

Pereskia and the Origin of the Cactus Life-Form

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ABSTRACT: The cactus life-form is cited as an example of a tight relationship between organism form and function: a succulent, long-lived, photosynthetic stem allows cacti to survive long periods of drought while maintaining a positive tissue water status. *Pereskia* (Cactaceae) comprises 17 species of leafy shrubs and trees that are thought to represent the original cactus condition. Recent phylogenetic work has shown that there are two separate clades of *Pereskia* species, which are basal and paraphyletic with respect to the rest of the cacti. We selected seven *Pereskia* species, representing both clades, and characterized their water relations by measuring a suite of physiological traits in wild populations. Additionally, we estimated basic climate parameters from collection localities for all 17 *Pereskia* species. Extant *Pereskia* species exhibit ecological water use patterns that are very similar to those of the leafless, stem-succulent cacti. Ancestral trait reconstruction for the physiological and environmental data provides a preliminary assessment of the ecology and water relations of the earliest cacti and suggests that several key elements of the cactus ecological niche were established before the evolution of the cactus life-form. We interpret these ecological traits as potentially important drivers of evolutionary innovation in the cacti.

Keywords: key innovation, *Pereskia*, Cactaceae, ancestral trait reconstruction, water relations, ecological niche, character evolution.

The cactus life-form is often heralded as a striking example of adaptive evolution in plants. Most cacti have the ability to survive extended periods of extreme drought, which has allowed the lineage to radiate extensively throughout the New World's arid and semiarid ecosystems (Gibson and Nobel 1986; Nobel 1988; Anderson 2001). Their drought

survival is conferred by a suite of anatomical and physiological specializations. All investigated cacti exhibit extensive, shallow rooting systems, which allow for the rapid absorption of water from the top layer of soil after brief desert rains. Most cacti possess enlarged pith and stem cortical layers, which contain large mucilaginous cells that aid in long-term water storage. Additionally, most cacti exhibit crassulacean acid metabolism (CAM) photosynthesis, a specialized pathway that temporally separates atmospheric CO₂ uptake from photosynthetic light reactions, allowing stomata to open during nighttime when the transpirational water loss is reduced (Kluge and Ting 1978). Finally, most cacti are functionally leafless and have transferred their primary photosynthetic activities to their long-lived, specialized stem cortical tissue layer.

The elimination of leaves is thought to be among their most important adaptations to drought: leaves are temporary structures with large surface areas that allow for excessive water loss to the atmosphere. Creating a long-lived photosynthetic tissue system in the stem minimizes the exposure of hydrated tissue to the atmosphere while at the same time extending the potential lifetime carbon assimilation of photosynthetic cells, which allows them to be more conservative about opening their stomata. Taken together, these traits promote a highly successful "ecological water use strategy": cacti live in environments characterized by extreme drought but maintain positive tissue water status by being exceptionally good at acquiring and storing water and simultaneously exhibiting high photosynthetic water use efficiency (Nobel 1977, 1988; Barcikowski and Nobel 1984; Gibson and Nobel 1986).

Pereskia (Cactaceae) consists of 17 species of leafy shrubs and trees that have long been considered the best living representation of the "ancestral cactus" (fig. 1). Several phylogenetic studies have questioned the monophyly of *Pereskia*, though they are all limited by taxon sampling and/or resolution (Wallace 1995; Nyffeler 2002; Butterworth and Wallace 2005). A new phylogenetic hypothesis of basal cactus relationships based on molecular data resolves "*Pereskia*" as a paraphyletic assemblage of species at the base of the cacti and confirms that their leafy habit and nonsucculent stems were not secondarily derived (Edwards et al. 2005). Further, the *Pereskia* species united with

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Figure 1: *Pereskia* growth form and morphology. *Pereskia* species range from woody scramblers and shrubs to taller trees, reaching over 20 m in height in *Pereskia lychnidiflora*. A, The shrub *Pereskia portulacifolia*. B, The tree *Pereskia guamacho*. C, Leafy stem of *Pereskia sacharosa*, showing the use of the areole short-shoot system to produce leaves as well as spines. D, Succulent leaves subtending the spiny cactus areole during new stem growth in *Pereskia weberiana*.

cactoid, opuntoid, and *Maihuea* lineages (referred to as the “core cacti”) have stem stomata and exhibit delayed bark formation, two traits that are critical in the development of the stem as a long-lived photosynthetic organ (Edwards et al. 2005). This substantiates the usefulness of *Pereskia* species for inferring early events in the transition to the cactus life-form and allows us to address several key questions regarding the evolution of the specialized cactus water use strategy. How did the cacti regulate their water use before the evolution of efficient water storage tissue systems and stem-based photosynthesis? What sorts of environments did they live in, and what levels of drought could they tolerate? Are there particular aspects of their ancestral water relations that may have promoted the evolution of the specialized cactus life-form?

Background and Objectives

In spite of the long-standing interpretation of *Pereskia* as a model of the ancestral cactus, there has been little work done to characterize basic *Pereskia* ecology and physiology. Historically, *Pereskia* has been described as inhabiting a range of dry tropical forest areas but not subject to or surviving severe water stress. They are generally considered to be drought-deciduous, nonsucculent woody plants, with primarily C3 photosynthesis and weak CAM-cycling abilities (Rayder and Ting 1981; Gibson and Nobel 1986; Leuenberger 1986; Nobel and Hartsock 1986, 1987; Mauseth and Landrum 1997; Mauseth 1999; Martin and Wallace 2000). CAM cycling refers to a variant of CAM photosynthesis wherein the plant opens stomata by day and closes them at night but uses the CAM metabolic pathway to reassimilate internally respired CO₂ (Kluge and Ting 1978). Investigations of photosynthetic pathway variation have primarily utilized greenhouse-grown plants, and field studies of wild *Pereskia* populations have been limited (but see Diaz 1984; Diaz and Medina 1984; Luttge et al. 1989).

Even less is known regarding how *Pereskia* species regulate their water use. General characteristics of the plant water transport pathway and the processes involved in regulating plant water loss are illustrated and explained in figure 2 (see table 1 for terminology abbreviations and definitions). Plants living in extremely water-limited environments exhibit several water use strategies that differ in phenology, leaf life span, rooting depth, minimum tolerated tissue water deficits (= minimum tissue water potential, Ψ_{\min}), maximum transpiration rates, and growth form.

Edwards and Diaz (2006) recently investigated the eco-physiology of *Pereskia guamacho* in northwestern Venezuela and found that the water relations of *P. guamacho* are strikingly different from those of co-occurring woody, leafy plants. *Pereskia guamacho* maintained high leaf water

