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A PHYLOGENETIC VIEW OF LOW-LEVEL CAM IN *PELARGONIUM* (GERANIACEAE)[1]

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Crassulacean acid metabolism (CAM) is common in several plant families and is often associated with succulence. Few studies have examined the occurrence of CAM from a phylogenetic perspective. The genus *Pelargonium* is promising for such a study because members are characterized by dramatic variation in growth form (including geophytes, shrubs, and stem succulents) and because growth form diversity is expressed to the greatest extent in a monophyletic group comprising 80% of *Pelargonium* species. This clade, predominantly from the winter rainfall region of southern Africa, likely proliferated in response to Miocene or Pliocene aridification. We present a survey for CAM across *Pelargonium*, emphasizing the winter rainfall clade. Dawn/dusk fluctuations in titratable acidity were examined in 41 species, with detailed measurements of carbon uptake and stomatal conductance under progressive water stress in four species. No species exhibited obligate CAM. When well-watered, most species exhibited stomatal conductances and acid fluctuations characteristic of C_3 photosynthesis, though some exhibited more pronounced increases in nocturnal acidity, suggesting CAM cycling. In four species examined during dry-down, water stress led to increased nighttime acid levels and decreased daytime stomatal conductance. Ultimately, stomata closed and external carbon uptake ceased, consistent with CAM idling. These results are discussed from the perspective of the evolution of CAM flexibility.

Key words: CAM idling; crassulacean acid metabolism; Geraniaceae; growth form; *Pelargonium*; phylogeny; southern African plants; succulence.

Crassulacean acid metabolism (CAM) is a mode of photosynthesis usually characterized by a suite of traits: CO_2 fixation first by phosphoenolpyruvate carboxylase (PEPc), significant diel fluctuations in titratable acids, and nocturnal stomatal opening. Crassulacean acid metabolism is thought to be adaptive in environments where water is scarce. Research has revealed intriguing mixed patterns of succulence and the occurrence of CAM with, for instance, some South African succulents being strongly CAM, some apparently intermediate or CAM-flexible, and some exhibiting only typical C_3 carbon assimilation via Rubisco and daytime stomatal conductance (Schütte, Steyn, and van der Westhuisen, 1967; Mooney, Troughton, and Berry, 1977; Rundel, Esler, and Cowling, 1999; Veste, Herppich, and von Willert, 2001).

The presence of high numbers of succulent plant species in the South African Cape Floristic region (Goldblatt, 1978) has inspired interest in the occurrence of CAM in the southern African flora. Numbers of plant species in the succulent karoo and fynbos regions exceed Amazonia in some areas (Cowling and Richardson, 1995) and are accompanied by high endemism, estimated at nearly 70% for the Cape Flora (Bond and Goldblatt, 1984) and 40–50% in Namaqualand (Cowling, Esler, and Rundel, 1999). This extraordinary species richness in several clades has been hypothesized to result from explosive, recent radiations in response to increasing aridification caused by the altered flow of ocean currents in the Miocene (Bakker, Culham, and Gibby, 1999; Richardson et al., 2001). Consistent with this hypothetical radiation is the large number of species in these regions that are stem succulent, leaf succulent, or geophytic—growth forms whose presence is generally attributed to predictable but limited rainfall. The rapid radiation of species, possibly since the late Miocene and Pliocene, combined with the common occurrence of variations on succulent growth forms, suggests that the South African flora may be particularly useful for studying not only the patterns of occurrence of CAM, but also the evolution of CAM photosynthesis within genera whose phylogenetic relationships are well described.

Pelargonium L'Hérit. ranks as the third largest plant genus in the Cape flora (Goldblatt and Manning, 2000). It is the sister group to the remainder of the Geraniaceae clade (Price and Palmer, 1993; Albers, 1996). Based on limited data (e.g., Thomas and Beevers, 1949; Schütte, Steyn, and van der Westhuisen, 1967; Kluge and Ting, 1978), the Geraniaceae are considered a ''minor'' CAM family, defined as one in which only a small number of species have CAM photosynthesis or one that contains species that show only weak CAM activity (Winter and Smith, 1996). Sampling for CAM activity in the family has not been systematic or extensive. The apparent radiation of species within the genus *Pelargonium* offers unusual potential for exploring the evolution of CAM photosynthesis within a known phylogenetic framework. Species of *Pelargonium* fall into two main groups that can be distinguished on the basis of chromosome size, biogeographical distribution, and mitochondrial and chloroplast DNA sequences (Bakker et al., 1999, 2000; Bakker, Culham, and Gibby, 1999). The majority of the approximately 280 species of *Pelargonium* are concentrated in the winter rainfall region of southern Africa, and over 80% of the species in the region are members of a single clade (Clade A in Fig. 1). These species are characterized by growth forms ranging from evergreen shrubs to deciduous subshrubs, stem succulents, and geophytes (for illustrations, see van der Walt, 1977; van der Walt and Vorster, 1981, 1988). The evolution of stem succulence in shrubs and

