Distribution of leaf dissection and sclerophylly along microhabitat gradients in pteridophytes of the Cape Peninsula

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Dissertation presented in partial fulfilment of the requirements for the degree of

Bachelor of Science (Honours) in the Department of Botany

University of Cape Town

November 2010

Abstract

Plant species with small leaves and sclerophylls have been reported to occur mainly on dry, low-nutrient soils in situations of high insolation. However, a number of physiological functions have been proposed for the two sets of traits. Ferns are well-suited to the study of leaf structure and its impact in different environments, as they show remarkable variation in leaf dissectedness and sclerophylly, and are able to inhabit a variety of marginal habitats.

In this study, ecological and leaf trait data were collected for 17 fern species occurring in Skeleton Gorge, Table Mountain, in the Western Cape. Correlations between the traits and regressions of leaf traits on potential environmental determinants was carried out using both species averages and phylogenetically independent contrasts. The habitat and leaf traits were also subjected to a test of evolutionary trait conservatism.

Sclerophyllous plants were found to be have thicker leaves, containing less chlorophyll, but sclerophylly was poorly correlated with leaf dissection. Plants occurring in high-light environments tended to be more sclerophyllous and have more dissected leaves, although these environments also were nutrient-poor.

Leaf dissection appears to be primarily a means of dissipating heat by convection, rather than evaporative cooling, but it may also improve nutrient acquisition in low-nutrient soils. Sclerophylly in these ferns was not directly associated with nutrients; instead low sclerophylly seems to be favoured in low-light environments, perhaps because of lower metabolic costs or to reduce self-shading.

ture stress (Schimper, 1903) and to reduce the loss of nutrients associated with leaf senescence (Orians & Solbrig, 1977; Reich et al., 1997; Wright et al., 2002; Wright et al., 2004). Thus, sclerophylly may offer distinct benefits in environments that are arid and/or nutrient-deficient.

In shaded environments such as forest understoreys, the availability of light energy is one of the primary limitations on growth (Finzi & Canham, 2000), and plants occurring in low-light environments have well-known responses in their leaf properties: decreased LMA (Lusk & Warton, 2007), increased chlorophyll concentration, and lower *chl a:b* ratios (Saldaña et al., 2010). Lusk et al. (2008) noted that, although low LMA is favoured as it reduces leaf construction and maintenance costs under conditions of low carbon assimilation as well as reducing self-shading, shade-tolerant evergreens tend to have higher leaf longevity in order to retain the acquired resources, which entails having higher LMA to resist physical and herbivore damage. Understorey species also compensate for the lower insolation by producing more chlorophyll, allowing them to maximize their photosynthetic potential (Curran et al., 1990; Saldaña et al., 2010).

The pteridophytes of the Cape Peninsula display remarkable variation in leaf form, where the dimensions of individual leaflets range from about 5 × 1 mm in *Hymenophyllum peltatum* to about 80 × 15 mm in *Blechnum capense* (Roux, 1979). Frond shape is also highly variable, ranging from the simple, entire fronds of epilithic and epiphytic *Elaphoglossum* spp. and *Pleopeltis macrocarpa* to the highly dissected, quadripinnate fronds of *Hypolepis sparsisora* (Roux, 1979). In addition, fronds in some taxa are tough and hard (e.g. *Blechnum attenuatum* var. *giganteum, Rumohra adiantiformis*), while others are thin and delicate (e.g. *Hymenophyllum* spp. and *Adiantum capillus-veneris;* pers. obs.).

Ferns occur in a wide variety of habitats, including rainforests, temperate forests, grasslands, and semi-deserts. However, they mainly dominate forest understoreys as these environments are moist enough for reproduction and the light availability is low, reducing competition from angiosperm species (Page, 2002). A number of species (e.g. *Pteridium aquilinum* and *Cheilanthes* spp.) nevertheless thrive in open fynbos vegetation, which is characterized by low soil water and nutrient content and high solar radiation.