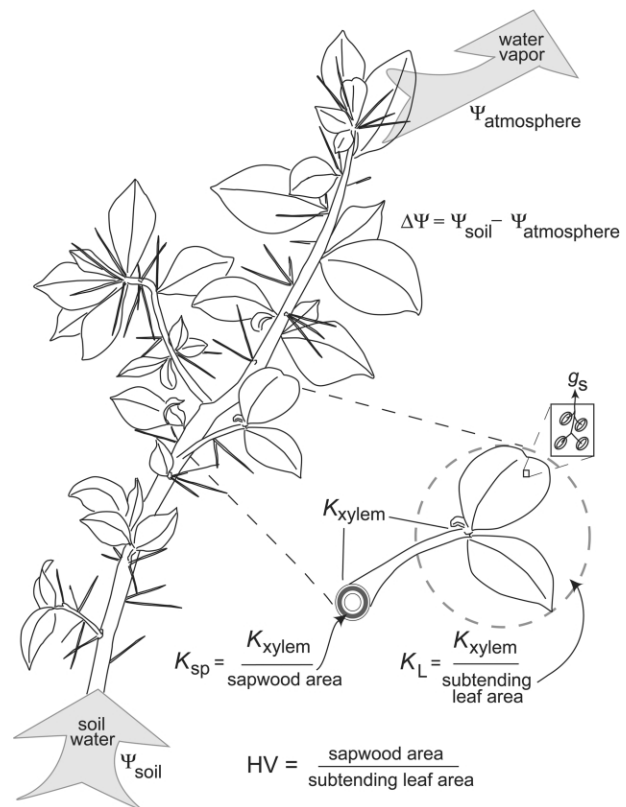


Figure 2: Basic parameters of plant water relations. Water flux through the soil-plant-atmosphere continuum is generally modeled after Ohm's law, where the driving force behind water movement is the difference between soil and atmospheric water potentials ($\Delta\Psi$). The rate of water flux is a function of $\Delta\Psi$ and the hydraulic conductivity of the pathway. Plant hydraulic conductivity (K_h) is often compartmentalized into root, stem, leaf (K_{lamina}), and leaf boundary layer components, each of which may vary independently. To compare K_h of xylem (the primary plant water-conducting tissue) across plant species, researchers usually normalize stem K_h by either the amount of conductive tissue measured (sapwood cross-sectional area, K_{sp}) or the amount of leaf area that the measured conductive tissue is supplying (one-sided leaf surface area, K_L). K_{sp} is an intrinsic value of the wood, largely governed by vessel length, diameter, and density, while K_L reflects both K_{sp} and Huber value (HV), which is the sapwood/leaf area ratio. A high HV means that plants are investing in large amounts of wood per leaf, which is a relatively expensive (in terms of both carbon and energy costs) allocation pattern.

potentials year-round because of a high leaf-specific xylem conductivity (K_L) conferred by a large wood-to-leaf carbon allocation pattern (HV). Stomatal behavior of *P. guamacho* was conservative and afforded a high photosynthetic water use efficiency. Additionally, *P. guamacho* exhibited complex and unpredictable leaf phenological patterns; different populations were asynchronous with one another as well as with their respective plant communities. Rather than shedding its leaves in response to drought, one population of *P. guamacho* performed drought-induced CAM pho-

Table 1: List of abbreviations used in text

Abbreviation	Definition	Unit
K_{sp}	Sapwood-specific xylem hydraulic conductivity	$\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$
K_L	Leaf-specific xylem hydraulic conductivity	$10^{-4} \text{ kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$
HV	Huber value	$10^{-4} \text{ m}^2 \text{m}^{-2}$
SPI	Stomatal pore index	$10^{-2} \mu\text{m}^2 \mu\text{m}^{-2}$
SLA	Specific leaf area	$\text{m}^2 \text{kg}^{-1}$
Ψ_{\min}	Minimum bulk leaf water potential	MPa
$\delta^{13}\text{C}$	Leaf carbon isotope discrimination ratio	‰

tosynthesis, while a second retarded leaf expansion and kept stomata closed day and night. Edwards and Diaz (2006) speculated that *P. guamacho* is not strictly drought deciduous and may instead perform drought-induced CAM photosynthesis as a means of lengthening leaf life span. They concluded that in many ways, the water use strategy of *P. guamacho* is similar to that of the leafless, stem-succulent cacti: it too maintains a positive tissue water status while surviving in water-limited environments, it exhibits water storage (in leaves), and its stomatal behavior is conservative and tightly regulates water loss. To put it simply, *P. guamacho* may not look like a cactus, but it behaves like one.

If *P. guamacho* is representative of all *Pereskia* species, this suggests that the morphological and anatomical specializations exhibited by the core cacti are not directly responsible for their ecological water use strategy per se; rather, key elements of this strategy were more or less established in the Cactaceae before the evolution of stem succulence and the evolutionary loss of functional leaves. Here we characterize the physiological ecology of other *Pereskia* species, focusing especially on traits pertaining to water use, and we use this information to infer the ecology and water relations of the first cacti. We extend the sampling of *Pereskia* ecophysiology to include six additional species, representing both major *Pereskia* clades. In order to more accurately characterize the climate regimes of extant *Pereskia*, we gathered climate data from specimen collection localities for all *Pereskia* species. We then reconstructed both ecophysiological and climatic variables at two specific nodes in the basal cactus phylogeny to help infer the ecological water use strategy of ancestral *Pereskia*, and we used these reconstructions to construct a preliminary hypothesis of the ecological and physiological conditions that preceded the evolution of the typical cactus life-form.

Methods

Ecophysiological and Anatomical Data

To characterize the water relations of *Pereskia* species, we focused on a suite of traits relating to plant hydraulics and

water use. For each of the seven species, we measured sapwood (K_{sp} , $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) and leaf-specific xylem hydraulic conductivity (K_L , $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$), Huber value (HV), minimum leaf water potential (Ψ_{\min} , MPa), and daily patterns of stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$). All field measurements were made in natural populations during the growing (rainy) season on healthy mature plants growing in full sun, using methods identical to those described by Edwards and Diaz (2006). In *Pereskia*, it proved difficult to measure K_{lamina} directly because of succulence and the lack of a petiole in many species. However, Sack et al. (2003) demonstrated a highly significant correlation between stomatal pore index (SPI; guard cell length² \times stomatal density [$\text{mm}^2 \text{mm}^{-2}$]) and leaf laminar conductance (K_{lamina}) across a diverse collection of species, so we instead calculated the SPI of three field-grown leaves from multiple individuals from each species, and we here use SPI as a proxy for K_{lamina} .

Stable carbon isotope ratios of leaf tissue are routinely used as a time-integrated measure of photosynthetic water use efficiency for C3 plants, with lower levels of ^{13}C discrimination indicative of higher water use efficiency (Farquhar et al. 1982). They are also used to differentiate between photosynthetic pathways because the enzymes used in the first carboxylation step of atmospheric CO_2 differ strongly in their discrimination of ^{13}C (Kluge and Ting 1978). Multiple leaves from each individual ($n = 3$ individuals for each species) were dried, bulk ground with mortar and pestle, and subsampled. Using a Finnigan MAT delta E isotope ratio mass spectrometer, $^{13}\text{C}/^{12}\text{C}$ ratios were determined on CO_2 collected from the samples after combustion. Numbers here are expressed relative to the PDB standard using the equation

$$\delta^{13}\text{C} = 1,000 \left(\frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}}}{^{13}\text{C}/^{12}\text{C}_{\text{standard}}} - 1 \right).$$

We also estimated specific leaf area (SLA, $\text{m}^2 \text{kg}^{-1}$), calculating bulk leaf surface area and dry weight of multiple leaves for eight individuals of each species. SLA is a function of leaf water content, leaf thickness, and leaf density and has been shown to correlate negatively with leaf life

span and nutrient use efficiency and positively with maximum photosynthetic rate (Reich et al. 1992; Ackerly and Reich 1999). It is often used as a proxy for carbon investment per leaf and as a good predictor of ecological growth strategy (Westoby et al. 2002).

Climate Data

To create a more accurate picture of current *Pereskia* ecology, we collated all specimen collection location information in Leuenberger (1986) and from the collections of the Missouri Botanical Garden (St. Louis, MO) that are available online. We then translated the descriptive location data into latitude/longitude coordinates using the free Web-based tool BioGeomancer (<http://www.biogeomancer.org>). We used DIVA-GIS (<http://diva-gis.org/>) to map these points and extract climate information for each species distribution. DIVA-GIS uses the WorldClim global climate data set, available at <http://www.worldclim.org>. We calculated mean and standard error of each climate variable for each species and used these as tip values in our phylogenetic climate reconstructions.

