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Research

Correlated evolution of stem and leaf hydraulic traits in *Pereskia* (Cactaceae)

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Summary

- Recent studies have demonstrated significant correlations between stem and leaf hydraulic properties when comparing across species within ecological communities. This implies that these traits are co-evolving, but there have been few studies addressing plant water relations within an explicitly evolutionary framework.
- This study tests for correlated evolution among a suite of plant water-use traits and environmental parameters in seven species of *Pereskia* (Cactaceae), using phylogenetically independent contrasts.
- There were significant evolutionary correlations between leaf-specific xylem hydraulic conductivity, Huber Value, leaf stomatal pore index, leaf venation density and leaf size, but none of these traits appeared to be correlated with environmental water availability; only two water relations traits – mid-day leaf water potentials and photosynthetic water use efficiency – correlated with estimates of moisture regime.
- In *Pereskia*, it appears that many stem and leaf hydraulic properties thought to be critical to whole-plant water use have not evolved in response to habitat shifts in water availability. This may be because of the extremely conservative stomatal behavior and particular rooting strategy demonstrated by all *Pereskia* species investigated. These results highlight the need for a lineage-based approach to understand the relative roles of functional traits in ecological adaptation.

Key words: evolution, independent contrasts, leaf size, *Pereskia*, stomatal pore index, venation density, water relations, xylem hydraulic conductivity.

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Introduction

A suite of physiological and anatomical traits are generally used to characterize plant water relations. These include stem xylem sapwood-specific hydraulic conductivity (K_{sp}) and leaf-specific hydraulic conductivity (K_L), sapwood to leaf allocation ratio [Huber Value (HV)], wood density (ρ_{wood}), operating minimum leaf water potential (Ψ_{min}), leaf lamina conductance (K_{lamina}), stomatal conductance (g_s) and photosynthetic water-use efficiency (WUE) (see Table 1 for the definition of trait abbreviations used in this article). These traits are primary components of whole-plant water use, and it is clear that they can (and do) respond to the transpirational demand and water availability imposed on a plant by its environment (Tyree & Ewers, 1991; Sperry *et al.*, 2002). Many recent studies have demonstrated significant correlations between these traits when comparing both within species and across distantly related species within ecological communities. For example, K_L has shown strong positive correlations with g_s and leaf photosynthetic capacity, and ρ_{wood} has been suggested to be a good predictor of both water transport capacity and leaf drought tolerance (Sperry & Pockman, 1993; Brodribb & Feild, 2000; Nardini & Salleo, 2000; Stratton *et al.*, 2000; Hubbard *et al.*, 2001; Brodribb *et al.*, 2002; Bucci *et al.*, 2004; Macinnis-Ng *et al.*, 2004; Santiago *et al.*, 2004a,b; Preston *et al.*, 2006). These results

Table 1 List of abbreviations used in the text

Symbol	Definition	Units
K_{sp}	Sapwood-specific xylem hydraulic conductivity	kg m^{-1} s^{-1} MPa^{-1}
K_L	Leaf-specific xylem hydraulic conductivity	10^{-4} kg m^{-1} s^{-1} MPa^{-1}
HV	Huber value	10^{-4}
SPI	Stomatal pore index	10^{-2}
SLA	Specific leaf area	m^2 kg^{-1}
Ψ_{min}	Minimum bulk leaf water potential	MPa
$\delta^{13}C$	Leaf carbon isotope discrimination ratio	‰
ρ_{wood}	Wood density	g cm^{-3}
MAP	Mean annual precipitation	mm
VPD	Leaf to air vapor pressure deficit	kPa
I_M	Moisture index	

support the view that plant water use is governed by a set of general relationships between stem and leaf properties. Recent syntheses have suggested that these relationships place a significant constraint on the number of different ways in which a plant might adapt to a given environment, resulting in plant functional convergence at a grand scale, where species' trait combinations uniformly sort out along a shared response curve (Meinzer, 2003; Bucci *et al.*, 2004).

This 'functional convergence' hypothesis implies that stem and leaf traits are co-evolving, but there have been few investigations explicitly testing this. It is important to do so, as species do not represent independent data points but rather share an evolutionary history, and thus can variously misrepresent the strength of evolutionary correlations (Felsenstein, 1985; Harvey *et al.*, 1995). For instance, Ackerly & Reich (1999) demonstrated that an apparently strong relationship between specific leaf area (SLA) and leaf size is reduced when analysed using phylogenetically independent contrasts (PIC), because most of the signal in the original analysis reflected only the very large differences between conifers and angiosperms. Similarly, using independent constrasts, Maherali *et al.* (2004) showed that a negative relationship between K_{sp} and xylem resistance to drought-induced cavitation (Pockman & Sperry, 2000) exists within evergreen angiosperms and conifers, but not within deciduous angiosperms. Their results clearly reject the hypothesis that there is a mechanistic trade-off between xylem vessel hydraulic conductivity and resistance to embolism; instead, the correlation between these traits in some species, but not in others, probably represents a general co-ordination related to possessing a particular water-use strategy.

In general, ahistorical and PIC analyses are more likely to provide similar results when traits exhibit greater homoplasy (Ackerly & Donoghue, 1998). It is often assumed that plant eco-physiological traits are evolutionarily labile and 'adaptable' because of their observed plasticity in response to environmental perturbations. However, when analysed within a historical framework, plant functional traits and ecological preferences have shown both high and low levels of homoplasy (Ackerly & Donoghue, 1998; Prinzing *et al.*, 2001; Cavender-Bares *et al.*, 2004; Feild *et al.*, 2004; Preston *et al.*, 2006). Although we know, to some degree, how traits pertaining to plant water relations are distributed across ecological communities, we currently know very little about how they are distributed across lineages. Similarly, as there is generally a range of values of water relations parameters found within any particular environment, it is difficult to predict how this set of traits may be involved in ecological adaptation.

