Full Length Article

Evolutionary Associations of Leaf Functional Traits in Nine Euphorbiaceae Species

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Abstract

Leaf functional traits are determined by both environment and phylogeny, especially within closely related species. However, many former studies investigated the environmental drivers of the traits, while only a few studies addressed the effects of phylogeny. Euphorbiaceae is ideal for detecting the relationship of phylogeny with trait variations, correlations, and plant adaptation because of the family's wide distribution and diverse appearances. We tested phylogenetic association on leaf functional traits and examined traits correlations within nine Euphorbiaceae species to elucidate plant adaptations strategies. The results showed that all phylogenetic signals of leaf functional traits were <1, indicating their high liability. Leaf area was phylogenetically independent of leaf physiological attributes, while leaf length to width ratio was negatively correlated with stomatal conductance. Stomatal density and vein density were positively correlated, while leaf mass per area, stomatal length, leaf thickness, palisade thickness, and spongy thickness were negatively correlated with photosynthetic rate, stomatal conductance, and transpiration rate for species values. Phenotypically and phylogenetically, stomatal traits and vein density were significantly correlated with tissue thickness and cuticle thickness, indicating their phylogenetic coordination. These results reflected the adaptive strategies of Euphorbiaceae species to diverse environments, which is one of the significant reasons for the wide distribution of this family. © 2018 Friends Science Publishers

Keywords: Anatomy; Adaption; Correlated evolution; Morphology; Phylogenetic signal; Physiology

Introduction

Both current environmental conditions and evolutionary history are powerful determinants of traits that drive plant responses to environmental change; thus, phylogenetic effects cannot be neglected, especially in species with close relationships (Ackerly *et al.*, 2000; Baker *et al.*, 2017). However, while many previous studies investigate the environmental drivers of the traits and their coordination, only a few studies address the association of phylogeny with plant traits (Santiago *et al.*, 2009). Moreover, many previous studies have explored traits correlations using broadly diverse species, and trait relations within closely related species have not been frequently tested (Zhao *et al.*, 2016), despite growing realization that relations among the closely related species may provide more meaningful insights into plant adaptation (Edwards, 2006).

The use of leaf functional traits to study plant phylogenetic adaptations needs to answer two questions: which factor, phylogeny, or environment, has the largest effect on leaf trait evolutionary styles? How do leaf morphology and anatomy influence leaf physiological functions to drive plant adaptation through trait correlations? The Phylogenetically Independent Contrast (PIC) method can effectively test the evolutionary styles of

leaf functional traits, and thus be widely used in ecological studies, because this method can isolate phylogenetic effects (Blomberg *et al.*, 2003). PIC is designed to explore coordinated evolution, which means the relation of evolutionary changes in two traits (Price *et al.*, 2012). In PIC research of traits coordination, species values correlations have been always good guides to PIC contrast correlations, as no difference between these two correlations exists when phylogenetic effect is powerful (Price *et al.*, 2012). However, differences between these two correlations can also be biologically significant, indicating that correlations changing as proceeds of adaptive radiation (Price, 1997). This method so far has developed desirably in its calculation and models.

Previous researches into plant physiological functions reflected by leaf morphology and anatomy have been conducted during the past decades, and have indicated some general correlations (Wright *et al.*, 2004; Brodribb *et al.*, 2013; Zhang *et al.*, 2014). For example, across nearly all terrestrial plants, leaf morphological traits such as leaf size and shape are closely related to light harvesting, transpiration, and net carbon gain (Givnish *et al.*, 2004); leaf biomass per area and leaf tissue thickness are negatively correlated with carbon accumulation and respiration (Wright *et al.*, 2004); and leaf vein density and stomatal density are

both positively correlated with leaf hydraulic transport and transpiration loss (Brodribb and Jordan, 2011). Furthermore, because of similar effects on leaf physiological functions, the leaf morphology and anatomy may exhibit coordinated shifts across species. In fact, some studies have already suggested that leaf morphology, especially leaf size, can act as an easily measured alternative for a wide range of structurally, functionally, and developmentally linked anatomical traits that influence leaf photosynthetic capacity (Niinemets *et al.*, 2007; Kembel and Cahill, 2011). For instance, plants in environmentally stressful conditions usually consistently have small, dense and thick leaves (Cunningham *et al.*, 1999), always with high stomatal frequency as well as vein density (Dunbar-Co *et al.*, 2009; Brodribb and Jordan, 2011). These coordinated shifts among leaf morphology, anatomy, and physiology are important to our understanding of plant adaptability to environmental stress; however, their evolutions are rarely revealed (Sun *et al.*, 2014).