Ancestral Trait and Climate Reconstruction

Figure 3 depicts the basal cactus phylogeny described by Edwards et al. (2005). The current lack of trait data from cactus outgroups as well as appropriate members of the core cacti imposes important limitations on how confidently we can infer the character states of our particular physiological traits for the basal nodes in Cactaceae. In some instances, traits that are highly relevant to *Pereskia* water use are irrelevant to or even impossible to measure in the core cacti; leaf-related traits, such as SLA or K_{lamina} , for example, cannot be measured in a plant with no functional leaves. For this reason we chose to reconstruct the basal nodes of the two *Pereskia* lineages separately, and we use these values to infer the ecological setting and water relations of ancestral *Pereskia* (fig. 3, nodes A and B). Our fundamental assumption is that a general ecological water use strategy that is shared by both *Pereskia* clades and the core cacti is likely to be ancestral for Cactaceae. This would present a working hypothesis, to be tested as we gather more physiological data from portulacaceous outgroups and appropriate core cacti, and as basal Cactoideae and Opuntioideae phylogenetic relationships become better resolved.

We used COMPARE 4.6 (Martins 2004) to explore different methods of ancestral trait reconstruction for continuous characters. COMPARE employs a generalized least squares model of ancestral state estimation (Martins and Hansen 1997), which in its simplest form is analogous to the maximum likelihood reconstruction method of Schl-

ter et al. (1997). Branch lengths, intraspecific trait variation, and assumptions regarding models of character evolution can all have significant consequences in estimating ancestral values (Felsenstein 1985; Donoghue and Ackerly 1996; Martins and Hansen 1997; Cunningham et al. 1998; Butler and King 2004). To explore the sensitivity of our reconstructions to each of these variables, we generated sets of 500 trees with randomized branch lengths (using the “generate trees” function in COMPARE 4.6) and ran multiple analyses across all trees, using different models of phenotypic trait evolution. In all analyses, we included standard errors of our trait means as estimates of intraspecific trait variation. We employed two primary evolutionary models: a Brownian motion (BM) model of trait evolution, which corresponds to Schluter et al.’s (1997) maximum likelihood method and assumes that traits evolve by drift; and a simple Ornstein-Uhlenbeck (OU) model with one evolutionary optimum, which may be more appropriate for traits evolving under stabilizing selection (Hansen 1997). Butler and King (2004) present a compelling argument for exploring more complex OU models with multiple evolutionary optima; however, our limited taxon sampling ($n = 3-9$ taxa per tree) prevents us from estimating such parameter-rich models with any confidence. We did explore effects of selection strength on ancestral reconstruction, however, by employing four different values for α (0.5, 1.0, 5.0, and 10.0). For each of the *Pereskia* clades (separately), we ran BM and OU models across 500 trees with randomized branch lengths to reconstruct seven physiological traits and eight climate variables for nodes A and B (fig. 3). For the physiological traits, we used trees that consisted of only the seven focal *Pereskia* species (two trees, one of four and one of three species), and for the climate reconstructions, we used trees that included all 17 *Pereskia* species (two trees, one of eight and one of nine species).

Using environmental parameters of extant species to infer ancestral climates is a relatively novel endeavor (Graham et al. 2004; Hardy and Linder 2005), and the conceptual basis of this approach has not yet been fully developed. For example, most environmental parameters are continuous variables, such as mean annual precipitation, and a given species distribution will encompass a range of values for this variable. Graham et al. (2004) and Hardy and Linder (2005) reconstructed minimum and maximum values for species independently and used these to delimit “ancestral niche envelopes.” This may be overly conservative because the density curves of sampled environmental parameters should be similar to those of other continuous organismal traits (e.g., with appropriate sample size, they are normally distributed). Such is the case for many of our climate variable distributions (see fig. 4). Because we are most interested in the probable climates

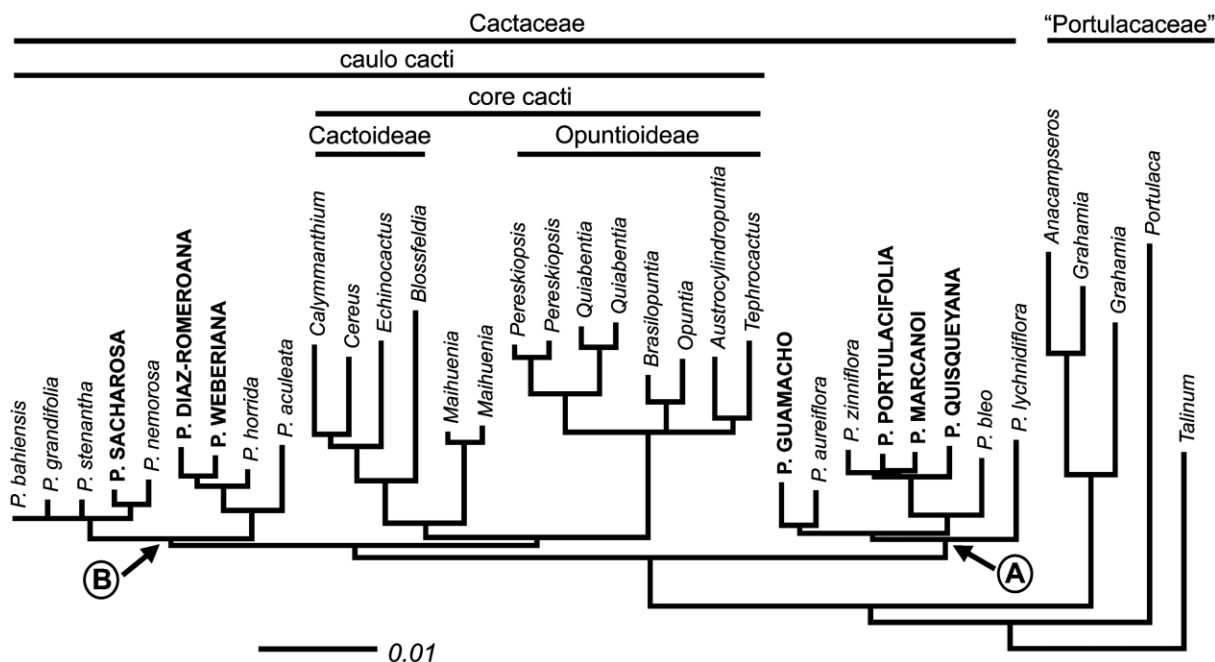


Figure 3: Basal cactus phylogeny, adapted from Edwards et al. (2005). Phylogram generated from a maximum likelihood search using a concatenated five-gene-region data set of 6,450 characters. *Pereskia* is paraphyletic, with nine *Pereskia* species united with the core cacti in the “caulocactus” clade. Species chosen for ecophysiological field studies are shown in boldface capital letters. We reconstructed ancestral values of nodes A and B for seven physiological traits, using measured values from the seven focal *Pereskia* species. For reconstructed climate estimates of nodes A and B, we used data from all 17 *Pereskia* species.

at nodes A and B, as opposed to the full range of potential climates, we have used means and standard errors for the extant species in our reconstructions.

Results

Ecological Physiology of Extant Pereskia Species

Means and standard errors of seven ecophysiological traits for the focal *Pereskia* species are reported in table 2, together with values culled from the literature to provide context for the *Pereskia* values. When possible, studies from tropical dry forest plant communities were used for comparison; unfortunately, this was not possible for SPI because this trait has not yet been reported from species living in these systems. Also, while there are many studies that measure xylem hydraulic properties, the methods used to do this are not standardized, making comparisons across studies difficult. For this reason, the range of values we present for K_{sp} , K_L , and HV are from one study in a Costa Rican dry forest (Brodribb et al. 2002) whose methods were the same as those used here.

