/spectrophotometric method of Gutmann and Wahlefeld (1974). Large amounts of mucilage in many of the species created difficulties in accurate micropipetting required by this technique, occasionally increasing the level of variability in the data obtained. For this reason, tissue from the pereskioid species was ground and titrated with 0.01 N NaOH to determine acidities. Dry weights of all tissues were determined as described above. Data are presented as mmol H^+ per kg dry mass; thus, malic acid concentrations were multiplied by 2.

Results

Taxa in both subfamilies were arranged in phylogenetic order in all data sets presented in this study (fig. 1). These determinations were based on morphological and distributional data for the Pereskioideae (Leuenberger 1986) and molecular data for both subfamilies (Wallace 1995; R. Wallace, unpublished data). With the exception of the clearly ancestral species *Maihuenia poeppigii*, taxa in the Pereskioideae were less easily ordered phylogenetically, in part resulting from their greater degree of relatedness.

All species of *Pereskia* had relatively narrow nonsucculent green stems and large flat leaves that were only partially succulent, at least in comparison with taxa in the Opuntioideae (cf. descriptions and tissue water contents in tables 1 and 2). An exception to this among the pereskioid taxa was *M. poeppigii*, which is a diminutive plant with a short thick stem and very small terete leaves. Although both the leaves and stem of this plant appeared "fleshy," their water contents were low (table 1). Results of the physiological measurements were

Table 4

Morning, Evening, and Diel Change (Morning–Evening) in Tissue Proton Concentrations (mmol H^+ kg^{-1}) for Leaves and Stems of 10 Species of Pereskioideae

Species and tissue	Morning	Evening	Difference
Maihuenia poeppigii:			
Leaf	131	77	54
Stem	88	55	33
Pereskia aculeata:			
Leaf	602	619	<0
Stem	153	159	<0
Pereskia horrida:			
Leaf	636	195	441
Stem	301	183	18
Pereskia ziniiflora:			
Leaf	344	267	77
Stem	437	407	30
Pereskia quisqueyana:			
Leaf	248	192	56
Stem	216	196	20
Pereskia bahiensis:			
Leaf	412	433	<0
Stem	365	378	<0
Pereskia sacharosa:			
Leaf	314	157	157
Stem	156	99	57
Pereskia bleo:			
Leaf	396	407	<0
Stem	365	380	<0
Pereskia aureiflora:			
Leaf	304	157	147
Stem	251	273	<0
Pereskia lychnidiflora:			
Leaf	1988	2012	<0
Stem	83	96	<0

Note. Corresponding diel CO_2-exchange data are given for *P. sacharosa* and *P. horrida* in figs. 2 and 3; day- and night-integrated CO_2 exchanges are given in table 3. Data are expressed on a dry mass basis. Because net nighttime CO_2 exchange was always negative (see table 3), all diel acid fluctuations resulted from recycling of internally generated CO_2. Species are arranged in assumed phylogenetic order from more ancestral to more derived. Values shown are for one individual of each species; measurements for a second individual yielded similar results.

nearly the same for all species in the Pereskioideae. Net CO_2 uptake was restricted to the daytime and to the leafy shoots, whereas leafless stems exhibited only CO_2 release day and night (table 3; data for *Pereskia sacharosa* given in fig. 2). An exception to the latter was observed in *Pereskia horrida*, the stems of which absorbed CO_2 during the day (fig. 3; table 3). Values of daytime water use efficiency (WUE) were low relative to nighttime values of the opuntioid taxa, yet comparable with daytime values of the latter (cf. tables 3 and 5). Tissue acidity increased to a small degree at night in leaves and stems of five species and in leaves only of one other pereskioid species (table 4). Because nighttime CO_2 uptake was never observed in any of the pereskioid taxa, all diel acid fluctuations resulted from recycling CO_2 generated within the tissues.