The present study selected 9 species of Euphorbiaceae as our objects. Euphorbiaceae is among the largest plant family of angiosperms with about 300 genera and 8910 species. This distribution of Euphorbiaceaeis nearly global, primarily in the tropics and subtropics, as it is often the second most abundant family in tropical rainforests of Southeast Asia and Africa. Wide distribution of Euphorbiaceae species, known for their diverse habitats, growth forms, morphology, structure, and function, make this family an ideal selection for examining the association of phylogenetic changed on plant trait variations, trait correlations, and plant adaptation (Duputié *et al.*, 2011; Horn *et al.*, 2012). However, previous studies of Euphorbiaceae species often focus on their well-known economic, ornamental, and medical values, as people historically select them for large scale planting as biodieseland drug extraction feedstock (Ye *et al.*, 2009). Meanwhile, the ecological significance of this family is neglected, even though ecological adaptability is the base and guide of utilization.

In view of the above information, we investigated the association of phylogeny on leaf functional traits and detected traits correlations within a family to understand plant ecophysiological adaptations. We measured 15 leaf traits and applied PIC method to explore evolutionary styles and coordinated relationships of leaf functional traits in Euphorbiaceae. We hypothesized that (1) at least some of the studied leaf functional traits may have phylogenetic signals; (2) both leaf morphological and anatomical traits may influence leaf photosynthetic functions in species values and evolutionary context; and (3) coordinated patterns may exist among leaf functional traits. Our study is likely to reveal some basic results of the correlation of phylogenetic changes with the leaf functional traits in order to understand the leaf eco-photosynthetic adaptive functions reflected by leaf morphology and anatomy in Euphorbiaceae.

Materials and Methods

Study Site and Plant Materials

Our study was managed at Xishuangbanna Tropical Botanical Garden (XTBG; 21°41′ N, 101°25′ E; 570 m a.s.l.) in southwestern Yunnan Province, China. The climate here is dominated by the East Asian monsoon and the Indian Ocean monsoon with an obvious contrast between wet season and dry season. Based on the dataset of Xishuangbanna ecological station, the mean annual temperature is 21.7ºC with the coldest monthly mean temperature is 14.8ºC (January), and the hottest monthly mean temperature is 25.5ºC (July). The mean annual precipitation is nearly 1560 mm, of which only 20% occurs during the dry season (November to April). In this study, we took all measurements in the beginning of dry season (November 2011 to January 2012).

To minimize the confounding effects of environment, nine species in Euphorbiaceae [*Acalyphahispida*; *A. wilkesiana*; *Baccaurea ramiflora*; *Breynia fruticosa*; *Codiaeum variegatum*; *Euphorbia neriifolia*; *Excoecaria cochinchinensis*, *Jatropha podagrica*; and *Plukenetia volubilis*], grown in XTBG for many years, were selected for our study. Each species had 15 individuals, at least, distributed in the garden. Based on availability, we selected eight healthy individuals of each species as our study objects.

Leaf Physiology

Leaf photosynthetic traits were measured *in situ* in the garden using a portable photosynthesis system (LI-6400; LI-COR Bioscience INC., Lincoln, NE, USA). The red-blue LED light source in the photosynthesis system was used to support steady photosynthetic photon flux density (PPFD). Three fresh, undamaged, and mature leaves per individual (24 leaves per species) were selected to measure leaf photosynthetic traits. All the measurements were conducted between 09:00am and 11:00 am when plant was most likely to have its optimum photosynthetic rate. Leaves were initially illuminated at a PPFD of 1000 µmol m^{-2} s^{-1} until a steady net CO_2 fixation was reached. The PPFD was then increased to 1800 µmol m^{-2} s^{-1}for 5 min as a steady state of photosynthesis approached. The maximum photosynthetic rate (A_{max}), stomata conductance (G_s) and transpiration rate (T_r) were thus recorded. During the measurements, the CO_2 concentrations, temperature and vapor pressure in the leaf chamber of the photosynthesis system were 380 µmol mol^{-1} CO_2, 25°C and 1.5 kPa, respectively.

Leaf Morphology and Anatomy

After measurement of leaf photosynthesis, we collected eight similar growing leaves from each individual. These leaves were cut, sealed in plastic bags with a wet paper ball

and taken to the laboratory, where they were numbered and divided into two parts. Five leaves per individual (40 leaves per species) belonged to the first part and the other three leaves per individual (24 leaves per species) belonged to the second part.

Each of leaves from the first part was scanned with a scanner (Hp Scanjet G4050, China). Three leaf characters including leaf area (LA), leaf length (LL) and leaf width (LW) were measured manually by ImageJ software (v.1.48; http://rsbweb.nih.gov/ij/) using the scanned images. To quantify leaf shape, leaf length to width ratio (LL/LW) was calculated. After scanning, leaves were dried for 72 h at 80ºC, and each leaf dry mass (LDM) was recorded. Leaf mass per area (LMA) was calculated as LDM divided by LA.

Leaves of the second part were cut in two halves along leaf mid-vein. The abaxial midpoints of one half were cleaned, pasted onto pellucid enamels, and the enamels were then transferred to glass slides after drying. The stomata that printed on the enamels were photographed for three digital images per leaf (72 images per species) (Fig. 1). Stomatal density (SD) was measured as the number of stomata per unit area, and it was calculated as the average of 60 digital images, while stomatal length (SL) was mean value from 60 randomly selected stomata for each species. All the stomatal photographs were taken using a light microscope (Leica DM2500, Bensheim, Germany). Stomatal traits were measured via the ImageJ software.