Despite large morphological differences between the seven focal *Pereskia* species (fig. 1), their ecophysiological characteristics are generally quite similar, and all species

exhibit the same general water use pattern described for *Pereskia guamacho* by Edwards and Diaz (2006). Values of K_L and HV are high, allowing for an efficient water supply system to transpiring leaves (this is taken to the extreme in *Pereskia portulacifolia*, with K_L and HV values among the highest reported for any woody broad-leaved plant). The value for SPI, our proxy for K_{lamina} , is among the lowest recorded in the literature. A coupling of high K_L and low K_{lamina} implies that whole-plant water use is being regulated primarily at the leaf level.

Pereskia minimum leaf water potentials are remarkably high for woody plants of semiarid tropical environments. The $\delta^{13}C$ values indicate very high photosynthetic water use efficiencies, with extremely high values in *Pereskia diaz-romeroana* and *Pereskia sacharosa*. In a survey of CAM plants that exhibit plasticity in the proportion of atmospheric carbon fixed during the day or night, Winter and Holtum (2002) found a strong linear relationship between tissue $\delta^{13}C$ and the percentage of carbon uptake occurring at night, with $\delta^{13}C$ values of -21‰ and -22‰ corresponding to approximately 20% nocturnal carbon uptake. This suggests that *P. diaz-romeroana* and *P. sacharosa*, like *P. guamacho* (Edwards and Diaz 2006), are using the CAM photosynthetic pathway to some degree. Values for SLA

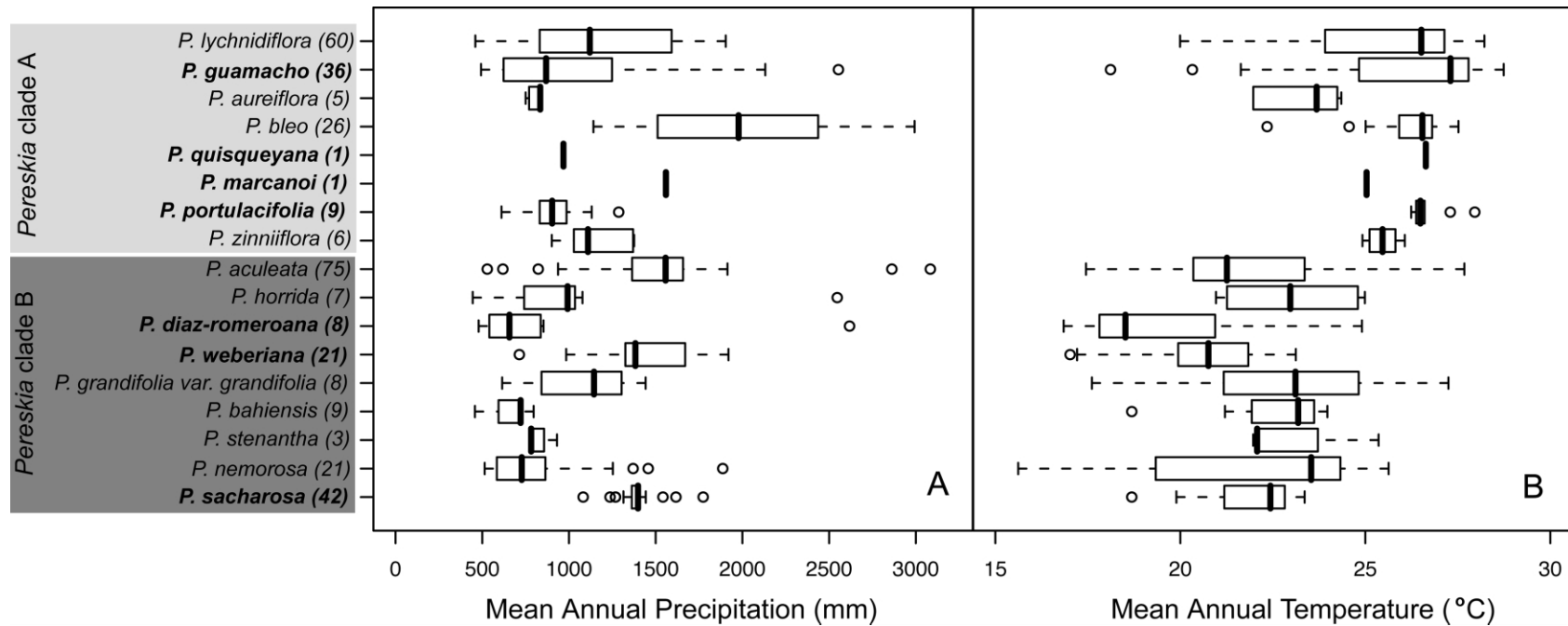


Figure 4: Box plots of two environmental parameters of extant *Pereskia* species. Values inside boxes represent 75% of data points, and tails include 95% of data points; line inside box is median. The closer the median line is to the center of the box, the more normally distributed are the data. Numbers next to species names are the total number of collection localities representing that species. Species names in boldface were sampled for ecophysiological trait characterization; the selected species encompass the majority of climate variation experienced across all *Pereskia* species. A, Mean annual precipitation; B, mean annual temperature. Most climate regimes correspond to the tropical dry or very dry forest Holdridge life zone (Holdridge 1967).

Table 2: Values of ecophysiological traits measured for seven *Pereskia* species and reconstructed nodes A and B

Species	Study site location	Latitude and longitude	K_p ($n = 8$ branches) ^a	K_L ($n = 8$ branches) ^a	HV ($n = 8$ branches)	SPI ($n = 3$ leaves)	SLA ($n = 8$ trees)	Ψ_{min} ($n = 5$ trees)	^{13}C ($n = 3$ trees)
<i>Pereskia diaz-romeroana</i>	Bolivia	18.10113°S, 64.45551°W	1.69 ^{AB} ± .30	7.81 ^{BC} ± .97	5.54 ^B ± 1.18	3.38 ^{BC} ± .21	13.80 ^B ± .39	−.88 ^{AB} ± .09	−21.39 ^A ± .42
<i>Pereskia guamacho</i>	Venezuela	11.28126°N, 69.69042°W	1.03 ^B ± .26	5.91 ^{BC} ± 1.44	5.79 ^B ± .66	2.74 ^C ± .26	15.09 ^B ± 1.27	−1.00 ^A ± .04	−25.83 ^B ± .31
<i>Pereskia marcanoi</i>	Dominican Republic	19.0895°N, 71.68403°W	2.06 ^{AB} ± .20	11.16 ^B ± 1.75	5.62 ^B ± .91	5.18 ^{AB} ± .53	24.80 ^A ± 2.07	−.60 ^C ± .05	−26.17 ^B ± .58
<i>Pereskia portulacifolia</i>	Dominican Republic	18.42655°N, 71.76983°W	2.69 ^{ABC} ± .27	27.62 ^A ± 2.37	10.82 ^A ± 1.54	6.02 ^A ± .89	11.45 ^B ± .84	−.84 ^{AB} ± .04	−24.3 ^{BC} ± .33
<i>Pereskia quisqueyana</i>	Dominican Republic	18.36913°N, 68.84258°W	1.76 ^{AB} ± .14	3.60 ^C ± .54	2.20 ^B ± .56	3.98 ^{ABC} ± .30	19.36 ^A ± 2.43	−.71 ^{BC} ± .03	−25.43 ^B ± .58
<i>Pereskia sacharosa</i>	Bolivia	18.27388°S, 64.15802°W	2.01 ^{AB} ± .25	8.85 ^{BC} ± 1.44	4.65 ^B ± .63	3.45 ^{BC} ± .02	13.58 ^B ± 1.90	−.84 ^{AB} ± .04	−22.54 ^{AC} ± .28
<i>Pereskia weberiana</i>	Bolivia	16.54096°S, 67.39115°W	1.91 ^{AB} ± .97	4.56 ^{BC} ± 2.60	1.96 ^B ± .12	3.36 ^{BC} ± .04	13.29 ^B ± .72	−.73 ^{BC} ± .03	−26.12 ^B ± .72
Reconstructed ancestral values:									
Node A			1.76 ± .001	8.89 ± .110	5.35 ± .003	4.06 ± .003	16.16 ± .005	−.82 ± .000	−25.41 ± .000
Node B			1.87 ± .000	7.54 ± .002	3.33 ± .005	3.40 ± .000	13.69 ± .000	−0.82 ± .000	−22.99 ± .002
Comparable values for other broad-leaved C3 angiosperms			1–3.8	.48–4.26	.54–1.7	3–20	4.8–26.8	−1.0 to −5.0	−22 to −30
Sources of comparable values			Brodribb et al. 2002	Brodribb et al. 2002	Brodribb et al. 2002	Sack et al. 2003	Eamus and Prior 2001; Vendramini et al. 2002	Sobrado 1986; Eamus and Prior 2001; Brodribb et al. 2002; E. Edwards, unpublished data	Mooney et al. 1989; Smith et al. 1997; Eamus and Prior 2001