In general, plants living in drier environments tend to have smaller, thicker leaves with lower SLA and wood with narrower vessels (thus a lower realized K_{sp}), suggesting that plants respond to drought stress by evolving tissues that are more resistant to water loss (Gibson, 1996; Smith *et al.*, 1997; Fonesca *et al.*, 2000; Carlquist, 2001; Reich *et al.*, 2003; Wright *et al.*, 2004). However, there are many processes governing the assembly of ecological communities, and interpreting such broad ecological patterns as evidence of de facto adaptive evolution can be misleading (Ackerly, 2004). While Cunningham *et al.* (1999) demonstrated repeated reduction in SLA associated with drier climates across 10 species pairs in Australia, Preston & Ackerly (2003) found inconsistent responses of K_{sp}, K_L, HV and stomatal density to environmental water availability across six lineages of California chapparal plants. This suggests that the response of the hydraulic pathway to more arid environments is lineage specific and probably depends on the physiological characters present in the lineage before the change in habitat.

Shifting the focus of comparative studies from ecological communities to closely related species that span different habitats allows us to test for the correlated evolution of stem and leaf hydraulic parameters and whole-plant water use, as well as the relative importance of these traits in ecological adaptation. This study explores the evolution of a suite of traits pertaining to plant water relations in seven species of *Pereskia* (Cactaceae), addressing the following specific questions.

(1) Is there co-ordination between stem and leaf water transport capacity, such that evolutionary changes in stem and leaf hydraulic traits are positively correlated?

(2) Are changes in any of these hydraulic traits associated with shifts in environmental water availability?

(3) Finally, do two traits recently established as important predictors of (i) habitat (SLA) and (ii) hydraulic architecture and drought tolerance (ρ_{wood}) correlate with these variables in *Pereskia*?

Ideally, tests of this sort would be undertaken in a controlled environment (e.g. a common garden or glasshouse experiment) to ensure that the interspecific differences measured are not merely the result of plastic responses to variable growth conditions. The data used here are derived from natural populations with the assumption that any large measured interspecific differences in traits would be maintained in a