After complete removal of the enamels, the same half of the leaves were boiled for 20 min in 7% NaOH aqueous solution, cleaned indistilled water for 30 min, bleaching in 5% sodium hypochlorite for 30 min, cleared for another 30 min in distilled water, stained with 1% toluidine blue for 1 min, and mounted on glass slides. These leaves were then photographed for three digital images per leaf (also 72 images per species) under the light microscope (Fig. 1). Vein density (VD) was calculated as total vein length per unit area with the total vein length was manually calculated using the ImageJ software.

The other leaf halves of the second part were hand-cut in transverse sections at the midpoint. After staining for 1 min with 1% toluidine blue, the sections were rinsed with distilled water slightly, mounted on glass slides, and then photographed under light microscope (Fig. 1). Leaf thickness (LT), palisade tissue thickness (PTT), spongy tissue thickness (STT), upper epidermal thickness (UET), and lower epidermal thickness (LET) were measured from the photographs with the ImageJ software.

Phylogenetic Tree

Internal Transcribed Spacers (ITS) and *rbc*L gene regions were downloaded from the website of GenBank (http://www.ncbi.nlm.nih.gov). The phylogenetic tree was reconstructed by the concatenated sequence of the two regions. Multiple alignments were performed automatically using MEGA v.5.0 with CLUSTALW module, and then manually performed slight corrections. The best-fit model was selected using the Model test software by the Akaike information criterion, and the GTR (generalized time reversible) +G (Gamma shape) model was finally chosen as best fit this data. Phylogenetic analysis was carried out using Mr Bayes software by Bayesian analyses with running Metropolis-coupled Monte Carlo Markov (MCMC) chains and sampling one tree every 1000 generations for 1,000,000 generations. The nodal robustness was estimated by posterior probability.

Data Analysis

The trait value of an individual was calculated as average of all leaf values from the same individual. The trait value of a species was calculated as average of all plant individual values. Before analysis, all data were log10 transformed to improve the normality and homoscedasticity.

We used *K*-statistic to test phylogenetic signal in each trait by the "picante" package in R program (v.3.01; http://ftp.ctex.org/mirrors/CRAN/) (Blomberg *et al.*, 2003; Kembel *et al.*, 2010). From this analysis, $K<1$ indicate that a trait is variable, while $K>1$ means that trait is more conserved than presumed from a Brownian expectation (Blomberg *et al.*, 2003). Relationships among studied traits were evaluated by both species values and contrasts. The contrasts were calculated by PICs using the "analysis of traits (AOT)" module in Phylocom software (Webb *et al.*, 2008). We explored correlations of contrasts to evaluate whether two traits were consistently correlated during their evolution (Kembel and Cahill, 2011).

Bivariate relationships among leaf functional traits were detected with *Pearson's* correlations with the R program. To analyze the relations among leaf traits, a principal component analysis (PCA) was also performed with the "vegan" package in R program. Figures were constructed by the software SigmaPlot (v.10.0, http://www.sigmaplot.com).

Results

The Euphobiaceae species varied extensively in many leaf functional traits (Table 1), with the largest variation observed in leaf area (LA, 32.6 fold). Taxa varied strongly in photosynthesis, from 7.57 fold in maximum photosynthetic rate (A_{max}) to 11.96 fold in transpiration rate (T_r). Taxa also varied strongly in leaf tissue thicknesses, from 6.98 fold in leaf thickness (LT) to 12.39 fold in palisade tissue thickness (PTT). Stomatal density (SD) also varied substantially with taxa varied 7.71 fold. Leaf mass per area (LMA), leaf length to width ratio (LL/LW), vein density (VD), and upper and lower epidemical thicknesses (UET and LET) varied moderately with taxa, from 2.0 fold to 4.54 fold. Taxa did not differ significantly in absolute water content (AWC, 1.53 fold) and stomatal length (SL, 1.82).

Table 1: Fifteen leaf functional traits of nine Euphorbiaceae species examined in this study