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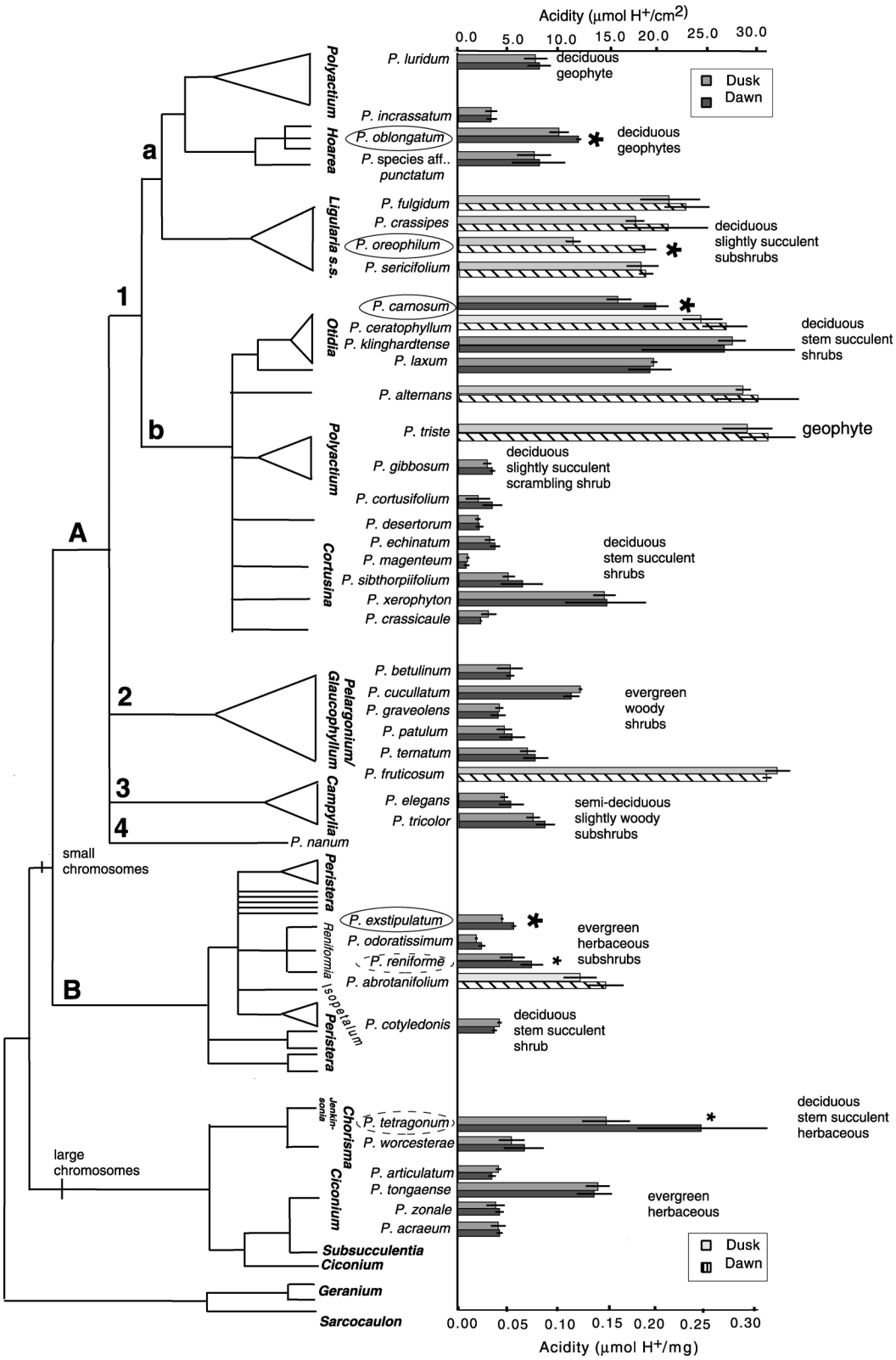

Fig. 1. Phylogenetic relationships among sections of *Pelargonium* determined by Bakker, Culham, and Gibby (1999) and Bakker et al. (1999, 2000). Species names are in the central column of the figure. To the left of these names, section names are shown in boldface type. Growth forms within sections are shown on the far right. Bars indicate amounts of titratable H^+ on a per area basis (dark gray bars = nighttime accumulation measured at dawn; light gray bars = daytime accumulation measured at dusk) or per mass basis for species with finely dissected leaves (hatched bars = nighttime accumulation measured at dawn; very light gray bars = daytime accumulation measured at dusk) (means ± 1 SD). Large asterisks and continuous ovals indicate species with significant differences ($P < 0.05$) between daytime and nighttime accumulations. Small asterisks and broken ovals indicate species with slightly less significant differences ($P < 0.10$) between daytime and nighttime accumulations.

subshrubs appears to have occurred at least three times in *Pelargonium* (C. S. Jones, unpublished data).