Thus, the pteridophytes were chosen as a study group to investigate the significance of leaf dissection and sclerophylly in determining the environments in which different species can survive. We gathered data on the microhabitats and leaf traits of fern species found along a longitudinal transect on Table Mountain, Western Cape, in order to carry out a correlative analysis to identify which environmental factors were associated with leaf dissection and with sclerophylly.

Species with more dissected leaves were expected to occur in conditions where solar radiation and evaporative demand were higher and where soil nutrient and water status were lower. Sclerophylly was expected to show the same trends. However, maximum stomatal conductance was hypothesized to be lower on dry soils, as this would allow plants to react more rapidly to changes in moisture availability and prevent dessication. In addition to reduced LMA, shade-tolerant species were expected to contain more chlorophyll and proportionally more *chl b* than light-tolerant species.

Methods

Study site

This study was carried out in Skeleton Gorge, Table Mountain National Park, South Africa. To determine the fern species composition and the distributions and abundances of species in Skeleton Gorge, an initial survey was undertaken along a 20-m-wide transect, divided into 21 verticals bands of 50 m. The lower limit of the study area was the Contour Path, while the two highest sites were in the fynbos vegetation above the forest at the top of the Gorge. The relative abundance of all fern species found in each band were recorded, so that samples could be chosen that represented the full distribution range of each species in the study area. Lithophytic species were excluded from the study as their substrates did not permit nutrient or soil moisture measurement. Finally, 17 species were selected for the study, and at least three individuals per species were sampled when possible.

Habitat characteristics

The leaf area index (*LAI*) of the forest canopy was measured with an LAI2000 Plant Canopy Analyzer (LI-COR, Lincoln, Nebraska) as a measure of the amount of light available to the plants (with high values indicating low light). This method involves measuring the amount of incident light above and below the canopy and calculating how much penetrates the leaf cover. For the "above" values, readings were taken in the fynbos area away from any obstructing trees or boulders, while "below" readings were taken at each plant.

Temperature (*T*) was recorded over a 24-hour period at thirteen locations throughout the study site using DS-1921 Thermochron iButtons (Maxim Integrated Products, Inc., Sunnyvale, California). Relative humidity (*RH*) was recorded at four locations over the same period with DS-1923 Hygrochron iButtons. The iButtons were kept in the same environment for 24 h before and after the measurement period, allowing them to be calibrated against each other post hoc.

Vapour pressure deficit (VPD) was calculated from T and RH using the following equation (Buck, 1981):

$$VPD = 0.61121 \times e\text{^}[(18.678 - T) / 234.5 \times T / (257.14 + T)] \times (1 - RH) \qquad (1)$$

where VPD is in kPa, T is in °C, and RH is in %. T and VPD were then averaged over the 24-h period for each location and interpolated for sites where measurements were not taken.

Anemometer readings are instantaneous and can vary widely from one minute to the next, so to obtain a measure of wind speed over an entire day, evaporative water loss was measured by placing a petri dish (Ø 88 mm) containing 40 ml of water at each plant for the same 24-hour period as above. An aluminium foil cover was fixed over the petri dish to avert direct sunlight and keep out debris. The water was collected the next day and weighed to determine the water loss gravimetrically. This was converted to "A" pan evaporation by scaling water loss by the ratio of the evaporative surface areas (i.e. $D_1^2/D_2^2 = 1207^2/88^2$). The wind speed at each plant was then calculated by rearranging the FAO Penman–Monteith equation for evaporation (Allen et al., 1998) after eliminating the Radiation term (which was nil thanks to the foil covers):

$$W = E \times (\Delta / \gamma + 1) / (900 \times VPD / T - 0.34 \times E) \qquad (2)$$

where W is wind speed in $m.s^{-1}$, E is "A" pan evaporation in $mm.day^{-1}$, Δ is the slope of the vapour pressure curve in $kPa.°C^{-1}$, and γ is the psychrometric constant in $kPa.°C^{-1}$. Δ depends on T and was determined separately for each plant by looking up the value corresponding to its T in Annex 2 Table 2.4 of Allen et al. (1998). γ varies with altitude; appropriate values were found in Table 2.2 of the same text. Altitudes were derived from contour lines on the map *Approved Paths on Table Mountain* (The Mountain Club of South Africa).