Unlike the various species of *Pereskia* included in this study, the opuntioid species examined here were more morphologically variable (table 2). Plants of *Austrocylindropuntia subulata* had extremely thick succulent green stems with long terete highly succulent leaves. Unlike most species of the more derived genus *Opuntia*, the large leaves of *A. subulata* remain attached to the stem for long periods of time. The green stems of individuals of all six species of *Quiabentia* and *Pereskiopsis* were relatively thick and somewhat succulent, whereas the leaves were smaller and more succulent than those of the pereskioids (cf. water contents in tables 1 and 2). Unlike all the above taxa, *Cylindropuntia spinosior* had ephemeral leaves which were small, terete, and highly succulent. Stems of these plants were green, thick, and also highly succulent (table 2).

Rates of net CO_2 exchange and stomatal conductances of *A. subulata* were much lower than those of the other opuntioid species examined in this study (cf. fig. 4 with figs. 5–7; table 5). Both the leafy shoot and the leafless stem exhibited similar

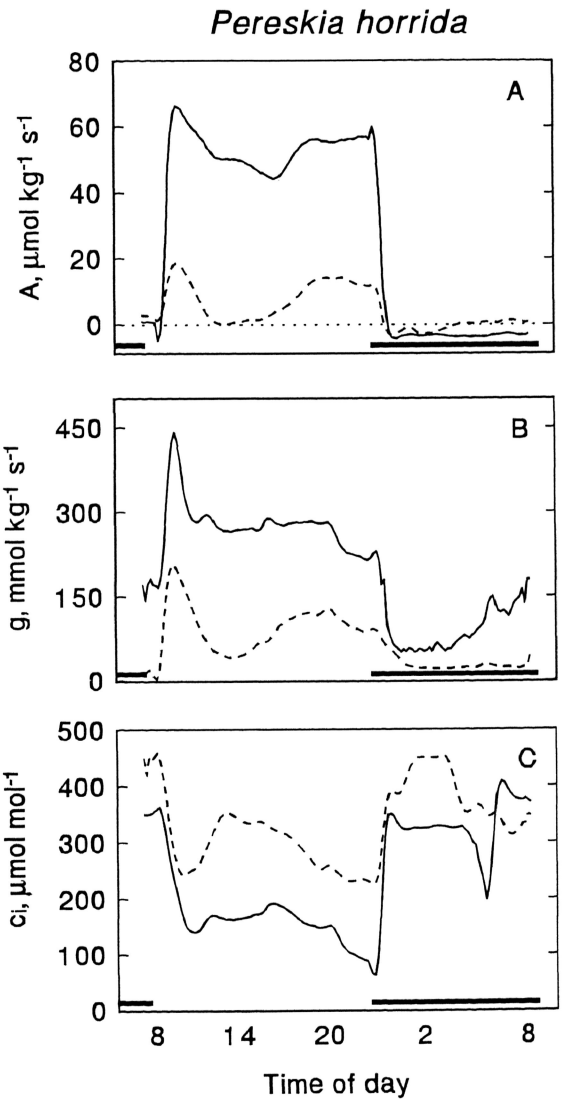

Fig. 3 *A*, Diel patterns of net CO_2 exchange, *A*; *B*, conductance, *g*; and *C*, internal CO_2 concentration, c_i for leafy shoots (solid lines) and leafless stems (dashed lines) of *Pereskia horrida*. For more information, see legend to fig. 2.