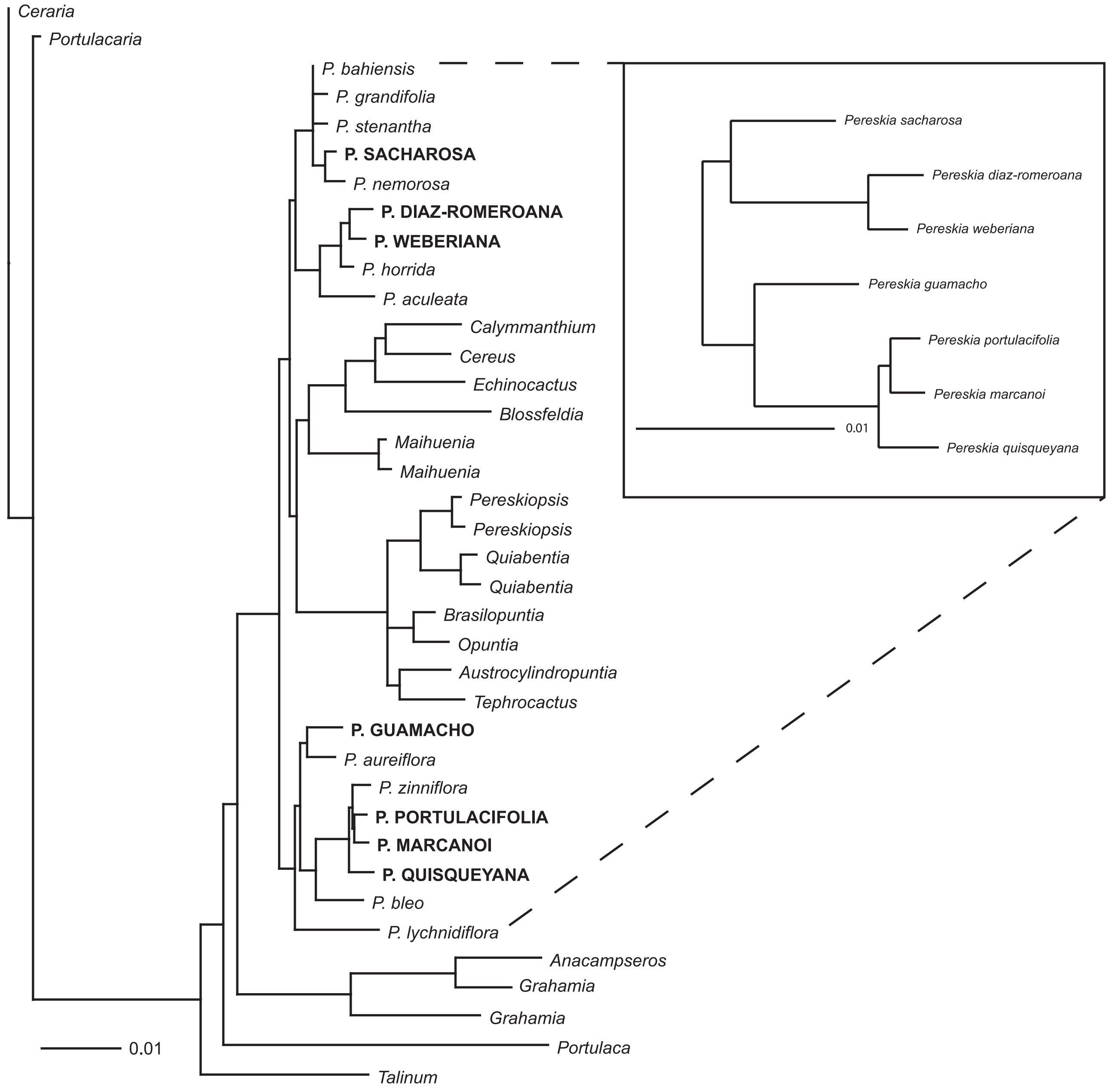

Fig. 1 Basal cactus phylogeny, modified from Edwards *et al*. (2005), with permission from the American Journal of Botany. The boxed inset is the pruned tree of seven *Pereskia* species used for correlated evolution analyses.

common garden. Informal observation of several glasshouse-grown species of *Pereskia* support this assumption (E. Edwards, pers. obs.), and future work will investigate this further.

Materials and Methods

Study species

Pereskia (Cactaceae) traditionally comprises 17 species of leafy shrubs and trees that have long been thought to represent the ancestral, leafy cactus. A recent molecular phylogenetic study of basal cactus relationships (Edwards *et al.*, 2005) provided resolution of species-level relationships in *Pereskia*, and showed that *Pereskia* species are not monophyletic; rather, they are a paraphyletic assemblage of species at the base of the cacti (Fig. 1). Although they are clearly cacti, the *Pereskia* lineages diverged from the main cactus clades before the evolution of stem succulence and leaflessness. Accordingly, *Pereskia* species are morphologically and anatomically similar to a 'typical' woody plant: they produce photosynthetic leaves, exhibit primarily C3 photosynthesis, and transport water from soil directly to their leaves through secondary xylem. As such, any universal relationships between stem and leaf water-use properties in woody plants should extend also to *Pereskia* species.

Pereskia ecology and physiology has not been well studied. They are found throughout a range of drier forest and scrub environments in the Caribbean and in Central and South America (Leuenberger, 1986), and have been described as relatively mesic-loving, drought-deciduous plants (Mauseth & Landrum, 1997; Mauseth, 1999). In a recent survey, however, Edwards & Donoghue (2006) characterized basic ecophysiological parameters and climate preferences of seven species distributed across the two *Pereskia* lineages (Fig. 1) and found that *Pereskia* species are actually most commonly found in semi-arid, seasonally very dry environments, but exhibit a suite of water relations traits that allow them to avoid developing large water deficits in their tissues. Furthermore, they are not strictly 'drought deciduous', and exhibit complex leaf phenology that does not appear to be closely linked with seasonal precipitation patterns (Edwards & Diaz, 2006).

Ecophysiological and anatomical measurements

This study analysed the eco-physiology data set of Edwards & Donoghue (2006), with additional anatomical and environmental data from the seven species. All ecophysiological data [K_{sp}, K_L, HV, g_s, Ψ_{min}, SLA and leaf carbon isotope discrimination ratio ($\delta^{13}C$)] were collected on mature plants, growing in their natural environments, using methods described by Edwards & Diaz (2006). Leaf Ψ was monitored predawn to dusk, and occasionally over 24-h periods, at all field sites for 2–4 wk during the primary growing (rainy) season. Therefore, in this study, Ψ_{min} refers to the average daily Ψ_{min} recorded during that time, rather than to the lowest Ψ_{min} experienced throughout the year. Stable carbon isotope ratios of leaf tissue were used as a time-integrated measure of WUE, where higher values of $\delta^{13}C$ indicate higher plant WUE (Farquhar *et al.*, 1982).

Anatomical measurements were made on tissues collected from field-grown individuals ($n = 3$ individuals per species). Leaf and wood samples from distal branches (similar in diameter to branches used for hydraulic measurements) were fixed in 45% ethanol, 40% H_2O, 10% formalin, 5% glacial acetic acid (FAA) and then transferred to 70% ethanol for long-term storage. Wood samples were cross-sectioned, using a sliding microtome, at 30–40 µm thickness and stained with safranine. Mean vessel diameter (µm) was estimated from measuring 200–300 vessels per species. Mean vessel density (mm^2) was estimated by counting vessels in 25–35 1-mm^2 squares for each species. ρ_{wood} was calculated as dry weight/volume ($g\ cm^{-3}$) ($n = 6$ per species). The volume of the wood sample was determined by submerging preserved wood in water on a balance and measuring the displacement of water in g (1 g of $H_2O \approx 1$ ml of H_2O). Wood was then dried in a 50°C oven and dry weight was recorded. Mean leaf surface area (one-sided, cm^2) was estimated from pooled leaf samples on branches collected for hydraulics measurements (six to eight branches per species). Two anatomical leaf characteristics – vein density (vein length per area, mm) and stomatal pore index [(SPI) = guard cell length^2 × stomatal density], have recently been shown to be important correlates of whole-leaf K_{lamina} (Sack *et al.*, 2003; Sack & Tyree, 2005). K_{lamina} proved difficult to measure directly in *Pereskia* leaves because of succulence and lack of a petiole in many species, so SPI and vein density were used as proxies for K_{lamina}. SPI similarly reflects theoretical maximum stomatal conductance, g_{smax}. Stomata were measured from abaxial leaf surface impressions taken in the field ($n = 3$–6 leaves from three individuals per species) using a quick-hardening dental epoxy (President light; Coltene/Whaledent Inc., Hudson, MA, USA). To calculate SPI, 50–60 guard cells and 20–30 1-mm^2 areas for stomatal density were measured for each species. Leaves were cleared for venation density measurements in either a solution of 30% household bleach or in 47.5% ethanol and 5% NaOH. After clearing, leaves were stained with safranine, and total vein length was measured in 30–40 1-mm^2 sections for each species. All microscopic work was performed on a Nikon Eclipse E600 microscope using SPOT advanced software for measurements.