To date, very few studies have examined photosynthetic characteristics of species within *Pelargonium*. *Pelargonium crithmifolium*, a stem succulent, has shown nocturnal acid increase in two different studies (Schütte, Steyn, and van der Westhuisen, 1967; Kluge and Ting, 1978), but Rundel, Esler, and Cowling (1999) found that both *P. crithmifolium* and another stem succulent species, *P. klinghardtense*, had leaf carbon isotope discrimination values typical of C_3 photosynthesis. Von Willert et al. (1992) reported that at least one *Pelargonium* species they examined (listed as *Pelargonium* sp.) was a C_3 plant, whereas one species of geophyte (also listed as *Pelargonium* sp.) is ''likely to exhibit CAM or CAM cycling.'' Another species, *P. fulgidum*, a semisucculent subshrub, did not exhibit diel acid fluctuations (Schütte, Steyn, and van der Westhuisen, 1967). In order to more systematically evaluate CAM-related patterns of nocturnal acid accumulation and the evolution of carbon metabolism within *Pelargonium*, we examined dusk/dawn changes in titratable acidity sampled broadly across the genus. We emphasized those species from the winter rainfall region, using a total of 41 species grown under well-watered, greenhouse conditions. These observations were supplemented with investigations of photosynthetic rates and stomatal conductance in selected species and with investigations of plasticity of acid fluctuations and photosynthesis in response to withholding water. We found variation among sections in the tendency toward pronounced increases in nocturnal leaf and stem acidity; we did not find species that had evolved full CAM.

MATERIALS AND METHODS

Pelargonium species used in the study—*Pelargonium* spp. used in the study and their phylogenetic relationships are shown in Fig. 1. Nomenclature of groups is under revision (Bakker et al., 2000); the two major groups (defined by small and large chromosomes) will receive subgeneric status. The phylogenetic relationships among sections of the small chromosome clade are based on analyses of chloroplast encoded *trnL-F* sequences (Bakker, Culham, and Gibby, 1999; Bakker et al., 1999). Phylogenetic relationships among sections of the large chromosome clade are based on combined *nad1* b/c and *trnL-F* sequences (Bakker et al., 2000).

Year 1: survey of species—Forty-one species of *Pelargonium* were selected from those in greenhouse cultivation at the University of Connecticut, Storrs, Connecticut, USA. (See http://ajbsupp.botany.org/v90/ for accession information; voucher specimens for species examined are deposited in the UConn Herbarium.) All individuals examined had been under greenhouse cultivation for at least 2 yr prior to the first year of this study. Individuals from the UConn greenhouse collection of *Kalanchoe blossfeldiana* and *Crassula lactea* (known CAM species), as well as *Alternanthera ficoides* (a known C_3–C_4 intermediate), were selected as controls. Except where noted below, plants were watered ''normally,'' which was every 3–5 d during the sampling period in year 1. Plants were sampled for titratable acidity between 22 February 1999 and 30 April 1999. Because the expression of CAM is known to vary with developmental age of leaves of some species (Holthe, Sternberg, and Ting, 1987; Ting et al., 1993), only fully expanded leaves were sampled. Leaf disks were punched immediately after sunset and again the following dawn using standard #1 (ten disks per leaf), #2 (2–5 disks were punched per leaf depending on leaf tissue available per species), or #6 (one disk per leaf) cork borers. Three samples, each from different leaves of the same individual, were punched at sunset and sunrise for a total of six samples per plant. Some species of *Pelargonium* (indicated by hatched and light grey bars in Fig. 1) have leaves that are too finely dissected for disks to be punched so they were cut with scissors, kept fully hydrated on moist towels, and their fresh mass determined to the nearest 0.0001 g within 10 min. Each sample was immediately frozen and stored in liquid nitrogen after removal from the plant (or immediately after weighing).