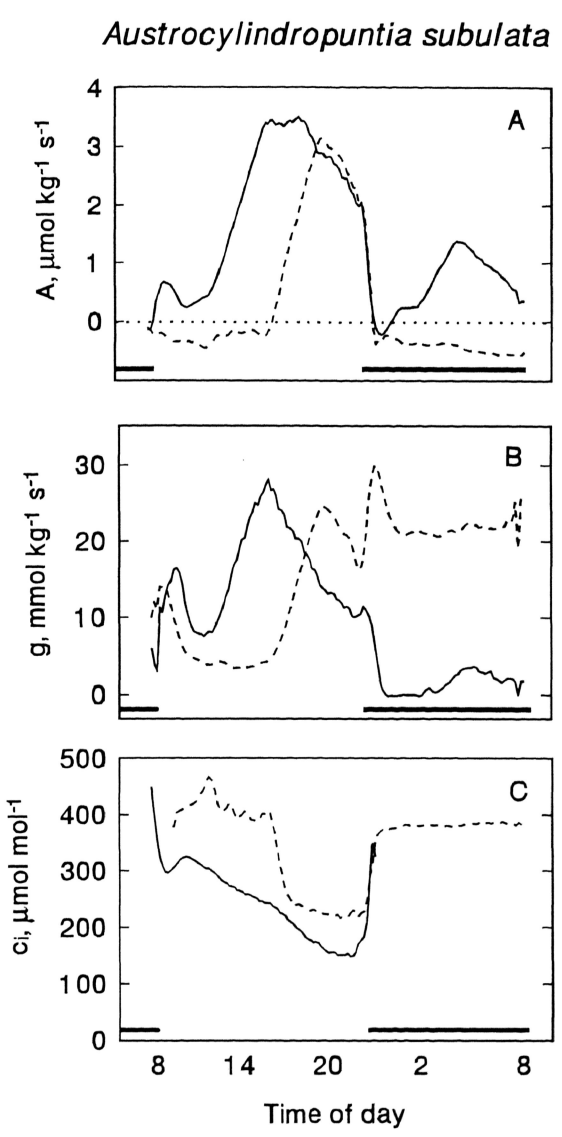

Fig. 4 *A*, Diel patterns of net CO_2 exchange, *A*; *B*, conductance, *g*; and C, internal CO_2 concentration, c_i (no c_i data presented for the second night because conductances were too low to allow accurate calculation of c_i) for leafy shoots (solid lines) and leafless stems (dashed lines) of *Austrocylindropuntia subulata*. For more information, see legend to fig. 2, except refer to table 5 for WUE and integrated CO_2-exchange data and to table 6 for malic acid data.

maximal rates of CO_2 uptake during the day; however, the shoot absorbed CO_2 for a greater proportion of the day (fig. 4). At night, net CO_2 uptake was observed only in the intact shoot, implying that the leaves were responsible for this uptake. The higher conductances observed at night for the stem relative to daytime conductances (fig. 4) were most likely attributable to the relatively large moist wound resulting from removal of tissue from the extremely thick stem for the determination of malic acid concentration at the end of the day. The maximal water use efficiency associated with CO_2 uptake by the shoot (leaves) at night exceeded that during the day by more than 10 times (table 5). Nocturnal increases in tissue acid concentrations were detected in both leaves and stems of *A. subulata* (table 6). Because no CO_2 uptake was observed in the stem at night, all of this acid necessarily resulted from the fixation of internally generated CO_2. Furthermore, the amount of acid generated at night in the leaves also greatly exceeded the amount of CO_2 absorbed from the atmosphere (table 6).

The results of gas exchange and acid determinations for the two species of *Quiabentia* were qualitatively similar, although overall rates of CO_2 uptake in *Quiabentia pflanzii* exceeded those of *Quiabentia verticellata* by nearly two times (table 5). In addition, the maximal rate of nocturnal CO_2 uptake by shoots of the latter species was nearly equal to the maximal daytime rate (fig. 5), whereas nighttime CO_2 uptake in *Q. pflanzii* was much lower than that measured during the day (table 5). Net CO_2 uptake during the day and night occurred in shoots of both species, whereas net CO_2 uptake was detected