Environmental data

At all field sites, daily patterns of atmospheric vapor pressure deficit (VPD) were monitored using a portable HOBO weather station (Onset Corporation, Bovene, MA, USA). Mean annual precipitation (MAP) and mean annual temperature (MAT), obtained from the WorldClim global climate data set (available at http://biogeo.berkeley.edu/worldclim/worldclim.htm), were used to calculate a moisture index, I_M, for each fieldsite. I_M is calculated as:

$$I_M = 100 \times (\text{MAP} - \text{PET})/(\text{PET}) \qquad \text{Eqn 1}$$

[PET, potential evapotranspiration, calculated from monthly mean air temperature estimates as adapted from Thornthwaite (1948)]. To estimate the seasonality of drought stress at each site, a wet season and a dry season I_M were calculated as the mean monthly I_M of the three consecutive wettest months ($I_M wet$) and the three consecutive driest months ($I_M dry$).

Trait evolution analyses

The AOT module in PHYLOCOM, version 3.22 (Webb *et al.*, 2005, available at www.phylodiversity.net/phylocom), was used to test for correlated evolution among traits. Independent contrasts (Felsenstein, 1985) are calculated as the difference in trait means of two daughter nodes or tips divided by the expected amount of change, which is the square root of the branch length separating the two taxa. This provides N–1

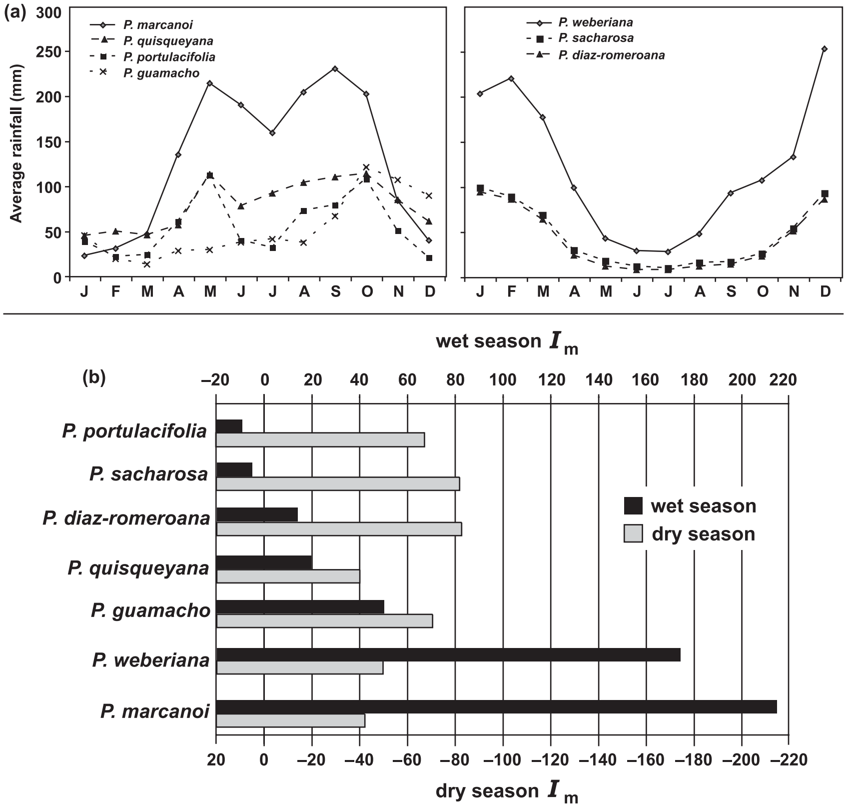

Fig. 2 Environmental parameters of field study sites. (a) Patterns of annual precipitation at the seven field sites. The left-hand graph includes sites in the northern hemisphere; the right-hand graph includes sites in the southern hemisphere. (b) Differences in calculated moisture index (I_M) for wet seasons (black bars) and dry seasons (gray bars), indicating that the study sites all experienced similar maximum levels of drought, but differed strongly in moisture availability during the primary growing season.

contrasts, where N is the number of tips in the phylogeny ($n = 7$). All trait data were logarithm-transformed before analyses, with the exception of I_M, which consisted of both positive and negative values; correlations between I_M and other traits were calculated with all data untransformed. Branch lengths were obtained from a maximum-likelihood tree, based on an analysis of molecular sequence data from all 38 taxa shown in Fig. 1 (Edwards *et al.*, 2005). As *Pereskia* is paraphyletic at the base of the cacti, it was necessary to prune a large number of taxa from the tree before analyses, because of missing data. Analysing the two *Pereskia* clades separately would have avoided pruning the core cacti from the tree, but would also have reduced the total number of independent contrasts from $n = 6$ to $n = 5$. Analysing the seven species together is appropriate because simulation studies have demonstrated that PIC analyses are generally robust to poor sampling schemes (Ackerly, 2000).