Leaf functional traits	Codes	Units	Mean (Minimum-Maximum)	Magnitudes
Physiology				
Maximum photosynthetic rate	A_{max}	$\mu mol\ m^{-2}\ s^{-1}$	8.50 (2.01-15.19)	7.57
Stomatal conductance	G_s	$10^2 \mu mol\ m^{-2}\ s^{-1}$	18.34 (2.94-33.62)	11.43
Transpiration rate	T_r	$mmol\ m^{-2}\ s^{-1}$	3.70 (0.58-6.90)	11.96
Absolute water content	AWC	%	74.23 (61.34-93.60)	1.53
Leaf mass per unit area	LMA	$g\ m^{-2}$	65.99 (45.48-90.89)	2.00
Morphology				
Leaf area	LA	cm^2	121.30 (11.15-363.53)	32.60
Leaf length to width ratio	LL/LW		2.09 (0.80-3.61)	4.54
Anatomy				
Stomatal density	SD	$no.\ mm^{-2}$	246.13 (46.57-358.95)	7.71
Stomatal length	SL	μm	29.61 (24.03-43.78)	1.82
Vein density	VD	$mm\ mm^{-2}$	9.58 (3.80-13.85)	3.64
Leaf thickness	LT	μm	368.88 (178.35-1245.31)	6.98
Palisade tissue thickness	PTT	μm	118.16 (39.34-487.39)	12.39
Sponge tissue thickness	STT	μm	208.70 (69.05-733.15)	10.62
Upper epidermal thickness	UET	μm	24.92 (15.38-39.00)	2.54
Lower epidermal thickness	LET	μm	21.30 (13.27-26.89)	2.03

Table 2: Phylogenetic signals (*K*-statistics), phylogenetically independent contrast (PIC) variance *p*-values, and correlations (*r*) of leaf functional traits with principle component analysis (PCA) axes 1 and 2 in nine Euphobiaceae species

Leaf functional traits	Phylogenetic signals		PCA of species values	
	K	*p*	*r* with axis1	*r* with axis 2
Maximum photosynthetic rate	0.28	0.33	**-0.90**[***]	0.36
Stomatal conductance	0.42	0.30	**-0.72**[*]	0.56
Transpiration rate	0.47	0.26	**-0.89**[**]	0.40
Absolute water content	0.45	0.24	0.64	0.65
Leaf mass per unit area	0.38	0.33	**0.75**[*]	-0.25
Leaf area	0.75	0.05	-0.31	**0.86**[**]
Leaf length to width ratio	0.20	0.49	**0.72**[*]	-0.58
Stomatal density	0.38	0.17	**-0.89**[**]	-0.13
Stomatal length	0.32	0.30	**0.82**[**]	0.24
Vein density	0.17	0.47	**-0.83**[**]	0.02
Leaf thickness	0.31	0.29	**0.94**[***]	0.25
Palisade tissue thickness	0.32	0.38	**0.86**[**]	0.46
Sponge tissue thickness	0.33	0.17	**0.93**[***]	0.16
Upper epidermal thickness	0.57	0.17	0.29	0.56
Lower epidermal thickness	0.29	0.52	0.36	**0.84**[**]

Significant correlations are showed in boldface. [*]$p<0.05$; [**]$p<0.01$; [***] $p<0.001$

Although numerous tested leaf functional traits varied strongly across studied species (Fig. 2), the *K* values of the 15 traits were all significantly lower than expected from $K<1$ (Table 2), meaning that these traits did not have significant phylogenetic signals. The result indicating the leaf functional traits of Euphobiaceae species were mainly influenced by environments.

Results of principle component analysis (PCA) showed that the first two axes explained 57.10 and 23.50% of the total variation, respectively (Fig. 3). The first PCA axis was positive with LMA, LL/LW, SL, LT, PTT and STT, but it was statistically negative with A_{max}, G_s, T_r, SD and VD. Leaf area (LA) and LET were both significantly positive with the second axis. The traits located at the first two axes were better able to explain plant adaptations to the environment. On the contrary, AWC and UET were not correlated with either of the first two axes (Table 2), indicating the negligible function of these two traits on plant environmental adaptation.
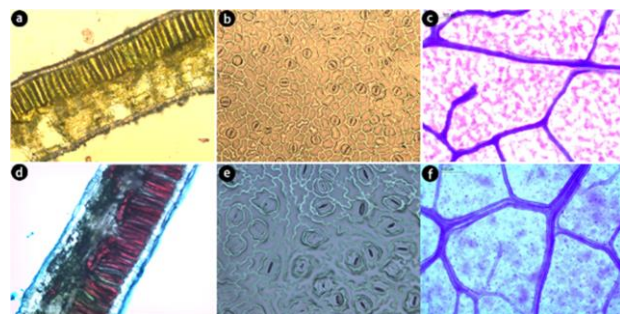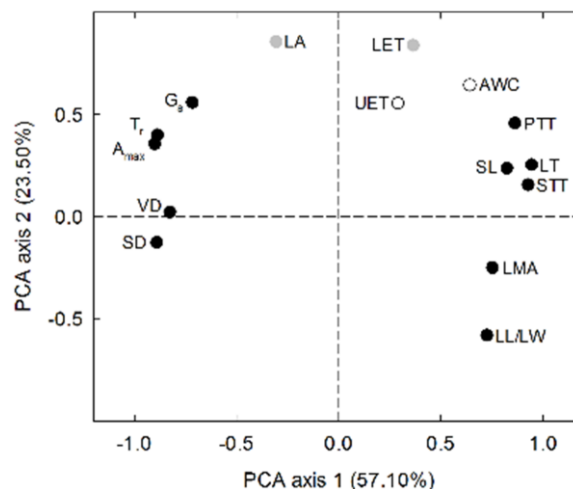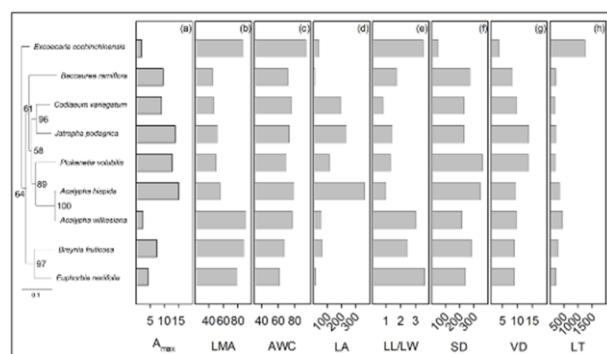
Photosynthetic characteristics including A_{max}, G_s and T_r were positively correlated with each other (Fig. 4), but after phylogeny was considered, A_{max} was only correlated with T_r (Table 3). Both LMA and LL/LW were negatively correlated with A_{max}, G_s, and T_r (Fig. 5, 6 and 7). After phylogeny was considered, these two traits were still correlated with G_s (Table 3). Species means for both A_{max} and T_r were also positively correlated with SD and VD, and they were negatively correlated with SL, LT, and STT (Fig. 5, 6 and 7), but all these correlations disappeared when correcting for phylogeny (Table 3). LMA was positively correlated with LL/LW, STT, UET and LET after correcting for phylogeny, although for their species values, a significant correlation was only observed between LMA and LL/LW, and between LMA and STT (Table 3). Species mean for AWC was significantly correlated with SD, SL, LT, PTT, and STT, but AWC was significantly correlated with VD when correcting for phylogeny (Table 3).