Note: Values are presented as mean ± SE. For extant species, values with different letters are significantly different from one another (all pairs Tukey-Hamer test, $P < .05$). For definitions of abbreviations, see table 1.

^a With the exception of *P. weberiana*, where $n = 2$ branches.

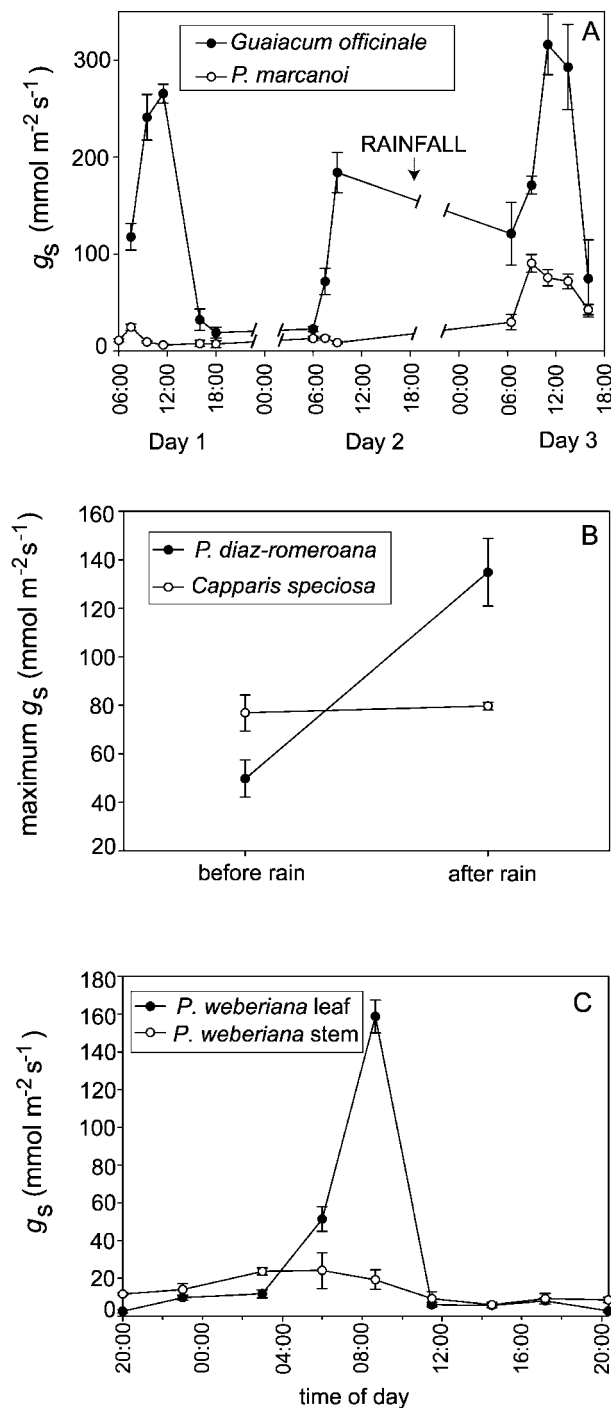


Figure 5: Patterns of stomatal conductance in *Pereskia*. A, *Pereskia marcanoii*, with co-occurring *Guaiacum officinale* as a control. Stomata of *P. marcanoii* minimally opened for only a brief period in the morning until there was a substantial rainstorm, after which stomata remained open for most of the day. Maximum g_s was quite low in comparison to that of co-occurring trees. Breaks in the curve represent periods where no measurements were made. B, Observed stomatal behavior in *Pereskia*

ranged from $11.45 \text{ m}^2 \text{ kg}^{-1}$ in *P. portulacifolia* to $24.8 \text{ m}^2 \text{ kg}^{-1}$ in *Pereskia marcanoii*, which fall in the middle to higher end of values for tropical dry forest trees (Eamus and Prior 2001) and are considerably higher than values reported for fully succulent plants (Vendramini et al. 2002).

In addition to these traits, we monitored daily patterns of stomatal conductance (g_s) for the seven species, which provided further evidence for conservative water use in *Pereskia* (fig. 5). Maximum g_s recorded varied between 90 and $183 \text{ mmol m}^{-2} \text{ s}^{-1}$ and usually peaked in midmorning, with stomata often closing for much of the afternoon. In several species (*P. guamacho*, *P. marcanoii*, *P. sacharosa*), stomata remained closed for entire days during their primary growing season, while co-occurring trees were transpiring freely (data not shown). *Pereskia* stomatal behavior is also remarkably sensitive to rainfall events, as witnessed in *P. marcanoii* and *P. diaz-romeroana* (fig. 5); this suggests that these species have extensive, shallow root systems similar to those of the leafless, stem-succulent cacti. A small degree of stem stomatal conductance was recorded in *Pereskia weberiana*, a member of the caulocactus clade characterized by stem stomata and delayed bark formation. Conductance values were so low in comparison to *Pereskia* leaves, however, that it is highly unlikely that significant carbon assimilation occurs in the stem. This is confirmed by the carbon isotope data; $\delta^{13}\text{C}$ values of leaf and stem cortical tissues within an individual plant were identical (data not shown), suggesting one site of carbon assimilation.

These results support the *Pereskia* ecological water use strategy hypothesized by Edwards and Diaz (2006) based on *P. guamacho*. The combination of water relations traits exhibited by *Pereskia* is unusual for woody, leafy plants. In particular, comparative studies have recently found significant coordination between plant hydraulic and photosynthetic capacity, such that a high K_L supports a high K_{lamina} , high g_s , and high photosynthetic rates (Brodribb and Feild 2000; Brodribb et al. 2002, 2003; Meinzer 2003; Sperry et al. 2003; Bucci et al. 2004; Santiago et al. 2004; Sack and Tyree 2005). While we do not have direct measures of photosynthesis, SLA values suggest that maximum net photosynthesis will be moderate in comparison with

diaz-romeroana was similar to that of *P. marcanoii*, with a more substantial stomatal response to rainfall events than in its woody co-occurring counterparts, here represented by *Capparis speciosa*. C, *Pereskia weberiana*, showing again a brief period of maximal g_s in the early morning, followed by stomatal closure for much of the day. Stems of *P. weberiana* also exhibited some stomatal conductance, though substantially less than the leaves. Stem g_s peaked just before dawn, indicating CAM-like patterns of stomatal opening.

other broad-leaved trees of tropical dry regions. *Pereskia* plants are hydraulically well built, and they could conceivably support high leaf transpiration rates, but stomatal behavior is conservative, and SPI (our proxy for K_{lamina} and a good measure of g_{max}) is low. In other words, the leaves of *Pereskia* are generally “oversupplied” with water.