Results

Species habitat characterization and trait values

Field studies of each species were conducted during their primary growing seasons between 2003 and 2005, at locations in Venezuela, the Dominican Republic and Bolivia (one field location for each species except for *P. guamacho*, Table 2). During field measurements, average daily VPD varied from 1.15 kPa in *P. marcanoi* to 1.73 kPa in *P. quisqueyana*, although there were larger differences in minimum VPD across species. The MAP at field sites varied from 477 mm yr^{-1} (*P. diaz-romeroana*) to 1578 mm yr^{-1} (*P. marcanoi*). Most of the variation in precipitation resulted from large differences in wet season rainfall, as evidenced by the very large spread in I_M*wet* among the seven species. This stands in stark contrast to the relatively little variation in I_M*dry* (Fig. 2). Although all field locations generally fall into the 'tropical dry forest' or 'tropical very dry forest' Holdridge life zones (Holdridge, 1967), the differences in MAP, VPD and calculated I_M corresponded with obvious differences in vegetation structure. The extremes were represented by *P. diaz-romeroana*, which grows as a scrambling shrub in dry thorn scrub of the inter-Andean valleys of central Bolivia, and *P. marcanoi*, which grows as a small subcanopy tree in drought-deciduous forests of central Hispaniola. With the exception of *P. marcanoi*, all species grew in full sunlight, in plant communities that did not form a closed canopy. For consistency, we only measured individuals of *P. marcanoi* growing in direct sunlight in canopy gaps.

Trait means for all variables are listed in Table 2. Some traits were considerably more variable than others; for example, Ψ_{min} only ranged between −0.6 MPa and −1.0 MPa across all species, while K_L varied by an order of magnitude, from 3.6×10^{-4} kg m^{-1} s^{-1} MPa^{-1} in *P. quisqueyana*, to 27.6×10^{-4} kg m^{-1} s^{-1} MPa^{-1} in *P. portulacifolia*.

Table 2 Species means and standard errors for anatomical and physiological traits, and locations and environmental parameters for study sites

	Species						
	P. diaz-romeroana Cardenas	*P. guamacho* F.A.C. Weber	*P. portulacifolia* (L.) D.C.	*P. marcanoi* Areces	*P. quisqueyana* Liogier	*P. sacharosa* Grisebach	*P. weberiana* K. Schumann
Study site location	Bolivia	Venezuela	Dominican Republic	Dominican Republic	Dominican Republic	Bolivia	Bolivia
Coordinates	S 18.10113 W 64.45551	N 11.93947 W 69.96241 N 11.28126 W 69.69084	N 18.42655 W 71.76983	N 19.0895 W 71.68403	N 18.36913 W 68.84258	S 18.27388 W 64.15802	S 16.54096 W 67.39115
MAP (mm)	482	638	662	1559	955	533	1433
Average daily VPD (kPa)	1.62	1.55	1.72	1.15	1.73	1.20	1.67
I_M	–65.8	–42.9	–68.4	50.3	–12.0	–92.3	36.9
Vessel density (mm^{-2})	56.67 ± 3.57	50.66 ± 2.30	63.59 ± 1.18	36.81 ± 1.92	37.87 ± 2.11	37.17 ± 1.22	60.67 ± 1.63
Vessel diameter (μm)	44.01 ± 0.78	39.18 ± 0.61	51.16 ± 0.86	46.82 ± 0.89	46.86 ± 0.75	52.34 ± 1.29	45.76 ± 0.70
K_{sp} ($kg\ m^{-1}\ s^{-1}\ MPa^{-1}$)*	1.69 ± 0.30	1.03 ± 0.26**	2.69 ± 0.27	2.06 ± 0.97	1.76 ± 0.20	2.01 ± 0.14	1.91 ± 0.25
$K_L \times 10^{-4}$ ($kg\ m^{-1}\ s^{-1}\ MPa^{-1}$)*	7.81 ± 0.97	5.91 ± 1.44**	27.62 ± 2.37	11.16 ± 1.75	3.60 ± 0.54	8.85 ± 1.44	4.56 ± 2.60
HV $\times 10^{-4}$*	5.54 ± 1.18	5.79 ± 0.66**	10.82 ± 1.54	5.62 ± 0.91	2.20 ± 0.56	4.65 ± 0.63	1.96 ± 0.12
SPI $\times 10^{-2}$*	3.38 ± 0.21	2.74 ± 0.26**	6.02 ± 0.89	5.18 ± 0.53	3.98 ± 0.30	3.45 ± 0.02	3.36 ± 0.04
SLA ($m^2\ kg^{-1}$)*	13.80 ± 0.39	15.09 ± 1.27**	11.45 ± 0.84	24.80 ± 2.07	25.14 ± 2.48	13.81 ± 1.57	13.29 ± 0.72
Ψ_{min} (MPa)*	–0.88 ± 0.09	–1.00 ± 0.04**	–0.84 ± 0.04	–0.60 ± 0.05	–0.71 ± 0.03	–0.84 ± 0.04	–0.73 ± 0.03
^{13}C (‰)*	–21.39 ± 0.42	–25.83 ± 0.31**	–24.30 ± 0.32	–26.17 ± 0.58	–25.43 ± 0.58	–22.54 ± 0.28	–26.12 ± 0.72
Wood density ($g\ cm^{-3}$)	0.61 ± 0.03	0.67 ± 0.03	0.60 ± 0.03	0.70 ± 0.01	0.62 ± 0.03	0.57 ± 0.01	0.64 ± 0.03
Venation density (mm^{-1})	7.75 ± 0.20	3.39 ± 0.16	6.89 ± 0.28	4.83 ± 0.12	4.44 ± 0.16	5.23 ± 0.35	4.83 ± 0.09
Leaf size (cm^2)	1.74 ± 0.17	6.52 ± 0.90	0.92 ± 0.21	3.84 ± 0.34	6.39 ± 0.58	12.40 ± 1.39	7.96 ± 1.11
Sapwood/leaf $\times 10^{-7}$ (m^2)	0.85 ± 0.14	3.78 ± 0.72	1.00 ± 0.28	1.99 ± 0.30	1.29 ± 0.24	5.35 ± 0.51	1.56 ± 0.30