Soil samples were collected at each plant. They were weighed, dried at 70°C for 48 h, and re-weighed to determine their moisture content. They were then analysed at Bemlab (Stellenbosch, South Africa) for total N and total P concentrations, as well as the T-value, a composite of the concentrations of the cations Na^+, K^+, Mg^{2+}, and Ca^{2+} in the soils. Soil pH was determined by shaking 2 g material in 20 ml 1-M KCl at 180 rpm for 60 min, centrifuging at 10 000 *g* for 10 min and measuring the supernatant pH. Nitrogen was determined by combustion using a FP-528 Nitrogen Analyzer. Phosphorus was extracted from 6.6 g soil in Bray II solution (Bray & Kurtz, 1945) before filtering and analyzing using ICP-AES (Varian Vista MPX). Exchangeable cations were displaced from 10 g of sample with 25 ml of 0.2-M ammonium acetate. The samples were filtered through Whatman No. 2 and made to 200 ml and K^+, Na^+, Ca^{2+}, and Mg^{2+} measured using ICP-AES analysis.

Leaf traits

Plant height was recorded in the field. Stipe diameter and leaf thickness were measured using a Waldo digital caliper. An entire frond from three individuals of each species was collected and photographed on a white background with a 1-cm scale bar. The area (*A*) and perimeter (*P*) of the leaf were determined using the image analysis software IMAGEJ v. 1.4.3 (Rasband, 1997). In this study, the ratio *P:A* was used as a measure of leaf dissection.

A subsample of each leaf was photographed and its area determined as above. It was then dried at 80 °C for 48 h and weighed. From the area and the dry weight, the leaf mass-to-area ratio (*LMA*) was calculated as a measure of sclerophylly. Leaf area was also scaled to stem area (*LASA*) to allow comparison of plants of different sizes.

Clear nail varnish was applied to the lower surface of each leaf sample. (No stomata were found on the upper surface of the leaves of any species.) The varnish was peeled off and examined at 400× magnification under a Galen III light microscope (Cambridge Instruments, UK): the number of stomata in three fields of view were counted and the stoma density (*SD*) was calculated by dividing the average count by the area of the field of view (Ø 0.464 mm). The dimensions of the stomata were also measured using the graticulated eyepiece and the area (*SA*) calculated (approximated to that of a perfect ellipse). The stomatal conductance to water vapour ($g_{w\,max}$) was then calculated using the formula of Franks & Beerling (2009):

$$g_{w\,max} = d/v \times SD \times SA \,/\, (l + \pi/2 \times \sqrt{(SA/\pi)}) \tag{3}$$

where d is the diffusivity of water vapour in air, taken to be 0.26 $cm^2.s^{-1}$ (Montgomery, 1947); v is the molar volume of water vapour = 24.465 × 10^{-3} $m^3.mol^{-1}$ at 25 °C and 1 atm from the Ideal Gas Law; and l is the stoma depth, estimated as the width of one of the guard cells (Franks & Beerling, 2009).

Chlorophyll was extracted from each leaf sample using dimethyl sulphoxide (DMSO), according to the method of Hiscox & Israelstam (1979). Samples were incubated for about 10 h to ensure that all chlorophyll had been extracted. The concentration of chlorophyll in the leaf tissue and the ratio of chlorophyll *a* to *b* was determined using a Multiskan Spectrum microplate spectrophotometer (Thermo Electron Corp., Vantaa, Finland) and taking absorbance readings at 646, 663, and 710 nm against DMSO blanks. Concentrations of chl *a* and *b* were calculated based on the measurements.

Statistical analysis

Correlation matrices were constructed for the soil nutrient variables, the environmental variables, and the leaf traits to assess covariation among the parameters. Since all nutrient variables were highly correlated, especially with Total Phosphorus, this variable was chosen to represent nutrient content, so as to reduce dimensionality and collinearity in the predictors.