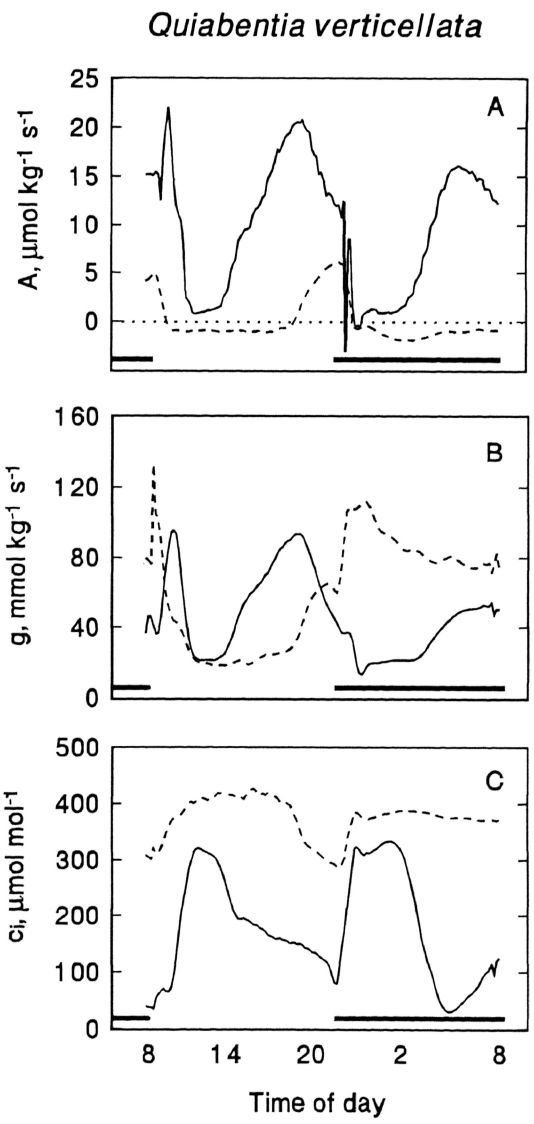

Fig. 5 *A*, Diel patterns of net CO_2 exchange, *A*; *B*, conductance, *g*; and *C*, internal CO_2 concentration, c_i for leafy shoots (solid lines) and leafless stems (dashed lines) of *Quiabentia verticellata*. For more information, see legend to fig. 2, except refer to table 5 for WUE and integrated CO_2-exchange data and to table 6 for malic acid data.

Table 5

Gains, Losses, and Net Exchange of CO_2 Integrated over a Day, Night, and 24-h Period (mmol CO_2 kg^{-1}) for Leafy Shoots and Leafless Stems of Eight Species of Opuntioideae

	Day			Night			24 h			WUE	
Species and tissue	Uptake	Loss	Net	Uptake	Loss	Net	Uptake	Loss	Net	Day	Night
Austrocylindropuntia subulata:											
Shoot	93	0	93	26	1	25	119	1	118	4	50
Stem	49	7	42	0	15	−15	49	22	27	3	...
Quiabentia pflanzii:											
Shoot	1277	0	1277	220	13	207	1497	13	1484	4	20
Stem	129	12	117	43	19	24	172	55	117	4	8
Quiabentia verticellata:											
Shoot	523	0	523	296	1	295	819	1	818	6	22
Stem	83	31	52	0	41	−41	83	72	11	2	12
Pereskiopsis aquosa:											
Shoot	1444	0	1444	548	0	548	1992	0	1992	10	12
Stem	186	4	182	181	1	180	367	5	372	3	13
Pereskiopsis diguetii:											
Shoot	1537	0	1537	392	1	391	1929	1	1928	6	21
Stem	104	0	104	233	6	227	337	6	331	2	15
Pereskiopsis gatesii:											
Shoot	1488	0	1488	323	2	321	1811	2	1809	7	6
Stem	195	1	194	440	0	440	635	1	634	3	9
Pereskiopsis porteri:											
Shoot	385	0	385	344	0	344	729	0	729	5	22
Stem	70	3	67	4	4	0	74	7	67	3	1
Cylindropuntia spinosior:											
Shoot	1259	0	1259	510	0	510	1769	0	1769	4	14
Stem	120	44	76	557	0	557	677	44	633	1	12

Note. Corresponding diel CO_2-exchange data for selected species are given in figs. 4–7. Water use efficiencies (WUE) in μmol CO_2 mmol H_2O^{-1}. For more information, see table 3 note.

only during the day and for a very brief period at the beginning of the night in the leafless stems (fig. 5; table 5). Thus, the leaves were responsible for nearly all the CO_2 uptake at night and the majority of uptake during the day as well. As was the case with *A. subulata*, exposure of moist stem tissue after sampling for malic acid concentrations at the end of the day most likely resulted in unusually high conductances of the stems at night (fig. 5). Regardless, nighttime WUE of the leafy shoots were much higher than daytime values (table 5). In both species, nocturnal accumulations of acid occurred in leaves and stems and in all cases greatly exceeded nighttime levels of CO_2 uptake (table 6). Thus, internal sources of CO_2 accounted for three-fourths or all of the malic acid synthesized throughout the night.