To determine titratable acidity, each sample was ground with 11 mL of pure water from a Millipore Milli-Q plus Ultrapure Water System (Millipore, Bedford, Massachusetts, USA). Ten mL of the resulting solution were then titrated using 0.01 mol/L NaOH to an endpoint of pH 7.00 with constant mixing. The remaining 1 mL of solution was used to rinse the end of the Orion pH meter probe (model 520A, Orion Research, Beverly, Massachusetts, USA) before beginning the titrations. Titratable tissue acidities are presented as micromoles of H^+ per square centimeter fresh tissue or micromole of H^+ per milligram fresh mass tissue.

For all data, reported values of $P < |t|$ between dusk (daytime accumulation) and dawn (nighttime accumulation) acidity samples were determined using *t* tests based on three leaves per plant (except for *P. laxum*, which was based on two leaves per plant) (PROC *t* test) (SAS, 1999–2000).

Year 1: test for CAM induction in leaves—Two stem succulent species, *P. carnosum* and *P. laxum*, were tested for acid fluctuations correlated with water stress. Six plants of *P. carnosum* were used: three were watered normally, and three were denied water for 9 d beyond the normal watering period (i.e., about 12 d). For *P. laxum*, five individuals were used; three were denied water for 12 d and two were watered normally. On day 12, plants in both groups were sampled at sunset and then the following morning.

Year 2: gas exchange measurements and acidity patterns in stems and leaves—Based on morphology and on acidity measurements taken during year 1, *P. carnosum*, *P. cortusifolium*, *P. reniforme*, and *P. tetragonum* were selected for gas exchange measurements during May of year 2. Stomatal conductance and photosynthetic rates were logged every 10 min over 24-h periods using two LI-COR 6400 photosynthesis systems (LI-COR, Lincoln, Nebraska, USA) equipped with red/blue light sources and powered by deep cycle marine batteries. Greenhouse air (varying between ~360 and 400 ppm CO_2 over the course of the day) was drawn from a large ballast volume to buffer short-term CO_2 fluctuations. Light inside the chamber was programmed to track greenhouse light levels, which were measured by quantum sensors mounted on each leaf chamber head. The LI-COR systems were programmed to keep leaf temperature constant at 25°C, but heating was insufficient to keep leaf temperature at this level overnight. Leaf temperatures dipped as low as 17°C for some plants some evenings and temperatures rose as high as 28°C for some plants on some hot days. Plants were well watered. Also during year 2, titratable acidity was measured in leaves and stem cores for *P. carnosum*, *P. crithmifolium* (not included in the year 1 study), and *P. tetragonum* under well-watered conditions. Stem cores approximately 2 mm thick were punched using a #6 core through the periderm and underlying tissues of branches that had been quickly transported to the laboratory. These pieces of tissue were weighed, immediately frozen, and processed as described above for dissected leaves.

Year 3: gas exchange and leaf acidity during dry-down from well-watered to water-stressed conditions—Because our results from years 1 and 2 suggested that CAM flexibility may influence diel acid accumulations, in June of year 3, both photosynthesis and H^+ levels were measured simultaneously in the same plants (different leaves) as they progressed from recently watered (i.e., watered that day or the day before) to water-stressed conditions. Diel photosynthetic responses were tracked every third day over the course of a week of sunny days in early June, when no additional water was applied. Acid levels were determined at dusk and dawn at the beginning of this experiment and again at the end. (Limited leaf material in *P. tetragonum* constrained us to measuring acids only under water-stressed conditions.)

RESULTS

Year 1: survey for nocturnal acid accumulation under normal greenhouse watering—The two known CAM species, *Kalanchoe blossfeldiana* (evening: 278.8 μmol H^+/cm^2; dawn:

1184.0 μmol H^+/cm^2) and *Crassula lactea* (evening: 701.0 μmol H^+/cm^2; dawn: 3127.5 μmol H^+/cm^2) showed nighttime accumulations in titratable tissue acidity that exceeded daytime values by 400%. No nighttime acid accumulations in *Pelargonium* species were similarly dramatic. Several species of *Pelargonium* showed higher nighttime accumulations, measured at dawn, than daytime amounts, measured at dusk (Fig. 1, species names circled). Note that in Fig. 1, darker, solid-colored pairs of bars report acid quantified per unit leaf area, whereas lighter striped bars report acid quantified per unit leaf mass because leaves were too finely divided to get accurate leaf area measures. Comparisons of overall acidity levels are thus only valid within groups of similarly colored bars.