Traits marked with an asterisk indicate that data have previously been reported (*Edwards & Donoghue, 2006; **Edwards & Diaz, 2006).
See Table 1 for definitions of abbreviations.

Table 3 Independent contrast correlations between selected water relations and environmental traits

Trait 1	Trait 2	Correlation coefficient (*R*)
K_{sp}	Vessel diameter	0.909**
K_{sp}	Vessel density	0.420
K_L	K_{sp}	0.681
K_L	Venation density	0.806*
K_L	SPI	0.798*
K_L	Leaf size	–0.863**
K_L	HV	0.941***
HV	Leaf size	–0.851**
HV	Wood per leaf	–0.349
Leaf size	Venation density	–0.879**
Leaf size	SPI	–0.637
Venation density	SPI	0.656
Ψ_{min}	MAP	0.875**
Ψ_{min}	I_M	0.882**
Ψ_{min}	I_M wet season	0.852**
Ψ_{min}	I_M dry season	0.843**
$\delta^{13}C$	MAP	–0.892**
$\delta^{13}C$	I_M	–0.851**
$\delta^{13}C$	I_M wet season	–0.772*
$\delta^{13}C$	I_M dry season	–0.875**
Wood density	Vessel density	–0.557
Wood density	Vessel diameter	–0.681
Wood density	K_{sp}	–0.513
Wood density	K_L	–0.478
Wood density	Ψ_{min}	–0.745*
SLA	MAP	0.590
SLA	I_M wet season	0.621
SLA	I_M dry season	0.546

Traits that are significantly correlated are marked: ***, $R \geq 0.917$, $P < 0.01$; **, $R \geq 0.811$, $P < 0.05$; *, $R \geq 0.729$, $P < 0.10$.
See Table 1 for definitions of abbreviations.

Correlation of leaf and stem hydraulic traits

Correlation coefficients and significance values for selected traits and environmental parameters are reported in Table 3. As predicted from the Hagen–Poiseuille equation used to describe water flow through xylem (Zimmermann, 1983), K_{sp} was significantly correlated with mean vessel diameter, but not with mean vessel density. K_{sp} and K_L were not significantly correlated; rather, the large differences in K_L resulted primarily from changes in HV. As HV is the ratio of sapwood area to supported leaf area, it is sensitive both to changes in individual leaf size (one-sided surface area, cm^2) as well as to the number of leaves that are produced per given cross-sectional area of stem. In this data set, HV was tightly correlated with mean leaf size, but not with the mean number of leaves per given amount of wood ('wood per leaf' in Table 2), although both traits were quite variable. Vein density and SPI were also negatively correlated with leaf size (Fig. 3), although the SPI leaf size correlation was not significant ($P > 0.10$). As expected, K_L was positively correlated with SPI and vein density (Fig. 3); however, there were no significant correlations between these variables and Ψ_{min} or $\delta^{13}C$.

Correlations with plant water-use traits and environmental water availability

Although traits pertaining to leaf and stem water transport capacity were correlated with one another, none was correlated with MAP or I_M. Only two traits – Ψ_{min} and $\delta^{13}C$ – showed any significant relationship with measures of environmental water availability. Ψ_{min} correlated positively with MAP, I_M, I_M*wet* and I_M*dry*, showing a slightly higher correlation with I_M*wet* than with I_M*dry*. In turn, $\delta^{13}C$ correlated negatively with all of these measures, showing a significantly higher correlation with I_M*dry* than I_M*wet*. No traits showed significant correlation with average or minimum daily VPD.

SLA and ρ_{wood} as predictor traits

Specific leaf area varied widely among the *Pereskia* species sampled (Table 2), but was not significantly correlated with any measure of environmental water availability. ρ_{wood} exhibited relatively little variation, and did not correlate significantly with changes in vessel diameter, vessel density, K_{sp}, K_L, or HV. While multiple studies have demonstrated a negative correlation between ρ_{wood} and Ψ_{min}, in this study the two variables were positively correlated, although it was significant only at $P < 0.10$ (Table 3).

Discussion

Correlated evolution of stem and leaf traits

These analyses indicate a significant evolutionary correlation between K_L and two leaf hydraulic traits (SPI and vein density) that have been shown to be primary components of whole-leaf K_{lamina} (Fig. 3). Surprisingly, other traits that have previously been correlated with K_L showed no significant relationships. For instance, there was an apparent independence between traits involved in water transport (K_L, vein density, SPI) and traits associated with tissue water deficits (leaf Ψ_{min}, $\delta^{13}C$). This decoupling has also been reported by Sack *et al.* (2003), and suggests that hydraulic capacity and drought tolerance may represent independent evolutionary axes of plant water use.