Patterns of net CO_2 exchange for whole shoots (including leaves) and leafless stems of the four species of *Pereskiopsis* were similar, and thus, diel patterns of gas exchange for only *Pereskiopsis diguetii* are presented here (fig. 6). Maximal rates of net CO_2 uptake of the leaves greatly exceeded those of the stems. Rates of daytime CO_2 uptake in leafy shoots were higher than those measured at night (fig. 6), except similar amounts of CO_2 uptake were observed day and night in *Pereskiopsis porteri* (table 5). In all species of *Pereskiopsis*, daytime rates declined precipitously at midday and then increased in the late afternoon. This midday decline was attributable primarily to a decrease in photosynthetic capacity in *Pereskiopsis gatesii*, reflected in an increase in tissue internal CO_2 concentration, whereas in all other species of *Pereskiopsis* the midday decline in CO_2 uptake was a function of limitations in photosynthetic capacity as well as decreases in stomatal conductance (data not shown). For stems of these species, maximal rates of net CO_2 uptake were similar day and night or were higher during the day and were typically restricted to the latter half of either time period (fig. 6; table 5). Very low rates of net CO_2 uptake were observed only at the start of the nighttime in *P. porteri* (table 5). The instantaneous WUE of the leafy shoots was much greater at night than during the day in *P. diguetii* and *P. porteri*; however, similar values were measured in *Pereskiopsis aquosa* and *P. gatesii* (table 5). Overnight increases in tissue acid concentrations were substantial in both leaves and stems of the four species of *Pereskiopsis* (table 6). The majority of the nocturnal increase in acid in both leaves and stems was not attributable to uptake of CO_2 from the atmosphere but reflected internal recycling of CO_2 instead (table 6). Degrees of this internal recycling ranged from 50% for the stems of *P. aquosa* to 100% for leaves of *P. gatesii* (table 6).

Net CO_2 uptake by shoots of *C. spinosior* occurred during both the day and the night, with higher rates observed during the day (fig. 7; table 5). In contrast, leafless stems of *C. spinosior* assimilated CO_2 primarily at night, with only a brief period of uptake occurring at the beginning and end of the day. Because such rates were comparable to those observed for the leafy shoots at night (fig. 7), it is likely that the night CO_2 uptake observed in the shoots reflected activity solely of

Table 6

Morning, Evening, and Diel Change (Morning−Evening) in Tissue Proton Concentrations (mmol H^+ kg^{-1}) for Leaves and Stems of Eight Species of Opuntioideae

Species and tissue	Morning	Evening	Difference	CO_2 recycled[a]
Austrocylindropuntia subulata:				
Leaf	1194	88	1106	0.95
Stem	342	0	342	1.00
Quiabentia pflanzii:				
Leaf	2804	806	1998	0.82
Stem	1066	34	1032	0.92
Quiabentia verticellata:				
Leaf	3078	394	2684	0.78
Stem	1050	206	844	1.00
Pereskiopsis aquosa:				
Leaf	4384	734	3650	0.80
Stem	2290	1560	730	0.50
Pereskiopsis diguetii:				
Leaf	3602	0	3602	0.91
Stem	6092	3584	2508	0.81
Pereskiopsis gatesii:				
Leaf	6870	1108	5762	1.00
Stem	4056	1260	2796	0.69
Pereskiopsis porteri:				
Leaf	2562	0	2562	0.73
Stem	612	444	168	0.95
Cylindropuntia spinosior:				
Leaf	1956	228	1728	1.00
Stem	7052	660	6392	0.83

Note. Corresponding diel CO_2-exchange data for selected species are given in figs. 4–7. For more information, see table 4 note.

