Leaf water relations in epiphytic ferns are driven by avoidance rather than tolerance mechanisms

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Running Head: Terrestrial and epiphtic fern functional traits

# Abstract

Key Words:

# Introduction

* Epiphytism, ferns and evoln. history

Within forests, epiphytes grow at many levels. Some are restricted to the dark understory, whereas others grow on the exposed twigs of emergent tree (Watkeins 2012)

* microhabitat differences
* Consequences of radiation of plants into canopy, seed vs spore
* Evolution of traits With tropical ferns, there is a fundamental evolutionary clade where one clade of ferns (Euploypoid I) had predomintaley radiated into epiphytic nihces, while a sister clade (Eupolypoid II) has mostley diversified on the forest floor (Watkings 2012). Previous works by Zhang shows how these traits influence gas exchange however, what lead to these initial success is unclear
* water relations and stomata

Terrestrial ferns may have reduced water use efficiency, which would be consistent with Brodribb and McAdam’s hypothesis of inefficient stomatal control.

if stomata are regulated by leaf water status, what traits realted to water relations allows ferns to succeed.

* this study seeks… looking within E1 and E2 Additionally, a hemi-epiphyitc life form co-exists with both these groups which may represent an evolutionary transition between terrestrial and epipythic life histories.

# Methods

## Study Site and Species Selection

The sites used for this study included two Costa Rican wet tropical forest locations at La Selva Biological Research Station in Heredia (84⁰00’12W, 10⁰25’52N) and Las Cruce Research Station in San Vito (8° 47′ 7” N, 82° 57′ 32” W). The La Selva site is a low elevation (ca 50 m) tropical forest, with a moderate dry season. The Las Cruces site is a premontane tropical forest located at a higher elvation (ca 1200 m). Both sites receive approximately 4m of annual rainfall (Holdridge, 1967, p. @gentry\_four\_1993).

A survey of morphological, stoichiometric, anatomical and leaf water relations parameters were conducted for six individuals from 39 fern species across three fundamentally distinct life froms (Table 1). Across both sites, 18 terrestrial, 15 epiphytic and 6 hemi-epiphytic species were collected and meausured. In this study, terrestrial life froms were all collected from shaded closed canopy understories in the forest floor. Epiphyitc life forms were sampled from trunks or within tree canopies,depending on the species. Epiphytic species were collected from canopy trees using single-rope climbing techniques. Hemi-epiphytic species were all collected along lower sections of trees trunks (1-3 m). Importantly, all sampled hemi-epiphtic species are known to have root connections to forest floor soils at some point in their life history. Individuals of species were collected across multiple populations but within similar microhabitat conditions. All sampled fern species were restricted to the eupolypod I and II clades. Vouchers for each species were deposited at the sites of collection at either the La Selva (LSCR) or Las Cruces (LCCR) herbariums.

## Plant Material

Two complete fronds from sampled individuals were field collected in the early morning (6-7:00 am). Stipes were cut at the base of the rhizome and cut ends were wrapped in wet paper towels and transported to the lab in black plastic bags. Stipes were re-cut under water adn rehydrated until time of hydaulic measurement (1-6 hours). Due to the difficulty in sampling some canopy species; whole epiphytic individuals were removed, maintained overnight in well-watered conditions in an abient air laboratory and sampled the following day. One frond from each individual was utlized for presssure volume curves, while the other was sampled for strucutral morphology, lamina stoichmetry and anatomical traits. ## Leaf Morphometric traits

Stipe length (cm) and lamina length (cm) were calculated from one sampled frond per indiviudal. Total lamina area for each frond was measured with a Li-3100 leaf area meter (LiCor Biosciences, ). Leaf mass per unit area (LMA, g cm-2) was calculated using the tissue punch method. For each individual, ten lamina punches (5 mm 2) were dried to a constant mass and LMA was calculated as the total dry mass divided by the total area of all leaf punches.

## Anatomical traits

Total **xylem vascular** area was determined for stipe segments from hand cross-sections. Each cross section was stained with toulune blue….for. Images under 10x were … Total vascular area was determined by measuring the area of all vascular bundles within a stipe cross section. Images were analyzed using Image J software (National Institutes of Health, Bethesda Maryland, USA).