ρ_{wood} is another trait that has been functionally linked to K_L, and it has been presented as a potential constraint on the evolution of the plant water transport pathway (Bucci *et al.*, 2004). In *Pereskia* this does not appear to be the case, in that ρ_{wood} varied very little, while K_L varied by over an order of magnitude, and changes in the two traits were not correlated. It appears that ρ_{wood} is not tightly linked to water use in these

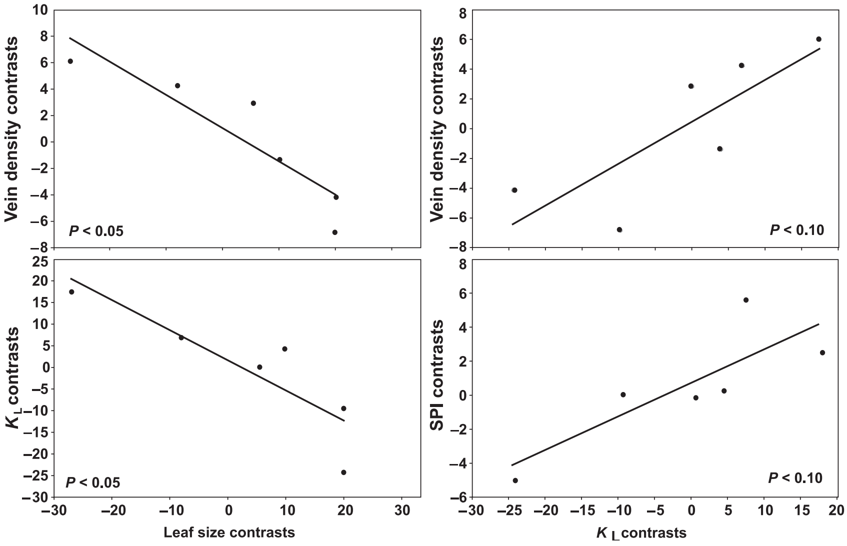

Fig. 3 Plots of indpendent contrast correlations for selected leaf and stem hydraulic traits and leaf size. Correlation coefficients for all examined trait-wise comparisons are listed in Table 3.

species, and it is more likely that the small differences in ρ_{wood} are the result of changes in xylem cells not directly involved in water transport.

Analysing trait correlations within an evolutionary context also allows for a finer deconstruction of functional relationships within suites of interdependent traits. For example, K_L is, by definition, a function of K_{sp} and HV. But are evolutionary transitions in K_L associated with changes in K_{sp}, or HV, or both? In these *Pereskia* species, K_L is primarily governed by HV, suggesting that changes in carbon-allocation patterns, rather than changes in xylem anatomy, are driving the large differences in leaf-specific hydraulic efficiency. This is not suprising, as HV has previously been shown to exhibit greater variation than other traits with respect to environmental water stress, both by intra- and interspecific differences in leaf and shoot allometry, as well as by individuals shedding leaves in response to drought (Mencuccini & Grace, 1995; Maherali *et al.*, 1997; Kolb & Sperry, 1999; DeLucia *et al.*, 2000; Cavender-Bares & Holbrook, 2001; Preston & Ackerly, 2003; Bucci *et al.*, 2005). Shedding leaves will immediately increase HV and, thus, K_L by reducing the number of leaves supported by a given amount of stem cross-sectional area, thereby affording the remaining leaves with a substantially greater water supply. In the case of *Pereskia*, however, there was no apparent leaf shedding observed during the study, and HV was tightly correlated with leaf size rather than leaf number.

The large variation in HV and K_L in *Pereskia* deserves mention; it is greater than that captured by other studies which included a phylogenetically broad sampling of species, and encompasses some of the highest K_L values reported for broad-leaved plants (Brodribb & Feild, 2000; Brodribb *et al.*, 2002). *Pereskia* species exhibit a short-shoot stem architectural system, which allows new leaves to be directly connected to older wood and alleviates the hydraulic bottleneck of current-year xylem. As such, *Pereskia* species may achieve very high values of K_L relatively cheaply in terms of construction costs, provided that their xylem remains functional for multiple years (discussed in Edwards & Diaz, 2006).

Pereskia hydraulic evolution and environmental water availability

In spite of the variation of water relations traits in *Pereskia* and the correlated evolution of a suite of traits that presumably are tightly linked to whole-plant water use, there was little evidence for the correlation of these traits with estimates of water availability experienced by the different *Pereskia* species. This could potentially be the result of an inaccuracy of the climate estimates, or the possibility that the study populations do not accurately represent the typical climate of each respective species. The second explanation is unlikely, as the MAP estimates of field sites are consistent with those recorded for each species across their current geographical ranges (Edwards & Donoghue, 2006). Similarly, the strong and classically predicted responses of Ψ_{min} and $\delta^{13}C$ to both MAP and I_M suggest that MAP and I_M are providing an accurate measure of environmental water stress (Ehleringer, 1993; Stewart *et al.*, 1995).