[a] Amount of CO_2 absorbed internally in the tissue, expressed as a fraction of the total acid accumulated. This was calculated by subtracting the nighttime CO_2 gain (see table 5; to obtain gas-exchange data for leaves, values for the stems were subtracted from values for whole shoots) from the diel change in acid content (assuming 2 H^+ per CO_2) and dividing by the latter. Thus, a value of 1 indicates that no CO_2 was absorbed from the atmosphere.

the stems. Likewise, the high daytime CO_2 uptake rates observed in the shoots were attributable solely to the leaves (fig. 7). Values of WUE in both shoots and stems were higher at night than during the day (table 5). Diel changes in tissue acid concentrations occurred in both organs, although to a much greater extent in the stem (table 6). As was the case with most of the other species of Opuntioideae examined here, nighttime CO_2 uptake could account for zero (leaves) or only a small percentage (stems) of the malic acid synthesized (table 6).

Discussion

A typical diel pattern of CO_2 exchange in an obligate CAM plant includes small amounts of daytime uptake, restricted to the early morning (CAM Phase II) and late afternoon (CAM Phase IV), but most CO_2 uptake occurs throughout the night (CAM Phase I; see Osmond 1978 for a description of these gas-exchange phases). Some plants, for example, species of *Sempervivum* (Wagner and Larcher 1981) and *Sedum* (Groenhof et al. 1986; Gravatt and Martin 1992) in the Crassulaceae, *Peperomia* (Sipes and Ting 1985) in the Piperaceae, *Clusia* (Franco et al. 1991) in the Clusiaceae, *Plectranthus* in the Lamiaceae (Herppich and Herppich 1996), and *Aptenia* in the Mesembryanthemaceae (Herppich and Peckmann 1997), absorb large amounts of CO_2 during both the day and the night; such plants are often referred to as "C_3-CAM intermediates." In addition, an increasing number of taxa exhibit a strictly C_3 pattern of CO_2 exchange with no CO_2 uptake at night, yet malic acid concentrations in their photosynthetic tissues undergo large diel fluctuations as in obligate CAM plants (Ting 1985; Griffiths 1988; Martin 1996). This phenomenon has been termed "CAM-cycling" (Ting 1985). All of these patterns were observed in the current investigation.

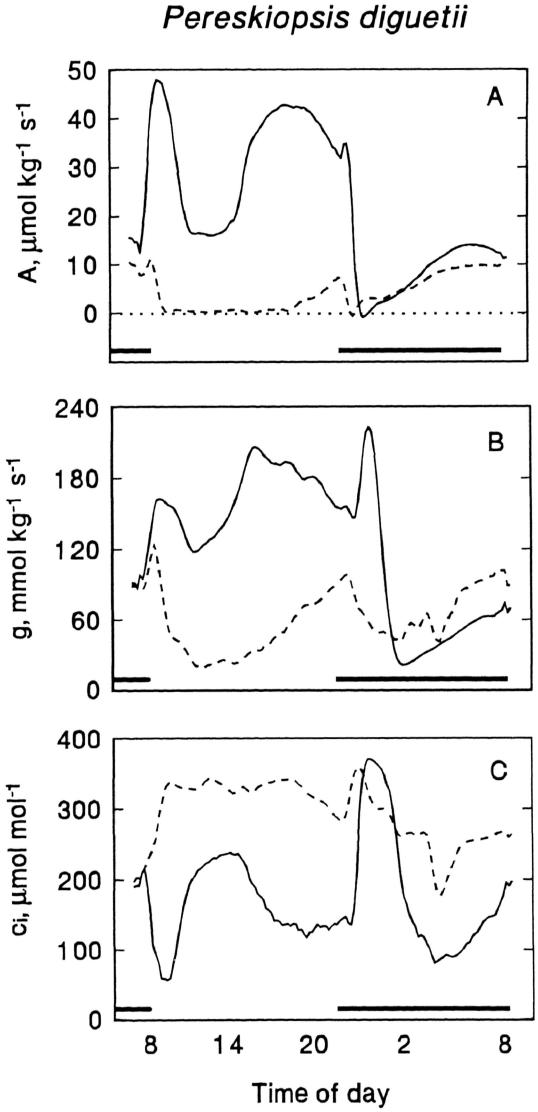

Fig. 6 *A*, Diel patterns of net CO_2 exchange, *A*; *B*, conductance, *g*; and *C*, internal CO_2 concentration, c_i for leafy shoots (solid lines) and leafless stems (dashed lines) of *Pereskiopsis diguetii*. For more information, see legend to fig. 2, except refer to table 5 for WUE and integrated CO_2-exchange data and to table 6 for malic acid data.