The two species occurring only in the fynbos had extreme and highly influential values compared to the rest of the data points. Analyses were thus performed for all species, and with these two species removed. In order to make the data more symmetrical and reduce the influence of extreme values, *LMA* and $g_{w\,max}$ were log-transformed prior to analysis. Regressions of *P:A*, *LMA*, $g_{w\,max}$, and chlorophyll concentration were performed on each predictor variable hypothesized to be influential. Due to the small sample size and the instability of parameter estimates due to collinearity, only simple regressions were performed and interactions between predictors were not estimated.

To account for phylogenetic trait covariance, regressions were also performed on phylogenetically independent contrasts (PICs; Garland et al., 1992), as implemented in the package APE in R v. 2.11.1 (R Development Core Team, 2010; Paradis et al., 2004). Contrasts were scaled to branch length, which led to one extremely high data point for all variables, as *B. australe* and *B. punctulatum* were separated by a negligible genetic distance. This point was thus removed prior to regression as it obscured the pattern in the rest of the data.

The phylogeny required to calculate the PICs was obtained by performing a Bayesian reconstruction in BEAST v. 1.5.4 (Drummond & Rambaut, 2007), using DNA sequences of each species for the *rbcL* and *trnL–F* gene regions, downloaded from GenBank. As no DNA data were available for any species of *Cheilanthes,* this taxon was not included in the tree or the PICs. Other taxa for which DNA data were unavailable were substituted for with another member of the same genus. The *Blechnum* clade was the only case in which more than one species was present in the data set and DNA data were unavailable. The closest relatives of each species were inferred based on their chromosome number, which has been used extensively as a character for reconstructing relationships in the ferns (Löve et al., 1977). For the phylogeny reconstruction, *B. occidentale* ($2n$ = 124) was substituted for *B. australe* ($2n$ = 124; Löve et al., 1977), and *B. cartilagineum* ($2n$ = 64) was substituted for *B. attenuatum* var. *giganteum* (2n = 128; Roux, 2009).

Model selection was performed using MRMODELTEST v. 2 (Nylander, 2004) and GTR+I+Γ was selected for *rbcL* and GTR+Γ for *trnL–F.* They were concatenated but parameter estimates were unlinked in the MCMC procedure. The prior probability distribution for the tree height was set arbitrarily to normal with a mean of 100 and s.d. of 0.1 in BEAUTI v. 1.5.4. The birth-death speciation model was used with the uncorrelated log-normal rate model (Drummond et al., 2006). The analysis was run for 5 million generations, saving the parameter estimates every 500 generations. TRACER v. 1.5 (Rambaut & Drummond, 2007) was used to calculate the effective sample size of each parameter; these were all near or above 200, indicating that the

MCMC algorithm had been run long enough. Burn-in was assessed by inspecting the trace for each run; all runs were deemed to have reached stationarity after 250,000 generations. The tree with the highest total clade support was annotated with the medians of the post-burn-in node height estimates in TREEANNOTATOR v. 1.4.8.

Conservatism of both leaf traits and habitat characteristics was assessed using Blomberg et al. (2003)'s test for phylogenetic signal. The test is based on permuting the trait values across the phylogeny to create a null distribution of "Brownian motion" evolution and then calculating the statistic K, where values of K greater than 1 imply that close relatives are more similar than expected (i.e. the trait is conserved), while values less than 1 indicate trait dispersion. The K value and its probability were calculated for each variable using the package PICANTE in R, with the number of permutations set to 1000.

Results

Trait correlations

The soil nutrient variables were all highly correlated with each other (Fig. 1), with less acidic soils having higher concentrations of all nutrients. This justifies the use of Total Phosphorus as a representative of overall soil fertility.

LAI was positively correlated with VPD and Total P (Fig. 2), indicating that plants under denser canopies inhabit richer soils and face greater evaporative demand. These sites are also cooler and less windy.

Sclerophyllous plants were less leafy and had thicker leaves (Fig. 3), although LMA was poorly correlated with leaf dissection. Sclerophyllous leaves also contained less chlorophyll. Stomatal conductance was not correlated with any other leaf traits.

Regressions

Leaf dissection was positively related to soil fertility when phylogenetic covariance was ignored. However, the regression on PICs reversed the relationship and showed that LAI was, in fact, a significant predictor of leaf dissection, with more dissected leaves occurring in higher-light environments (Fig. 4, Table 1, 2).