Stomatal density (SD) was measured by directly counting stomata on the abaxial leaf surface under 40x magnification. Three leaf punches (4 mm2 diameter) were sampled across random locations on different pinnae from each individual. The number of stomata in each field of fiew were counted in three random regions on each of three leaf punches. The stomatal density for each indivudal is presented as the mean SD across all 9 sampled regions

Indiviudal images of stomata were directly photographed under 40x magnification (AmScope FMA050) across all three leaf punches per individual. Stomatal length and width of both guard cells were calculated for 9 stomata for each individual using Image J. Stomatal size (SS) was calculated as guard cell length multipled by the combined width of each guard cell pair, as in Franks & Beerling (2009).

## Foliar chemistry

Subsamples of foliar tissue used for lamina area calculation were samples across muliple pinnae for each individual. These subsamples were dired to a constant mass and ground using a Wig-L-Bug (Sigma-Aldrich Co. St. Louis, USA). Nitrogen content and deltaC13 were measured using a Delta V isotope ratio mass spectrometer interfaced to a NC2500 elemental analyzer (Thermo Scientific) and corrected by comparison with certified standards.

## Pressure volume curves

We used a modified pressure–volume technique of Tyree and Hammel (1972) to estimate the following tissue-water relations: osmotic potential at full tissue hydration ( Ψ π , sat ), osmotic potential at the turgor loss point of tissues ( Ψ π , tlp ), bulk modulus of tissue elasticity ( ε ), and tissue-water capacitance ( C ). This method has been fully described by Davis and Mooney (1986).

For each pressure-volume curve we sampled top most intact pinnae for each fully hydrated frond. We generated pressure–volume curves by taking sequential water potential measurements ( Ψ ) as fronds air dried first in closed plastic bages (0-2 hrs) and then on a bench top. The fresh mass was recored immediately before and after each Ψ determination. Following each PV curve, samples were dried to a constant mass to calculate relative water content (RWC).

Lawren Sack’s = something here To determine Ψπ, tlp , a plot of 1/ Ψ vs. RWC was generated, and the point at which the graph linearized was taken as the Ψπ, tlp ( Davis and Mooney, 1986 ). Ψπ, sat was determined by extending the regression line to the point where x = 0 (RWC =100). ε was determined from the slope m of the line of Ψpvs. RWC and the RWC at the turgor loss point (RWC°), such that ε= m RWC ° , and C was determined as the slope of RWC vs. Ψ ( Table 2 ).

## Statistical analysis

Linear mixed-effect models were used to test responses of functional traits to categorical fixed effects of life form and site. The interaction between life form and site was tested to confirm any potential environmental or climate infunce on trait patterns. Generally, there were few life form x site interactions, so models with life form and site as main effects were compared to full models (AIC scores). Depending on the AIC scores, the most parsimonious model was selected. To test for broad differences among life forms, individual plant species were treated as random effects in each model. Tukey’s post-hoc test were performed in conjunction with ANOVA to determine which mean values of functional traits were different among fixed effect treatments with the ‘multcomp’ package (**???**). We utlized a type 3 ANOVA due to an unbalanced design with the limited number of hemi-epiphytes species available. When interactions were present, we computed pairwise comparisons with the ‘emmeans’ package (**???**) to investigate interactions between life form and site.

**A phylogenetic tree for these 30 fern species was constructed based on chloroplast rbcL sequences obtained from the GenBank website (**[**http://www.ncbi.nlm.nih.gov/genbank/**](http://www.ncbi.nlm.nih.gov/genbank/)**). Phylogenetic analyses for each matrix were carried out using the maximum likelihood method in PAUP\* v.4.0b10 [44]. Schneider et al. (2004) has integrated Colysis and major components of Microsorum into Leptochilus by using nucleotide sequences derived from three plastid loci [45]. For simplicity, the old Latin names of species in Colysis and Microsorum were used in the present study. The phylogenetic signal (K-statistic) for each trait was calculated using ‘picante’ based on the R package. Such K-statistics can express the conservatism of traits. Cases where the K-value is ,1 indicate convergent traits while K.1 represents that traits are more conserved than would be presumed from a Brownian expectation [47]. Relationships among variables were evaluated by both pair-wise Pearson correlations in the R package and a phylogenetically independent contrast (PIC). Possible evolutionary associations were assessed via PIC analysis, utilizing the molecular phylogenetic tree. This PIC analysis was examined with the ‘‘analysis of traits’’ module in Phylocom, which calculates the internal node values for continuous traits [48].**