It is currently not clear why *Pereskia* species do not take advantage of their high K_L and keep stomata open a greater proportion of the time; neither do we know why *Pereskia* species apparently avoid the development of lower Ψ_{min} in their leaves. In a sense, however, the unusual leaf-stem relationship in *Pereskia* bears a functional resemblance to that of the inner and outer cortical tissue of the core cacti: as a cactus stem dehydrates, the inner cortex behaves as a water reservoir for the outer, photosynthetic cortical layer, thus maintaining a steady and reliable water supply to transpiring tissues (Barcikowski and Nobel 1984; Lerda et al. 1992). While the xylem in *Pereskia* is not explicitly behaving as a capacitor, a high K_L and low g_s are ensuring a steady and reliable water supply to transpiring leaves.

Climate of Extant Pereskia Species

Not all of the *Pereskia* specimens that we collated from various sources had enough location information to accurately determine latitude/longitude coordinates; we were confident in using a total of 330 data points. Numbers of locations for each species varied, ranging from $n = 1$ in *Pereskia quisqueyana* and *P. marcanoi* (which are endangered and currently known only from single populations) to $n = 73$ in *Pereskia aculeata*. Most climate variables are similar for extant *Pereskia* species; figure 4 shows the species ranges of mean annual precipitation and mean annual temperature. The environmental parameters of most species correspond to the tropical dry and very dry forest life zones of Holdridge (1967). The only exception to this is *Pereskia bleo*, which lives in areas receiving considerably higher annual rainfall. All species live in climates with highly seasonal precipitation patterns, with the mean wettest month receiving 187 mm and the driest month 26 mm.

Ancestral Trait Reconstructions

Results from our reconstruction analyses are presented in tables 2 and 3. Estimated values were surprisingly robust to variations in both branch lengths and the model of evolution used in reconstruction (range of values reported in table 3; in table 2 we report only the mean value of all analyses because of the small variation between them; reported error estimates in table 3 are those associated with running one analysis across all 500 trees with randomized branch lengths). This is probably because of the relatively

small size of our trees and the similarity of tip values. Node B was consistently estimated with slightly lower K_L , SPI, HV, and SLA and higher K_{sp} and photosynthetic water use efficiency than node A (table 2). Node B habitat also may have had lower mean annual precipitation with more seasonal rainfall and cooler, more highly seasonal temperature patterns (table 3).

When viewed within a broad ecological context, however, the differences in reconstructed values for nodes A and B are relatively slight. In general, our results imply that ancestral *Pereskia* inhabited tropical, semiarid to sub-humid environments with discernible “wet” and “dry” seasons and relatively low mean annual rainfall, but they nevertheless maintained very high minimum water potentials in their photosynthetic tissues. They accomplished this in two ways: first, by allocating large amounts of water-conducting tissue (wood) per given amount of leaf area, and second, by exhibiting low leaf laminar conductance and extremely conservative stomatal behavior, opening stomata only when soil moisture was plentiful (after rains) or when transpirational demand was minimal (early mornings, occasionally at night). Since most investigated *Pereskia* have been shown to exhibit some degree of CAM cycling, it is probably this ability that enables such conservative stomatal behavior. Recycling respired carbon when stomata remain closed during the day allows the plant to maintain basic metabolic processes without relying heavily on carbon stores (Kluge and Ting 1978; Ting 1985; Cushman 2001). There is also some evidence of inducible CAM photosynthesis, where stomata open at night, in three of the seven *Pereskia* species examined in this study (*P. guamacho*, *P. diaz-romeroana*, and *P. sacharosa*). The distribution of inducible traits is not straightforward to reconstruct, however, because of the possibility of “false negatives”; just because the trait was not induced in the course of a given study does not mean that the trait is never induced. Since CAM cycling has been recorded previously in seven of 11 investigated *Pereskia* species, and here we present some evidence of inducible full CAM in *Pereskia* species from both major *Pereskia* clades, we conclude that the CAM photosynthetic pathway was also present in ancestral *Pereskia* and played a limited but potentially important role in allowing for conservative stomatal behavior and possibly in extending leaf life span.

Discussion

As our knowledge of phylogeny improves, taxa once thought to be monophyletic are sometimes found to be basal paraphyletic assemblages (see Donoghue 2005). Such is the case for “*Pereskia*” in relation to the core cacti. Discoveries of this sort provide us with opportunities to dissect the sequence of evolutionary events through major

Table 3: Estimated values for nodes A and B of environmental parameters, using different models of phenotypic evolution

Model of evolution	Mean annual precipitation (mm)	Precipitation of wettest month (mm)	Precipitation of driest month (mm)	Precipitation seasonality index (SD \times 100)	Mean annual temperature ($^{\circ}$ C)	Temperature seasonality index	Maximum temperature of warmest month ($^{\circ}$ C)	Minimum temperature of coldest month ($^{\circ}$ C)
Node A:								
BM (α = .0)	1,256 \pm .13	177 \pm .05	27.8 \pm .06	57.1 \pm .09	25.6 \pm .00	133 \pm .12	32.9 \pm .00	17.9 \pm .00
O-U (α = .5)	1,258 \pm .07	176 \pm .03	28.6 \pm .05	55.8 \pm .07	25.7 \pm .00	131 \pm .09	32.9 \pm .00	17.9 \pm .00
O-U (α = 1.0)	1,256 \pm .07	177 \pm .03	27.3 \pm .04	57.7 \pm .06	25.7 \pm .00	132 \pm .08	32.9 \pm .00	17.9 \pm .00
O-U (α = 5.0)	1,253 \pm .03	178 \pm .03	25.5 \pm .04	60.2 \pm .06	25.7 \pm .00	129 \pm .09	32.9 \pm .00	17.9 \pm .00
O-U (α = 10.0)	1,252 \pm .02	179 \pm .04	24.8 \pm .05	61.0 \pm .06	25.7 \pm .00	127 \pm .07	32.9 \pm .00	18.0 \pm .00
Node B:								
BM (α = .0)	1,102 \pm .08	174 \pm .04	19.9 \pm .08	69.2 \pm .05	21.8 \pm .00	175 \pm .07	30.6 \pm .00	12.2 \pm .00
O-U (α = .5)	1,100 \pm .03	173 \pm .02	18.0 \pm .05	70.3 \pm .03	21.8 \pm .00	174 \pm .04	30.6 \pm .00	12.2 \pm .00
O-U (α = 1.0)	1,100 \pm .03	173 \pm .02	19.7 \pm .03	69.0 \pm .03	21.8 \pm .00	175 \pm .05	30.6 \pm .00	12.2 \pm .00
O-U (α = 5.0)	1,099 \pm .03	173 \pm .01	20.4 \pm .01	68.1 \pm .01	21.9 \pm .00	175 \pm .04	30.6 \pm .00	12.3 \pm .00
O-U (α = 10.0)	1,098 \pm .02	172 \pm .00	20.2 \pm .01	68.3 \pm .01	21.9 \pm .00	175 \pm .03	30.5 \pm .00	12.4 \pm .00

Note: Values are presented as mean \pm SE of analyses run over 500 trees with identical topologies but randomized branch lengths. BM = Brownian motion; O-U = Ornstein-Uhlenbeck; α = strength of restraining force.