Nighttime acid accumulation was found scattered across the phylogenetic tree, and data suggest some section-specific patterns. For example, consider sections where four or more species were sampled. Species within *Ligularia* sensu stricto (s.s.) (in Clade A1a, Fig. 1) are deciduous, slightly succulent subshrubs, and those we studied had finely divided leaves. All species we examined within *Ligularia* showed a nighttime increase in mean acid levels, though because of variability in the data, fluctuations were statistically significant only for *P. oreophilum*. Similarly, all species we examined within section *Reniformia* (in Clade B, Fig. 1), which are evergreen, herbaceous subshrubs, exhibited nighttime acid accumulation. In contrast, no species within section *Ciconium* (in the large chromosome clade) showed any nighttime acid accumulation; these species are evergreen and herbaceous. Nor were significant nighttime accumulations observed in the clade of evergreen, woody shrubs (sections *Pelargonium* and *Glaucophyllum*). All other sections where four or more species were sampled showed mixed patterns of acid accumulation.

Comparisons among sections in overall leaf acidity also showed some section-specific patterns. Most obviously, in section *Otidia*, containing those species most prominently stem succulent among our collection, leaf acidity per unit area was high during night and day relative to acid levels in leaves of other groups. The only other species with similar leaf acidity levels was *P. tetragonum*, an herbaceous stem succulent in the large chromosome clade.

Gas exchange, years 2 and 3—*Pelargonium carnosum* (section *Otidia*), *P. cortusifolium* (section *Cortusina*), *P. reniforme* (section *Reniformia*), and *P. tetragonum* (section *Chorisma*) were used for gas exchange experiments during years 2 and 3. These species were chosen for several reasons: their leaf shape was amenable to gas exchange measurements, they are members of widely divergent groups within *Pelargonium*, and each exhibited nocturnal acid accumulation. Results from all species both years were similar, so only results from *P. carnosum* for year 3 are presented.

Figure 2 shows photosynthetic rates, stomatal conductance, and light levels immediately after watering (Fig. 2A, B), 3 d later (Fig. 2D, E), and 7 d later for *P. carnosum* (Fig. 2F, G). During dry-down, there was never evidence of nighttime CO_2 fixation in any of the four species examined (e.g., Fig. 2A, D, F). A decline in midday stomatal conductance was observed during intermediate stages of water stress (e.g., Fig. 2D); *P. reniforme* showed nearly complete midday stomatal closure under these conditions (data not shown). *Pelargonium carnosum* and *P. cortusifolium* exhibited slight nocturnal stomatal conductance (~1 μmol $H_2O \cdot m^{-2} \cdot s^{-1}$) under well-watered and intermediate conditions (e.g., Fig. 2A, D), but not under water stress (e.g., Fig. 2F). By the sixth day without water, plants had shut down completely.

Nocturnal acid accumulation under water stress in years 1, 2, and 3—Under well-watered conditions, two different years, no nocturnal accumulation of acid occurred in *P. carnosum* (Fig. 2C). Under water stress (Fig. 2H), higher mean nighttime acidity was observed; the increase in year 1 was statistically significant ($P < 0.01$). Similar results were obtained for *P. laxum*, another stem succulent species (data not shown). Results from *P. cortusifolium*, *P. reniforme*, and *P. tetragonum* also showed that nocturnal acid accumulation was enhanced with water stress (data not shown), but this enhancement was small in *P. cortusifolium* and *P. reniforme*. *Pelargonium tetragonum* exhibited the most dramatic nocturnal acid accumulation observed during any of these experiments under water stress during year 3; we measured an increase in acid accumulation of 183% (dusk: 10.39 ± 1.72 μmol acid/cm^2 [X ± SD]; dawn: 29.38 ± 7.23 μmol acid/cm^2) during the night relative to the day in this species.

Nocturnal acid accumulation in stems vs. leaves—In year 2, under well-watered conditions, acid fluctuations in both leaves and stems were measured for three species (Fig. 3). Nocturnal acid accumulations were proportionately greater in stems than in leaves in each species, although daytime and nighttime values were not significantly different (note that stem values were quantified per unit tissue mass).

DISCUSSION

The wide range of growth forms within *Pelargonium* and the relatively recent radiation within the group in response to aridification present an unusual opportunity to explore the potential evolution of CAM. We observed diel fluctuations in tissue acidity in leaves (Fig. 1) and stems (Fig. 3) that are similar in magnitude to those characterized by Martin and Wallace (2000) as ''low-level CAM cycling'' in Periskioideae, a subfamily of Cactaceae. Crassulacean acid metabolism cycling species exhibit typical C_3 daytime CO_2 fixation via Rubisco and stomatal conductance (e.g., Fig. 2), but also diel cycling of organic acids characteristic of CAM, associated with fixation of nocturnally respired CO_2 into organic acids (Ting, 1985; Patel and Ting, 1987; Martin, 1996). There is no nighttime stomatal conductance in CAM cycling. The low-to-moderate levels of nocturnal acid accumulation many *Pelargonium* species exhibited in year 1, when plants were receiving water in cycles supporting normal plant growth, may reflect intermediate levels of water stress that arise during this watering cycle (Fig. 1).