Table 3: Pairwise cross-species and phylogenetically independent contrast (PIC) correlations between the 15 leaf functional traits of nine Euphorbiaceae species

	A_{max}	G_s	T_r	LMA	AWC	LA	LL/LW	SD	SL	VD	LT	PTT	STT	UET	LET
A_{max}		0.21	0.80*	0.02	-0.41	0.15	0.28	0.28	-0.31	0.53	-0.03	0.01	0.08	0.01	0.25
G_s	0.89**		0.70	-0.88**	0.33	0.32	-0.85**	0.22	-0.56	-0.64	-0.44	-0.35	-0.44	-0.46	-0.46
T_r	0.98***	0.93***		-0.55	-0.17	0.21	-0.34	0.49	-0.59	0.07	-0.41	-0.32	-0.32	-0.35	-0.18
LMA	-0.80**	-0.73*	-0.76*		-0.18	0.11	0.90**	-0.39	0.65	0.65	0.70	0.61	0.72*	0.74*	0.75*
AWC	-0.33	-0.08	-0.31	0.09		0.58	-0.40	-0.66	0.47	-0.71*	0.51	0.57	0.43	0.40	0.28
LA	0.53	0.59	0.55	-0.32	0.28		-0.16	-0.38	0.33	-0.17	0.47	0.51	0.50	0.52	0.55
LL/LW	-0.83**	-0.86**	-0.85**	0.85**	-0.04	-0.71*		-0.28	0.51	0.81*	0.56	0.50	0.59	0.55	0.67
SD	0.73*	0.46	0.73*	-0.46	-0.72*	0.23	-0.51		-0.83*	0.22	-0.82*	-0.86**	-0.72*	-0.62	-0.66
SL	-0.72*	-0.54	-0.69*	0.44	0.73*	0.00	0.40	-0.85**		0.16	0.81*	0.85**	0.73*	0.75*	0.81*
VD	0.70*	0.39	0.67*	-0.49	-0.62	0.41	-0.52	0.85**	-0.55		0.11	0.08	0.18	0.26	0.44
LT	-0.72*	-0.51	-0.72*	0.64	0.79*	-0.09	0.54	-0.85**	0.75*	-0.82*		0.98***	0.98***	0.89**	0.88**
PTT	-0.58	-0.33	-0.56	0.42	0.92***	0.06	0.33	-0.87**	0.82**	-0.76*	0.95***		0.94***	0.87**	0.90**
STT	-0.73*	-0.55	-0.72*	0.69*	0.70*	-0.14	0.58	-0.78*	0.68*	-0.83**	0.97***	0.88**		0.86**	0.88**
UET	-0.08	0.04	-0.04	0.46	0.31	0.41	0.09	-0.10	0.16	-0.08	0.42	0.40	0.34		0.90**
LET	-0.06	0.14	0.00	0.25	0.63	0.67*	-0.11	-0.36	0.54	-0.11	0.51	0.64	0.42	0.76*	

Correlation data are given for species-based analyses below the diagonal and for PIC analyses above the diagonal. The sign of the significance for each correlation is indicated as: *$p< 0.05$; **$p<0.01$; ***$p<0.001$