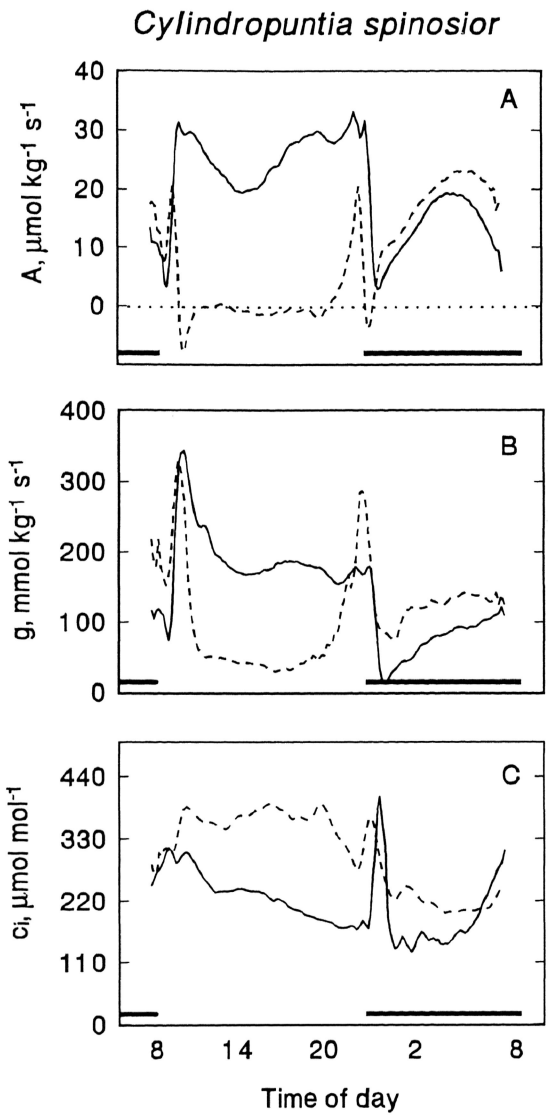

Fig. 7 *A*, Diel patterns of net CO_2 exchange, *A*; *B*, conductance, *g*; and *C*, internal CO_2 concentration, c_i for leafy shoots (solid lines) and leafless stems (dashed lines) of *Cylindropuntia spinosior*. For more information, see legend to fig. 2, except refer to table 5 for WUE and integrated CO_2-exchange data and to table 6 for malic acid data.

Patterns of net CO_2 exchange in shoots and stems of all species in the Pereskioideae were characteristic of C_3 photosynthesis only. Furthermore, the green stems of all but one species (*Pereskia horrida*) exhibited only net CO_2 release day or night. These results, coupled with a lack of diel acid fluctuations, indicated that four of the 10 taxa investigated were C_3 plants. Small diel acid fluctuations typical of CAM were observed in both the leaves and stems of five species and the leaves only of another pereskioid species. This combination of CAM-like acid fluctuations with C_3 gas exchange is characteristic of CAM-cycling, albeit at low levels in these pereskioids. Whether or not diel acid fluctuations or even CAM gas exchange might be stimulated in these or the other species by drought stress was not examined in this study. Rayder and Ting (1981) reported similar diel acid fluctuations for both well-watered and drought-stressed plants of two species of *Pereskia*, whereas Diaz and Medina (1984) found greater acid fluctuations in *Pereskia guamacho* during the dry, relative to the wet, season.