All four species of *Pelargonium* selected for gas exchange analyses exhibited C_3 photosynthetic traits under well-watered conditions, though *P. carnosum* and *P. cortusifolium* maintained low stomatal conductance during nighttime hours. All four species shut down stomatal conductance and C_3 photosynthesis during water stress; none exhibited a transition to CAM (e.g., Fig. 2). Nighttime acid accumulation became more pronounced under water stress in *P. reniforme*, *P. carnosum*, and *P. tetragonum*, suggesting a physiology such as CAM idling may be occurring during water stress. Crassulacean acid metabolism idling has been described in CAM species when water stress induces continuous closure of stomata and nocturnal cycling of low levels of organic acids through the CAM

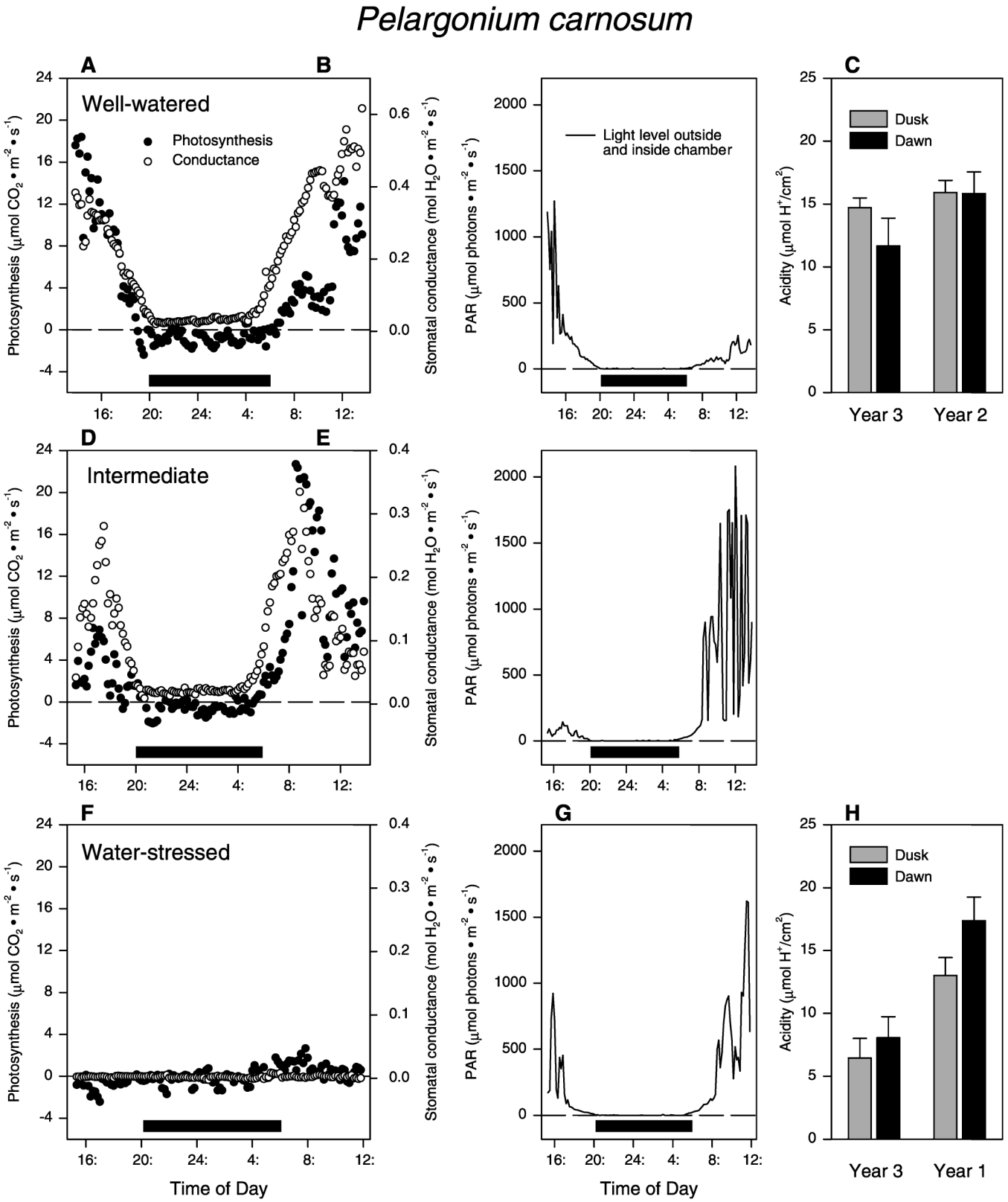

Fig. 2. Diel photosynthesis, conductance, light levels, and titratable acidity in *Pelargonium carnosum*. (A, D, F) Photosynthesis and stomatal conductance in year 3. (B, E, G) Photosynthetically active radiation (PAR) in the gas exchange chamber (programmed to track ambient light levels) in year 3. (A, B) Immediately following watering; (D, E) after 3 d of withholding water; (F, G) after 7 d of withholding water. (C) Levels of titratable acidity under well-watered conditions in years 3 and 2. (H) Levels of titratable acidity under water-stressed conditions in years 3 and 1 (means + 1 SD). Horizontal black bars indicate period of darkness in parts (A), (B), (D–G).

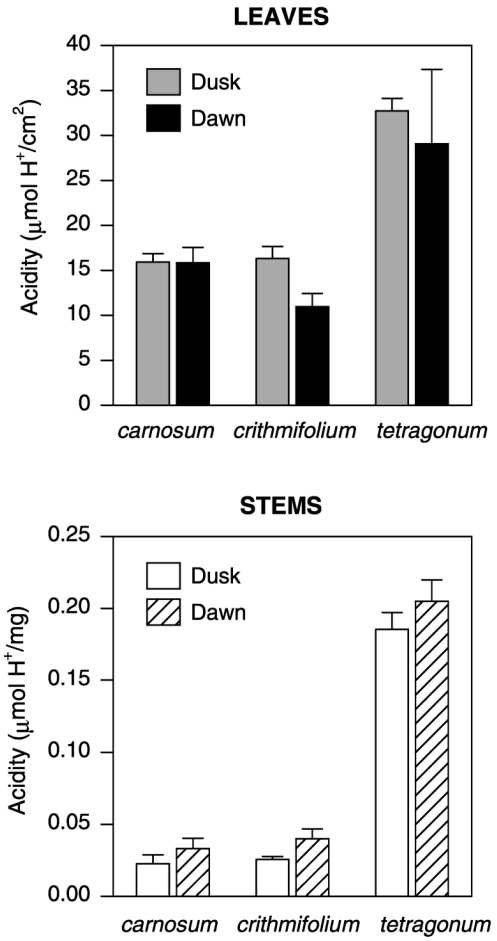

Fig. 3. Titratable acidity levels in *Pelargonium* leaves and stems under well-watered conditions. (A) Leaves: bars indicate amounts of titratable H^+ on a per area basis (black bars = nighttime accumulation measured at dawn; gray bars = daytime accumulation measured at dusk). (B) Stems: bars indicate amounts of titratable H^+ on a per mass basis (hatched bars = nighttime accumulation measured at dawn; open bars = daytime accumulation measured at dusk). Data are means + 1 SD.