A_{max}, maximum photosynthetic rate; G_s, stomatal conductance; T_r, transpiration rate; LMA, leaf mass per area; AWC, absolute water content; LA, leaf area; LL/LW, leaf length to width ratio; SD, stomatal density; SL, stomatal length; VD, vein density; LT, leaf thickness; PTT, palisade tissue thickness; STT, spongy tissue thickness; UET, upper epidermal thickness; LET, lower epidermal thickness

Fig. 1: Some leaf anatomical pictures of *Breyniafruticosa* (a-c) and *Acalyphawikesiana* (d-f). All pictures were amplified 200fold compared to original tissues

Fig. 2: Values for leaf physiological, morphological, and anatomical characters varying with phylogram in Euphobiaceae species. Numbers associated with nodes are maximum likelihood bootstrap values>50%. A_{max}, maximum photosynthetic rate; LMA, leaf mass per area; AWC, absolute water content; LA, leaf area; LL/LW, leaf length to width ratio; SD, stomatal density; VD, vein density; and LT, leaf thickness

Fig. 3: Principal component analysis (PCA) based on standardized traits values for 15 leaf functional traits of nine Euphorbiaceae species. Abbreviations for the leaf functional traits are defined in Table 1

For species correlations, LA was significantly positive with LET. For contrast correlations, LL/LW was significantly positive with VD. In addition to these two correlations, all the other phenotypic and phylogenetic correlations between leaf morphology and anatomy were insignificant (Table 3), meaning leaf morphology and anatomy were evolutionarily independent. Phenotypically and phylogenetically, leaf anatomical characteristics showed evolutionary coordination. After correcting for phylogeny, SD was negatively correlated, while SL was positively correlated with leaf histological thicknesses, including LT, PTT, STT, UET and LET. The histological tissue thicknesses were also positively correlated with each other.
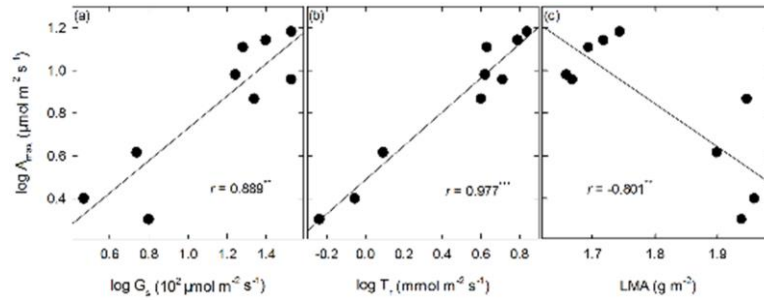
Fig. 4: Stomatal conductance (G_s) and transpiration rate (T_r) are positively correlated with maximum photosynthetic rate (A_{max}), while leaf mass per area (LMA) is negatively correlated with A_{max}. [**]$p<0.01$; [***]$p<0.001$
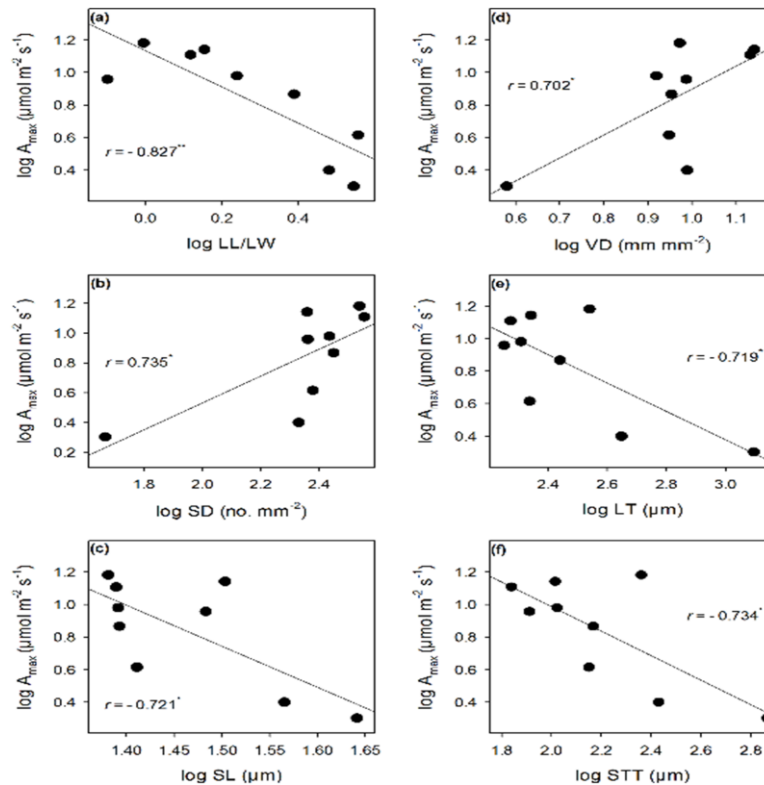

Fig. 5: Both leaf morphological and anatomical traits are statistically correlated with maximum photosynthetic rate (A_{max}).LL/LW, leaf length to width ratio; SD, stomatal density; SL, stomatal length; VD, vein density; LT, leaf thickness (LT); STT, spongy tissue thickness. [*]$p<0.05$; [**]$p<0.01$

SD was positively correlated with VD for species values, but these traits were found to be independent after including phylogenetic effects (Table 3).