In general, the findings of the current study support those of past studies of the Pereskioideae and expand them to include more taxa and more information differentiating photosynthesis in the leaves versus the stems. There was no apparent relationship between phylogenetic status of the species examined and the presence of CAM biochemistry, although nine of the 10 pereskioid species surveyed are in the same genus and their phylogenetic relationships are not fully understood. Regardless, it is clear that the biochemical capacity for CAM evolved in some of the most ancestral of taxa in the Cactaceae. Whether or not the low levels of CAM-cycling might benefit these plants is debatable and an area of active investigation (see Griffiths 1988; Monson 1989; Martin 1996).

In all eight species of Opuntioideae examined in this study, net CO_2 uptake during the day or night (usually both) was observed in both the leafy shoots and the leafless stems. In most cases, daytime rates of CO_2 uptake exceeded those at night, regardless of tissue type. In addition, daytime amounts of CO_2 uptake by the shoots always exceeded that by the stems, indicating that the leaves were responsible, at least in part, for the observed daytime uptake. The occurrence of stem photosynthesis in the Cactaceae is of no surprise (Gibson and Nobel 1986; Nobel 1988); however, it is notable that net CO_2 uptake by the stems constituted a considerable proportion of the whole-plant carbon budget even in taxa characterized by large long-lasting leaves and relatively diminutive stems.

Water use efficiencies of CAM plants typically exceed those of C_3 and C_4 taxa (Kluge and Ting 1978; Osmond 1978; Ting 1985; Helliker and Martin 1997). This difference is often ascribed to lower rates of transpiration resulting from a lower evaporative demand at night during nocturnal CO_2 uptake in CAM plants (but see Eller and Ferrari 1997 and Woerner and Martin 1999). Not surprisingly, nocturnal maximal WUE greatly exceeded values during the day in most comparisons in this study. On the other hand, two exceptions were found: shoots (mainly leaves) of *Pereskiopsis aquosa* and of *Pereskiopsis gatesii*. Reasons for this are presently unclear.

Photosynthetic gas exchange in leafy shoots (essentially the leaves) occurred during both the day and night in all opuntioid species except the most derived one, *Cylindropuntia spinosior*. In addition, overnight accumulations of malic acid, a diagnostic indicator of CAM, were measured in all species. Thus, the leaves of these taxa might be classified as C_3-CAM intermediates, or CAM-cycling in the case of *C. spinosior*. In comparison, the photosynthetic pathway type of the stems ranged from CAM-cycling in *Austrocylindropuntia subulata* and the two *Quiabentia* species to C_3-CAM intermediacy in *Pereskiopsis* to CAM accompanied by a high level of CO_2 recycling in *C. spinosior*. Thus, overall, there was a slight trend toward increased CAM in the stems and toward decreased CAM in the leaves. The results of the present study confirm those of Nobel and Hartsock (1986) and expand them by including more species and determinations of diel malic acid fluctuations. The latter provide added insight regarding the ability of these taxa to perform CAM.

Consideration of the whole shoot reveals that all eight taxa of the Opuntioideae included in this investigation absorbed CO_2 during both the day and night. All plants here were well watered. Presumably, daytime CO_2 uptake is suppressed under drought stress (Nobel and Hartsock 1987). Thus, these plants benefit from relatively high CO_2 uptake rates during the day at a considerable cost of water as a result of low WUE but then presumably continue to absorb CO_2 at night, albeit at low rates, during dry periods. Relative amounts of water loss during nocturnal CO_2 uptake were substantially lower than daytime values in most comparisons for these taxa. This combination of C_3 and CAM should prove advantageous in the tropical and subtropical habitats of these species of Opuntioideae (Gibson and Nobel 1986; Barthlott and Hunt 1993).

Overall, the results of this study indicate that CAM appears at low levels in the Pereskioideae, the most ancestral subfamily in the Cactaceae, and assumes increasing importance in the Opuntioideae. Furthermore, the increasing importance of CAM in the Cactaceae correlates with an evolutionary trend in a reduction in the physiological importance of leaves and an increase in the physiological importance of the stems. These evolutionary trends also correlate with a diminution in the size and longevity of leaves and an increase in proportional size of the stems in the taxa of these subfamilies of the Cactaceae.

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