For bivariate trait relationships, responses of dependent variables were analysed with linear mixed-effect models, with species as a random effect and plant group and/or canopy type as categorical fixed effects. Explained variance (R2) of mixed models were computed as in (**???**), in which the marginal R2 represents variance explained by fixed factors and the conditional R2 by both fixed and random factors. Tests of allometric relationships between log-transformed biomass components were implemented using standardized major axis regression in the ‘smatr’ package in R (**???**). Principal component analysis, utilizing the ‘vegan’ package (**???**), was used to explore how measured functional traits were distributed and co-varied among species (mean values) and life form. All tests of statistical significance were conducted at an level of 0.05. All analyses were performed with R 3.5.1 (**???**).

#Results

## Frond Morphology

Allomteric relationships between lamina area and total frond length had similar slopes across all three life forms (Figure 1a). This broad convergence in frond allometry suggests that tropical ferns build leaf structures in predicatble proportions, despite variation in frond structural traits. Total frond length was reduced by 26 % in epiphytic (59$$3.3) species, with frond length of hemi-epiphytes an intermediate between both groups (*P* = 0.021). The reduction in total frond length was driven by a large reduction (-54 %) of stipe length in epiphytic compared to terrestrial species (*P* = 0.001, Figure 1b). Leaf mass per unit area was 67% higher in epiphytic compared to terrestrial species, with hemi-epiphytic species intermediates between both life forms (*P* = 0.002, Figure 1C). Do differences were detected in total lamina area between any of the life form groups, due to the large amount of variation in lamina area across all species (R2 marginal = 0.17 & R2 conditional = 0.89).

## Anatomical traits (xylem and stomata)

Epiphytic and hemi-epiphytic species had 51% lower stomatal density (36$$3.1, *P* < 0.001).

\*\*no interaction with stomatal size, however, if only main effect model then

## Foliar Chemistry

Foliar nitrogen content was 29.8% lower in epiphytic ferns compared to terrestrial and hemi-epiphytic ferns (*P* = 0.007).

Foliar 13C for terrestrial and hemi-epiphytic species were more negative that epiphytic species (*P* = 0.004, Figure 2x). Additionally, foliar13C for fern species at the higher elevation Las Cruces site were less negative (-32.6 &permil) than fern species at the low elevation La Selva site (34.0 &permil,*P* = 0.015).

*chlorp hyll*? I had to perform a little data mining to get this result. I dropped one epiphytic species with way to variable data (400-800 mg m-2) and a few outliers. Not sure how meaningful these differences are.

## Foliar Hydraulic Traits

The turgor loss point (*tlp*)

## bivariate traits relationships:

leaf area with xylem lma and nitrogen density vs size

# Discussion

(Brodribb 2017): A passive linkage between leaf water status and guard cell turgor observed in extant basal vascular plants appears to be sufficient to prevent xylem cavitation during diurnal changes in evaporative demand (Martins et al., 2015). However, without more sophisticated mechanisms to reduce guard cell turgor and produce complete stomatal closure, it has been hypothesized that passive closure does not provide a sufficiently tight stomatal seal capable of preventing ferns and lycophytes from rapidly reaching critical leaf water potentials when soil water is depleted during drought (McAdam and Brodribb, 2013). As a result, ferns and lycophytes in dry environments rely on either a high plant capacitance or low stomatal density (McAdam and Brodribb, 2013), desiccation tolerance (Hietz, 2010), and in some cases, rather cavitation-resistant xylem (Baer et al., 2016).  
We foudn that cavaitaion-resistant xylem where not the case here (see review too)

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