It may be instead that the lack of hydraulic response to environmental water availability is caused by conservative stomatal behavior and a particular rooting strategy. Observations of daily patterns of g_s at all field sites indicate that *Pereskia* species often keep stomata closed for the majority of the day, even during the wettest parts of the year when neighboring plants are transpiring freely. This is reversed with a substantial rainfall event, and stomata will remain open from dawn to dusk on the day following a large storm (Edwards & Donoghue, 2006). This conservative, but opportunistic, stomatal behavior is indicative of a shallow and extensive root system that would allow for such a rapid response to rainfall. In addition, the relatively high Ψ_{min} exhibited by all *Pereskia* species suggests that *Pereskia* roots may become disconnected from the drying soil by mechanisms similar to the leafless, stem succulent cacti (see Edwards & Diaz, 2006 for further discussion). In a sense, *Pereskia* is maintaining its own internal 'mesic' environment, rather than adapting to new conditions, by minimizing transpiration and disrupting the soil–leaf pathway before large tissue water deficits can develop. This hypothesis is supported by the tight relationship beween δ^{13}C and MAP and I_M*dry*. A shift towards less negative δ^{13}C in *Pereskia* could be the result of an increase in instantaneous WUE, resulting from an elevated rate of carbon fixation for a given g_s. This is a common response of plants to increasing drought (reviewed in Reich *et al.*, 2003). Alternatively, in the two species with very high δ^{13}C values (*P. diaz-romeroana* and *P. sacharosa*, Table 2), it may be more likely that these values have resulted from periodic induction of drought-induced crassulacean acid metabolism photosynthesis (Winter & Holtum, 2002; Edwards & Donoghue, 2006). The very strong correlation between δ^{13}C and dry-season drought levels also indicates that these *Pereskia* species must be maintaining some photosynthetic tissue during the dry season. This lends support to Edwards & Diaz (2006), who hypothesized that *Pereskia* species are not strictly drought deciduous, as commonly described.

Pereskia hydraulics and leaf size

If K_L, HV, vein density and SPI are not evolving in response to environmental water availability, then why are they evolutionarily correlated? It is tempting to consider a mechanistic answer: perhaps at the genetic or developmental level, the evolution of these traits has become constrained such that they can no longer change independently. This seems unlikely, however, given the wide range of values (and combinations) of these traits observed across vascular plants, as well as their occasionally rapid and plastic responses to environmental perturbations. Alternatively, these traits could all independently be responding to a different, unmeasured environmental variable, or to another plant trait.

These analyses indicate that in *Pereskia*, each of these particular leaf and stem traits are also tightly linked to individual leaf size. Leaf size has been shown to respond to a multitude of environmental parameters, including drought, temperature, irradiance and soil fertility (reviewed in Givnish, 1987). In this case, the large interspecific differences in leaf size show no apparent relationship to environmental water availability. It is currently unclear what may be influencing the evolution of leaf size in *Pereskia*, although it does not appear to be temperature or light (E. Edwards, unpublished).

The relationship between leaf size and vein density has only rarely been investigated (Roth-Nebelsick *et al.*, 2001). It is unknown if the significant negative correlation demonstrated here in *Pereskia* is a common pattern within other lineages, although this relationship has been shown for sun and shade leaves within an individual plant (Uhl & Mosbrugger, 1999; Zwieniecki *et al.*, 2004). The degree of correlation between venation density and leaf size clearly depends on the relative timing of cell differentiation and expansion in a developing leaf, as any leaf expansion occurring after venation patterning has been set will necessarily cause a negative relationship between vein density and leaf size (Zwieniecki *et al.*, 2004). During leaf development in *Arabadopsis thaliana*, it appears that vein density remains relatively constant during leaf expansion because of the continued intercalation of higher-order veins (Kang & Dengler, 2004); however, it is unknown if this mechanism is present also in *Pereskia*, or if this process is maintained during evolutionary shifts in leaf size.

Differences in vein density among distantly related species were found to correlate with differences in leaf mesophyll structure and hydraulic architecture, but not with differences in leaf size (Sack & Frole, 2006). This study of *Pereskia* species is the first to address changes of vein density among very closely related taxa and suggests that at a population- or species-level scale, vein patterning may be constrained during leaf development, such that differences in expansion and final leaf size result in significant differences in vein density.

Patterns in plant traits: ecology vs evolution

This highlights an important point regarding the evolutionary interpretation of many broad-based comparative studies: even when using a phylogenetic 'correction' for species nonindependence, the patterns and trait correlations that we see at phylogenetically broad interspecific comparisons (what is most commonly sampled in ecological studies) may not always be applicable for understanding species-level responses to environmental change and ecological adaptation. This may be especially relevant when considering the evolution of traits that are influenced by a large suite of underlying anatomical and physiological variables, such as those involved in regulating whole-plant water use. Assume, for example, that both leaf size and ρ_{wood} are determinants of K_L. If ρ_{wood} is a relatively evolutionarily conserved trait (e.g. all or most members of particular plant lineages exhibit similar wood densities) then a covarying, but more evolutionarily labile, trait, such as leaf size, will be more likely to influence the evolutionary trajectory of K_L. In *Pereskia*, K_L was not governed by ρ_{wood}, shifts in SLA were not associated with shifts in habitat, and leaf size has emerged as a potential driver of plant hydraulic evolution. None of these results would be predicted from community-based ecological studies.

Conclusions

This study provides some evidence that in *Pereskia* many commonly measured stem and leaf hydraulic traits appear to be evolutionarily correlated with one another, but are not tightly linked to certain measures of environmnental water availability and drought stress. Instead, they are correlated with another trait, leaf size, that is highly labile and apparently also unlinked to habitat moisture regime. This lack of correlation may be a result of the particularly conservative stomatal behavior observed in *Pereskia*. These results are consistent with some studies (Preston & Ackerly, 2003), but not others (Cavender-Bares & Holbrook, 2001), suggesting that lineage-specific combinations of traits governing plant water relations will have a large effect on how those traits respond to environmental water availability. Accumulating more lineage-based comparative studies will allow for a closer evaluation of the relative importance of these traits in plant ecological adaptation.

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