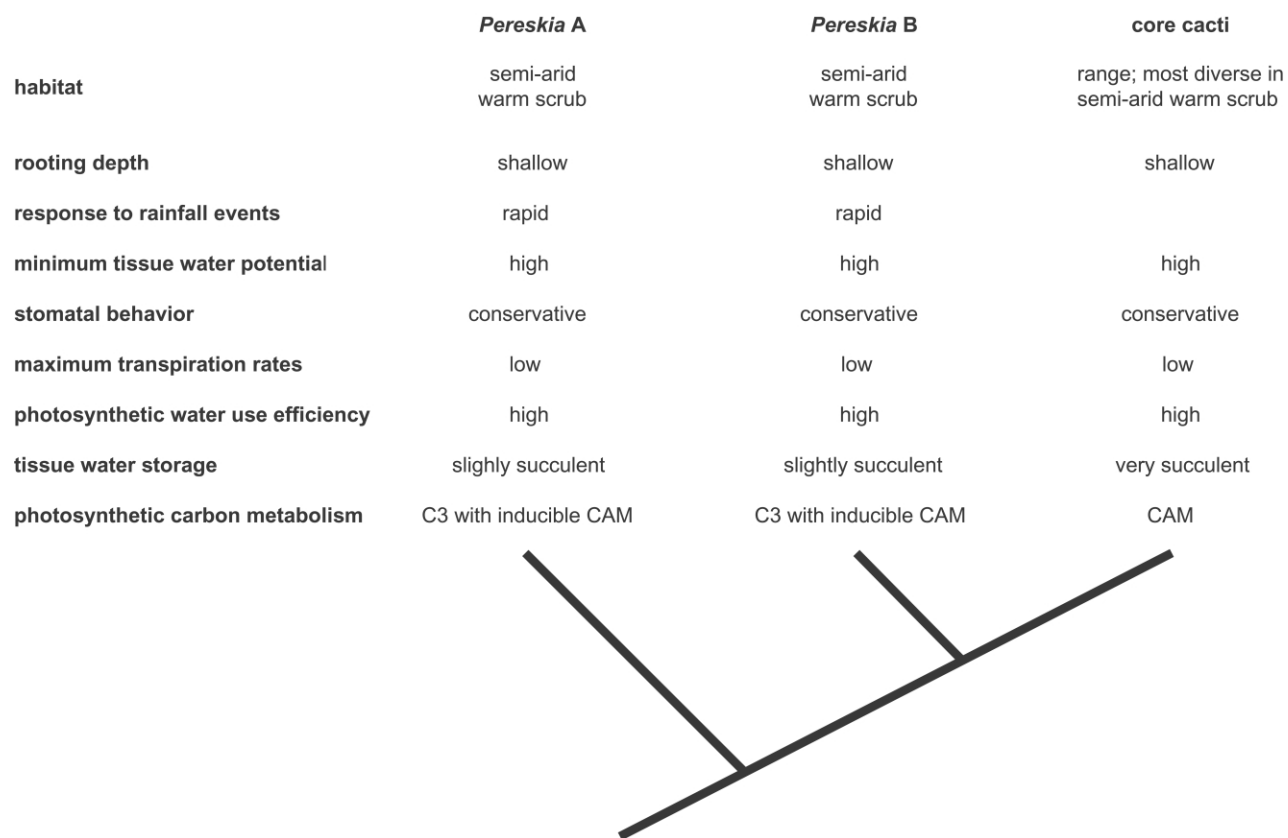


Figure 6: Ecological water use traits of ancestral *Pereskia* nodes and core cacti.

evolutionary transitions and therefore to analyze how changes in form and function were related to one another. Our analysis illustrates this approach and also highlights some of the difficulties encountered when trying to infer ancestral conditions with necessarily limited data and in lineages that have undergone radical morphological shifts during their evolution.

With our current knowledge of basal cactus phylogeny and character distributions in *Pereskia* and core cacti, our abilities to confidently infer the ancestral conditions of particular traits fall along a spectrum: some inferences are straightforward, some are effectively impossible at this stage, and others fall between these two extremes. For instance, based on the paraphyly of *Pereskia* and the nested position of Cactaceae within the Portulacaceae, we can now infer with considerable confidence that the first cacti were shrubs or small trees with photosynthetic leaves. At the other end of the spectrum, the lack of functional leaves in all extant Cactoideae and most Opuntioideae makes it unlikely that we will ever assess with great accuracy any particular physiological parameter of how those leaves functioned. Values of K_L , HV, and SLA, for example, will

be difficult to infer for the node subtending the core cacti because there are no Cactoideae with functional leaves to include in a broader sampling of these traits. Nevertheless, we may consider ancestral values of these leaf-related traits at *Pereskia* nodes A and B with regard to how they influence patterns of whole-plant water use, and we may compare these organismal-level (rather than organ-level) traits with what we know about the core cacti from the literature (fig. 6). Traits of this sort include habitat characteristics, tolerated tissue water deficits, stomatal behavior, and photosynthetic water use efficiency. Addressing the problem in this way, we are able to present a preliminary hypothesis of the ecological niche and water relations of the first cacti. Resolution of major relationships within the Cactoideae and Opuntioideae will provide a better basis for more targeted trait sampling of core cacti taxa to include (with additional physiological data from *Maihuenia* and Portulacaceae outgroups) in further tests of this hypothesis.

The reconstructed ecology and water use strategies of ancestral nodes A and B are remarkably similar to those that typify leafless, stem-succulent cacti (fig. 6), implying that the cacti had been inhabiting their particular ecolog-

ical niche long before they evolved the anatomical specializations that are often associated with their survival in water-limiting environments. In other words, it may not have been the evolution of leaflessness and stem succulence in cacti that yielded the cactus ecological water use strategy; instead, it seems that it was the origin of this strategy in these ancestors that promoted the evolution of leaflessness and stem succulence.

We are not suggesting that there are no ecological or physiological differences between *Pereskia* species and the core cacti. Certainly, the core cacti are superior to their leafy relatives both at storing water and in using it efficiently. They also occupy a greater diversity of habitats, with many species experiencing more extreme drought than either extant *Pereskia* or their reconstructed ancestors. Indeed, our work implies that the core cacti are so successful in these environments precisely because of their anatomical specializations. That is, the water use strategy shared by *Pereskia* and the core cacti, which we are arguing was also present in the first Cactaceae, would be much improved with a longer-lived photosynthetic tissue system (provided by stem-based, rather than leaf-based, photosynthesis) and larger reservoirs of stored water (allowing stomata to open for longer periods, in stronger drought situations).

Our results are also consistent with the view that ecological traits can be more highly conserved in evolution than generally assumed (Webb et al. 2002; Wiens and Donoghue 2004). Studies of adaptive radiations focus our attention on just how evolutionarily labile ecological traits can be under some circumstance (e.g., in lineages that have colonized relatively “open” islands). In contrast, some recent analyses have drawn attention to cases in which interspecific competition and ecological sorting have predominated over in situ evolutionary adaptation in the assembly of ecological communities (Peterson et al. 1999; Webb 2000; Ackerly 2004; Feild et al. 2004; Davis et al. 2005; but see Cavender-Bares et al. 2004). In the case of Cactaceae, we argue that the “cactus ecological niche,” at least with respect to water use, was occupied for some time before the evolution of the morphological and anatomical specializations that are often assumed to be responsible for their water use strategy. This niche conservatism may even predate the divergence of the cactus lineage from its portulacaceous ancestors because most Portulacaceae inhabit warm, water-limiting environments, are often succulent, and exhibit varying degrees of CAM photosynthesis (Martin and Zee 1983; Carolin 1987, 1993; Martin and Harris 1993; Eggli and Ford-Werntz 2001; Guralnick and Jackson 2001). When placed in this broader phylogenetic context, the evolution of leaflessness and stem succulence does not seem quite so extraordinary.