pathway (e.g., Holthe, Sternberg, and Ting, 1987; Ting et al., 1993; Kraybill and Martin, 1996). Considered to be a modification of CAM, CAM idling may be a mechanism for maintaining the plant in a physiologically ''poised'' state during the period of stress, preventing photodamage as well as recapturing respiratory CO_2 (Ting, 1985). More recent experimental analyses have supported this hypothesis (Griffiths et al., 1989; Martin, 1996). In *Pelargonium*, it appears that CAM idling-like behavior is found under water stress in species that otherwise perform C_3 photosynthesis under well-watered conditions.

Trends in CAM evolution in angiosperms—Numerous reports from recent studies of both major and minor CAM families (Winter and Smith, 1996) suggest that some form of CAM flexibility is common. Several investigators have proposed that the primary force driving evolution of the CAM pathway is its function as a CO_2 concentrating mechanism, perhaps in response to limited CO_2 availability resulting from midday stomatal closure, rather than solely as a mechanism for increasing water use efficiency (e.g., Winter and Smith, 1996). This is consistent with Ting's hypothesis (1985) that in the evolution of CAM, diel acid cycling, and the associated activity of PEPc would have preceded nighttime stomatal opening (see also Ehleringer and Monson, 1993). Full CAM activity then could have evolved further through the shift in the diel pattern of stomatal opening from one that is exclusively daytime (CAM cycling) to one that is predominantly nighttime (obligate CAM), with the potential intermediate step of nocturnal acid fluctuations occurring in conjunction with stomates that open during the day and again at night (also referred to as ''CAM intermediacy''). Thus, nocturnal fixation of respired CO_2 by PEPc that originated as a physiologically plastic response to drought may have become evolutionarily fixed to differing degrees, and only later may have become associated with nocturnal conductance to varying degrees in different groups of plants.