Discussion

We used a comparative phylogenetic approach to examine the evolutionary styles, or phylogenetic signals, of leaf functional traits and their correlated evolutions across nine Euphorbiaceae species in a common garden. Contrary to hypothesis 1, the present study showed all detected leaf functional traits exhibited significant adaptive features, indicating that the traits were weakly driven during phylogeny, and that variations of these traits likely played important roles for plant adapting to changing environments. Species values of leaf morphological and anatomical traits were both significantly correlated with leaf physiological attributes, and these results support our second hypothesis reflecting the adaptive stratagems of Euphorbiaceae species to varying environments. Moreover, supporting our third hypothesis, leaf anatomical characteristics were correlated with each other, meaning histological coordination exists between these traits.
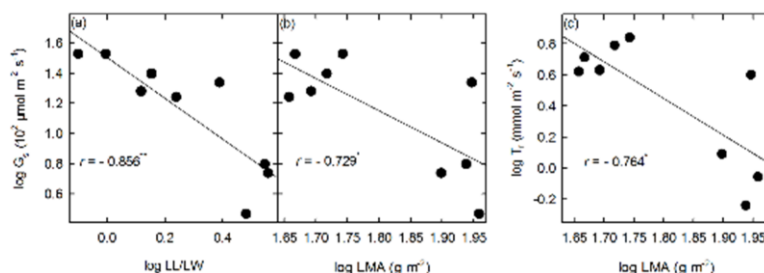
Fig. 6: Leaf length to width ratio (LL/LW) is negatively correlated with stomatal conductance (G_s), while leaf mass per area (LMA) is negatively correlated with both G_s and transpiration rate (T_r). $^*p<0.05$; $^{**}p<0.01$
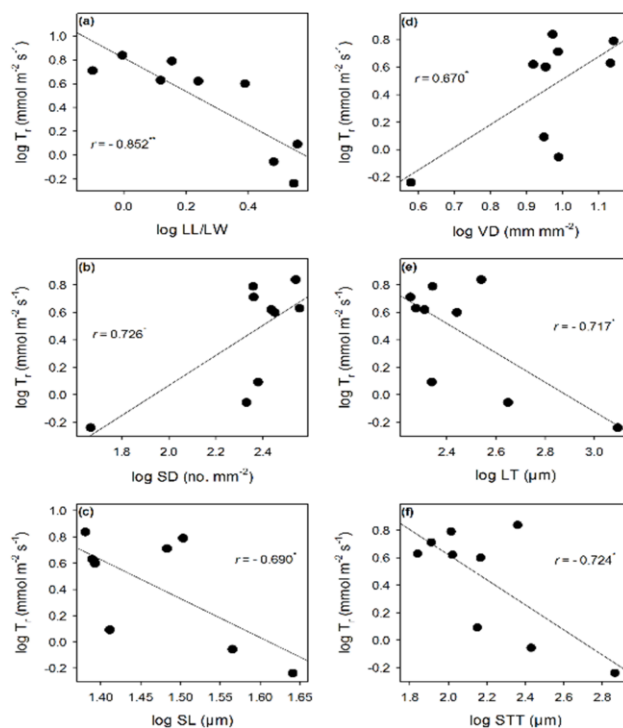

Fig. 7: Leaf morphological and anatomical traits are significantly correlated with the transpiration rate (T_r). LL/LW, Leaf length to width ratio; SD, stomatal density; SL, stomatal length; VD, vein density; LT, leaf thickness; STT, spongy tissue thickness. $^*p<0.05$; $^{**}p<0.01$

Most of the studied leaf functional traits varied strongly across Euphorbiaceae species (Table 1 and Fig. 2), indicating that these traits had obviously adaptive plasticity (Dunbar-Co *et al.*, 2009), which could potentially facilitate the high adaptive ability of Euphorbiaceae that has allowed for a nearly global distribution. In the studied leaf traits, leaf photosynthetic attributes, leaf mass per area, stomatal traits, vein density, and leaf tissue thicknesses are likely the main leaf functional traits supporting plant adaptations. The phylogenetic signals were weak for all of the examined traits (Table 2), possibly because of the traits was not correlated with phylogeny. Phylogenetic changes are slow perpetuating evolutionary processes, so they do not have effect rather associations with the traits. These results also reflected the selection in heterogeneous environments, allowing species to adapt to their growing conditions (Dunbar-Co *et al.*, 2009; Zhang *et al.*, 2014).