A Preliminary Sketch of Early Cactus Evolution

In fact, we can now begin to piece together the order of the early events that culminated in the evolution of the cactus life-form (fig. 7). We have argued that the first cacti lived in tropical, subhumid to semiarid environments and exhibited the water use strategy described above. After the first split within Cactaceae, the caulocactus lineage may have moved into drier and cooler climates with greater seasonality of both precipitation and temperature. During this time, the caulo cacti evolved delayed bark formation and stem stomata, key traits in the early development of the stem as a photosynthetic organ. Data from extant *Pereskia* suggest that these traits are not by themselves sufficient for significant stem photosynthesis (Gibson and Nobel 1986; Nobel and Hartsock 1986; Martin and Wallace 2000); it appears that modifications of the stem cortical tissue were also necessary (for discussion, see Edwards et al. 2005). Opuntioideae, Cactoideae, and *Maihuenia* all exhibit significantly greater water storage in leaf (leaves are present in some Opuntioideae and in *Maihuenia*, a specialized, leafy cushion plant of cold, arid Patagonia), stem, and/or root tissues than any *Pereskia* species (Gibson and Nobel 1986; Leuenberger 1997; Mauseth 1999), indicating a shift toward increased succulence before the divergence of the two major core cactus clades. It is within the Cactoideae that stem succulence is most fully developed, and it is only in this lineage that the stem cortex has evolved vascular bundles. It has been suggested (Mauseth and Sajeve 1992) that the vascularization of the cortex may have been a “key innovation” associated with the increased diversity and disparity of the Cactoideae, which contains the vast majority of cactus species ($\approx 1,250/1,600$ species) and exhibits the greatest morphological diversity. This remains to be tested, and the precise causal link between this feature and diversity/disparity is currently unclear to us. In any case, however, distributing vascular tissue throughout the inner and outer cortex allows for a more efficient connection between photosynthetic cells (outer cortex) and water and photosynthate storage cells (inner cortex, wood, and pith). This results in more rapid translocation of both water and photosynthetic assimilates throughout the cactus stem than could occur with cell-to-cell diffusion, and it may relax possible transport constraints on the ultimate width, shape, and water storage capacity of the cactus stem.

Reconstructing the evolution of perhaps the two most characteristic features of the cactus life-form—stem-based photosynthesis and a functional loss of leaves—is more complicated. Given the apparent lack of stem-based photosynthesis in *Maihuenia* (Martin and Wallace 2000), it is possible that this trait arose independently in Opuntioideae and Cactoideae. It is more certain that the functional

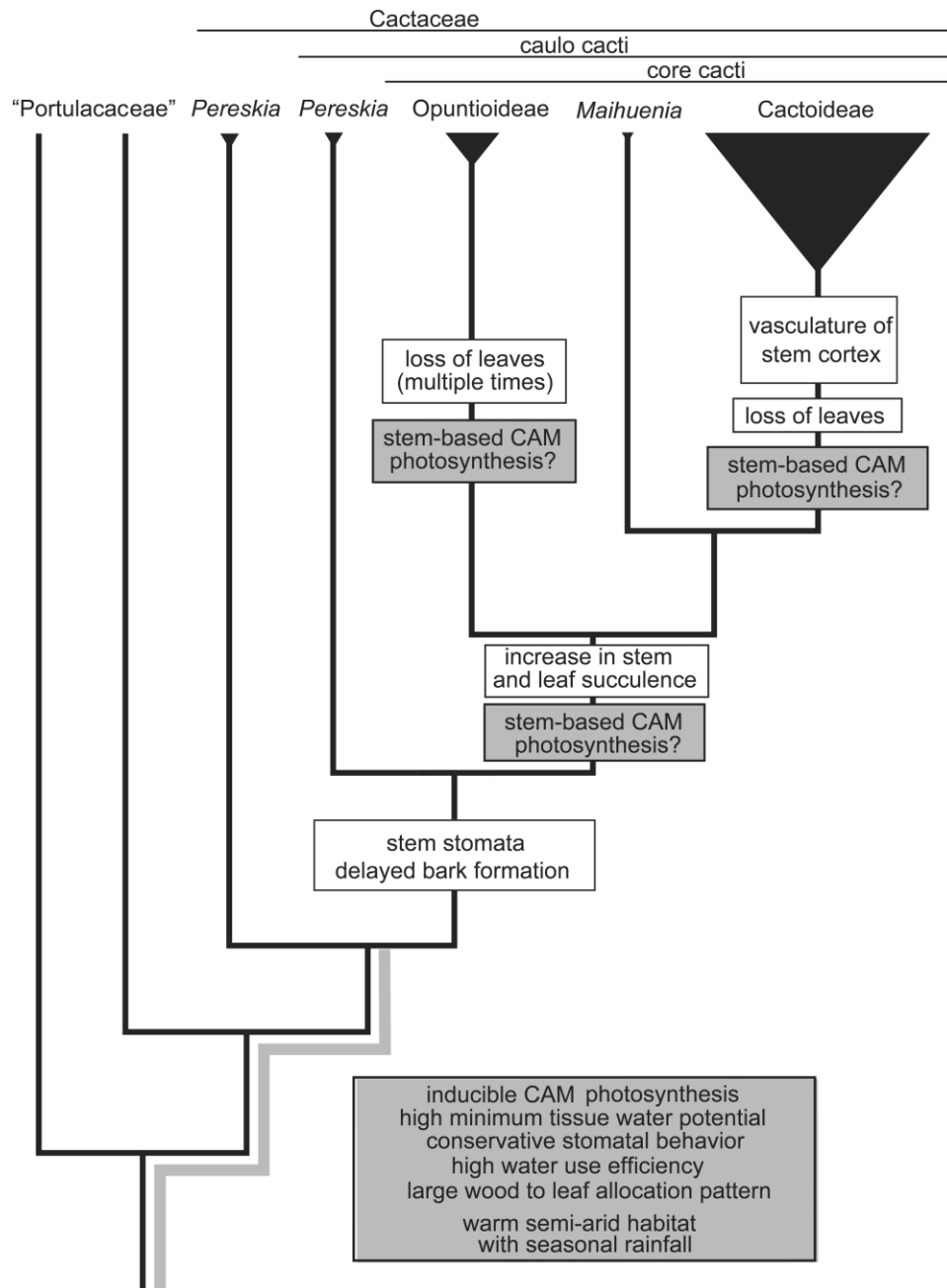


Figure 7: Overview of events in early cactus evolution. The placements of traits listed in white boxes are more certain; the placements of traits in gray boxes are more speculative. The gray line tracing several internal branches indicates that the traits in the gray box to the right could have evolved anywhere along those branches. Multispecies lineages are represented by triangles that are scaled to roughly represent relative lineage diversity (e.g., Cactoideae includes approximately 80% of all cactus species).

loss of leaves evolved independently in the two lineages, as evidenced by a number of species of Opuntioideae that produce functional leaves (along with photosynthetic stems) and are presumed to represent early-branching opuntioidean lineages (Wallace and Dickie 2002). It is the

Opuntioideae, then, that may hold the key to understanding the processes involved in completely transferring the photosynthetic function from the leaves to the stem. However, we currently lack adequate phylogenetic resolution of the major opuntioidean lineages, and we know little about

field water relations of leafy opuntoid taxa such as *Pereskopsis* and *Quiabentia*. Just as combining phylogenetic and physiological analyses of *Pereskia* has provided insights into early cactus evolution, we expect that such studies within the Opuntioideae will be especially helpful in understanding these “final steps” in the evolution of the cactus life-form.

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