Evidence for such a scenario is accruing from investigations of CAM activity within explicitly phylogenetic frameworks. In the genus *Kalanchoë* (Crassulaceae), the degree of CAM activity parallels evolutionary changes in growth form and habitat (Kluge and Brulfert, 1996). The phylogenetically more ancestral taxa exhibit CO_2 acquisition via a predominantly C_3 pathway, whereas the most derived taxa exhibit obligate CAM; intermediate taxa exhibit flexible CAM, with CO_2 uptake occurring during the night and day (Gehrig, Rösicke, and Kluge, 1997). In contrast to earlier assumptions, this phylogenetic analysis suggests a monophyletic origin of CAM activity, i.e., a singly acquired tendency toward carbon fixation via PEPc, present in the more basal taxa, becomes progressively strengthened and associated with nocturnal stomatal conductance in the more derived taxa in *Kalanchoë*. Pilon-Smits, 'T Hart, and van Brederode (1996) have drawn similar conclusions for *Sedum* and *Aeonium*, also in the Crassulaceae.

The pattern of CAM activity in other genera and families appears to be similar. The Cactaceae are described as a major CAM family, with many species of obligate CAM plants. Nevertheless, a recent study of two subfamilies of Cactaceae, Pereskioideae and Opuntioideae, showed C_3 patterns of carbon uptake and stomatal conductance, with limited nocturnal acid increases in some species. Thus strict C_3 photosynthesis or low levels of CAM cycling occurs in Pereskiodeae, a basal clade, under well-watered conditions. Under the same conditions, nocturnal carbon uptake, in association with proportional increases in nocturnal acid levels, occurs in the subfamily Opuntioideae, whose members exhibited obligate CAM, C_3-CAM intermediacy, and CAM cycling (Martin and Wallace, 2000). Members of the third and most highly derived subfamily Cactoideae are generally considered obligate CAM plants (Nobel and Hartstock, 1986).

Interestingly, a phylogenetic analysis of internal transcribed spacer (ITS) sequences suggests that the Portulacaceae is paraphyletic and that the Cactaceae arises as a clade within it (Hershkovitz and Zimmer, 1997). A recent analysis of CAM activity in the Portulacaceae shows that those species basal to the Cactaceae, previously considered to be C_3, actually exhibit CAM cycling at low levels (Guralnick and Jackson, 2001). More derived species of Portulacaceae show stronger nocturnal acid accumulations, and some of the most derived species exhibit facultative CAM, where full CAM is induced by water stress. We speculate that additional analyses of basal taxa in the Portulaceae, extending to the Aizoaceae, may reveal a singly acquired tendency toward low levels of CAM cycling as the basal condition in the clade of the Carophyllales containing

the Portulaceae, Aizoaceae, and Cactaceae. Multiple origins of nocturnal stomatal conductance and carbon fixation would then have led to the independent origin of full CAM in each of these groups.

CAM evolution in Pelargonium—In an investigation of the functional significance of CAM cycling, Martin (1996, p. 201) concluded that ''plants with CAM cycling may be viewed as C_3 plants when well-hydrated and CAM idling plants when stressed.'' Our observations indicate that this view accurately describes species of *Pelargonium*, with the caveat that the extent of nocturnal acid accumulation during CAM cycling that occurs prior to CAM idling is at a relatively low level compared to other genera, with the exception of *P. tetragonum*. However, our observations also suggest that some sections exhibit tendencies toward more pronounced increases in nocturnal acids than others. Species in sections *Ciconium* in the large chromosome clade, as well as sections *Pelargonium* and *Campylia* of Clade A, show very little if any increase in nocturnal acid levels under normal watering regimes. Species of the winter rainfall area in Clade A1 show considerable variation in their tendency to accumulate acids at night; more pronounced accumulations are not necessarily restricted to the stem or leaf succulent species. The more dramatic increases in nocturnal acids in individual species within sections *Chorisma*, *Reniformia*, *Otidia*, and Clade A1a suggest that an enhanced ability to recycle respired CO_2 via PEPc may have evolved, or is evolving, independently within each of the major clades within the genus. Whether such accumulations foreshadow the evolution of full CAM will depend on whether nocturnal conductance evolves in these species. Although not yet associated with measureable amounts of external carbon uptake or acid accumulation, very low levels of nocturnal conductance under well-watered conditions, seen in *P. cortusifolium* and *P. carnosum*, suggests that an evolutionary shift in carbon metabolism to full CAM within some species of *Pelargonium* may indeed be possible.

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