The trait-trait correlations implied a co-variation for integrated plant function, such as linkages of venation, stomata, and tissue thickness, supporting our results of phylogenetically independent contrasts. Our results showed coordinated diversification in leaf morphology, anatomy, and photosynthetic functional traits in an adaptation similar to previous studies of phylogenetically distantly related species (Reich, 2014). Trait associations may because of traits share a genetic, developmental or allometric basis, and may also be functionally coordinated if the traits share a common function (Sack *et al.*, 2003). Alternatively, the traits may be co-selected in a certain environment but be independent (Dunbar-Co *et al.*, 2009).

Leaf morphological shifts are closely related to light capturing and photosynthesis (Givnish, 1987). However, leaf area was independent with physiological traits in this study, but leaf length to width ratio was significantly negatively correlated with A_{max}, G_s, and T_r, and the leaf length to width ratio was also negatively correlated with G_s after correcting for phylogeny (Table 3 and Fig. 5, 6 and 7). Large and narrow leaves can accommodate increased self-shading and reduced maximize light capture, and they also correspond with low transpiration loss and gaseous exchange (Takenaka *et al.*, 2001). On the other hand, small and short leaves increase the thickness of leaf boundary layer, so that the physical convective boundary-layer conductance for heat and gaseous exchange from small leaves are more rapid than from large leaves (Sack *et al.*, 2003; Niinemets *et al.*, 2007; Nicotra *et al.*, 2011). According to the adaptive theories, the relationships between leaf area and physiological traits indicate that leaf area regulated the microenvironment within a leaf, such that leaf temperature and enzymatic activity act indirectly to steady leaf photosynthetic activity in species of family Euphorbiaceae. However, leaf shape (LL/LW) also can influence leaf transpiration and gaseous exchange (G_s) directly besides regulating leaf microenvironments.

Vein and stomata are two main channels determining leaf water supply and water loss (Fiorin *et al.*, 2016). In this study, denser leaf vein (high VD) can increase contact with mesophyll cells, reduce the hydraulic transmission path between the cells and transpiration sites (stomata), and consequently increase leaf hydraulic transport and photosynthetic rate (Sack and Frole, 2006). High SD and small SL will induce increased and quicker exchanges of water and CO_2, supporting a high photosynthetic carbon assimilation (Hetherington and Woodward, 2003; Franks and Beerling, 2009). The positive correlation between SD and VD for species values (Table 3) indicating a covariance of these traits, since both of the traits associated with hydraulic dynamics and photosynthesis (Brodribb and Jordan, 2011). The homeostatic balance between water supply and demand can be achieved by a close coupling of these two traits (Brodribb and Jordan, 2011). However, after phylogenetic correction, this correlation disappeared (Table 3), indicating that the correlation were not related to phylogeny, rather vein and stomata were biologically informative, supporting previous work on how adaptive radiation proceeds in Euphorbiaceae species (Price, 1997).

Plants may use other strategies (e.g. increasing leaf thickness to storage more water and prevent water loss) besides vein- and stomatal-based regulation when they face stress (Sun *et al.*, 2014; Zhang *et al.*, 2014). Thicker leaf and corresponding thicker mesophyll tissue increase the distance for water and CO_2 transport between xylem and stomata; therefore, high LT, PTT, and STT values may be negatively correlated with photosynthetic and transpiration rate (Brodribb *et al.*, 2007; Brodribb and Jordan, 2011). Both SD and SL were correlated with leaf tissue and cuticle thicknesses after considering phylogeny (Table 3). This confirmed that the correlations between stomatal traits and leaf tissue are related to phylogeny, suggesting functional associations among the traits (Dunbar-Co *et al.*, 2009; Brodribb *et al.*, 2013). Thick leaves with high leaf mass per area (LMA) suggest a longer leaf life span, as well as adaptation to resist low water and low soil nutrient environments related to high water use efficiency and high water retention (Cunningham *et al.*, 1999; Dunbar-Co *et al.*, 2009). A higher water use efficiency is consistent with a more conservative stomatal behavior or a higher mesophyll resistance (Niinemets and Sack, 2006). As it is well known that stomatal pores control the exchange of water and CO_2 between leaf and atmosphere, they act as the main channel for water loss from plants (Hack *et al.*, 2006). Plants increase water retention and/or storage by thicker lamina, and they adjust stomatal density and size to reduce water loss. The strong linkages among tissue thickness and stomatal traits provides strong evidence that plants coordinate these two groups of traits to achieve a balance between liquid and gas phase water conductance.

Conclusion

Evolutionary styles of leaf functional traits, including leaf morphology, anatomy, and physiological functions were weakly associated with phylogeny, and variations of these traits were mainly driven by environment. The effect of leaf shape on photosynthesis showed phylogenetic conservatism, since their species correlation and contrast correlation were both significant. Leaf anatomical characteristics were significantly informative in influencing photosynthesis to drive plant adaptation, because of their species correlations and contrasts correlations were different. Phylogenetic correlations also existed among leaf anatomical traits, especially among stomata, venation, and tissue thickness, reflecting their functional coordination. These results reflect the adaptive mechanisms of Euphorbiaceae species to diverse environments, which are likely drivers behind the worldwide distributions of this family.

Acknowledgements

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