LMA was not significantly predicted by any sampled variable using tip values, but the PICs showed a negative relationship with LAI: Plants with less sclerophyllous leaves were found in low-light environments.

Maximum stomatal conductance showed a weak relationship with VPD, but this relationship was not significant when taking phylogeny into account.

No effect of light intensity on chlorophyll concentration, or on the ratio of chlorophyll *a* to *b* was detected.

Trait conservatism

Leaf dissection was the only leaf trait that showed significant phylogenetic signal: the *K* value less than 1 indicates that this trait is overdispersed, i.e. that closely related organisms tend to have dissimlar levels of leaf dissection. Total P showed a similar trend.

Discussion

The results presented in this study conform to the trends noticed in other systems and plant groups, that smaller leaves are found in low-nutrient, high-light environments. In this case, it was not the overall size of the fern frond, but the level of dissection that was found to show this trend. The association of species with highly dissected leaves with low-nutrient soils might be due to the advantages conferred by small leaves in terms of nutrient acquisition by mass flow, as has previously been described by Cramer et al. (2008) and Yates et al. (2010).

However, the much stronger relationship with light intensity suggests a significant role for leaf size in preventing overheating. Since VPD was not found to have a significant effect on leaf dissection, the value of having dissected leaves, and therefore small boundary layers, appears to be in promoting sensible heat loss, rather than evaporative cooling.

The lack of a signal of trait conservatism indicates that leaf dissection is not simply a legacy of a plant's evolutionary history, but suggests plasticity in response to differences in habitat between related species. The fact that soil fertility was also significantly less conserved than expected due to chance indicates a possible role for niche differentiation between close relatives. If this is the case, the evolution of different leaf architectures may have been promoted by differences in substrate and nutrient availability, although it is not possible to determine the habitat variable to which leaf dissection was adapted from the data collected in this study.

The common association between small leaf size and sclerophylly was not found in this study, and the results are in agreement with the claim of Ackerly et al. (2002) that they are responses to different physiological stresses. The sclerophyllous ferns had smaller leaf areas overall (scaled by stem diameter) and thicker leaves, and they occurred in environments with low LAI. Their distribution thus appears to be determined by the light regime and not water or nutrient availability. (Although the open sites tended to having poorer soils as well, Total P did not significantly predict LMA.) The explanation that more delicate leaves occur in low light due to lower self-shading and construction and maintenance costs is favoured by these results.

It should be however noted that, both the results for sclerophylly and leaf dissection are influenced by the distinctiveness of the two species occurring only in the fynbos sites (*Pteridium* and *Cheilanthes*), while the majority occupied much more uniform habitats in the forest understorey. The strong effect of LAI on the results is largely due to this disctinction, as can be seen in Figures 5 and 6. This suggests that, under the forest canopy, the habitat gradients are much less steep and a large variety of ferns can occur here without special adaptation of their leaf structures. This is unsurprising, as the main limitation to fern distributions in general is known to be water availability (which is critical for reproduction), while low nutrients and light are more of a boon to ferns as they face less competition from angiosperm neighbours (Page, 2002).

The lack of an effect of VPD on maximum stomatal conductance may indicate that the stomata of these ferns are not especially adapted for particular microhabitats, perhaps because there was little variability in VPD through the study site. Alternatively, it may be that they have already converged on a solution that is optimal under all circumstances in this geological era: that of small and responsive stomata. It is impossible to reach firmer conclusions without an analysis of changes in stomatal dimensions and densities through evolutionary time, as performed on fossil plants by Franks & Beerling (2009).

Acknowledgements

I would like to express my gratitude to the following:

- Tony Verboom, Mike Cramer, Adam West, Rob Skelton, and Tim Moore for advice on methodology;
- Natasha Hardcastle, Stuart Hall, and Tony Verboom for invaluable assistance in the field;
- the technical staff of the Department of Botany for providing equipment;
- the National Research Foundation, the Council of the University of Cape Town, and the Department of Botany for financial assistance.

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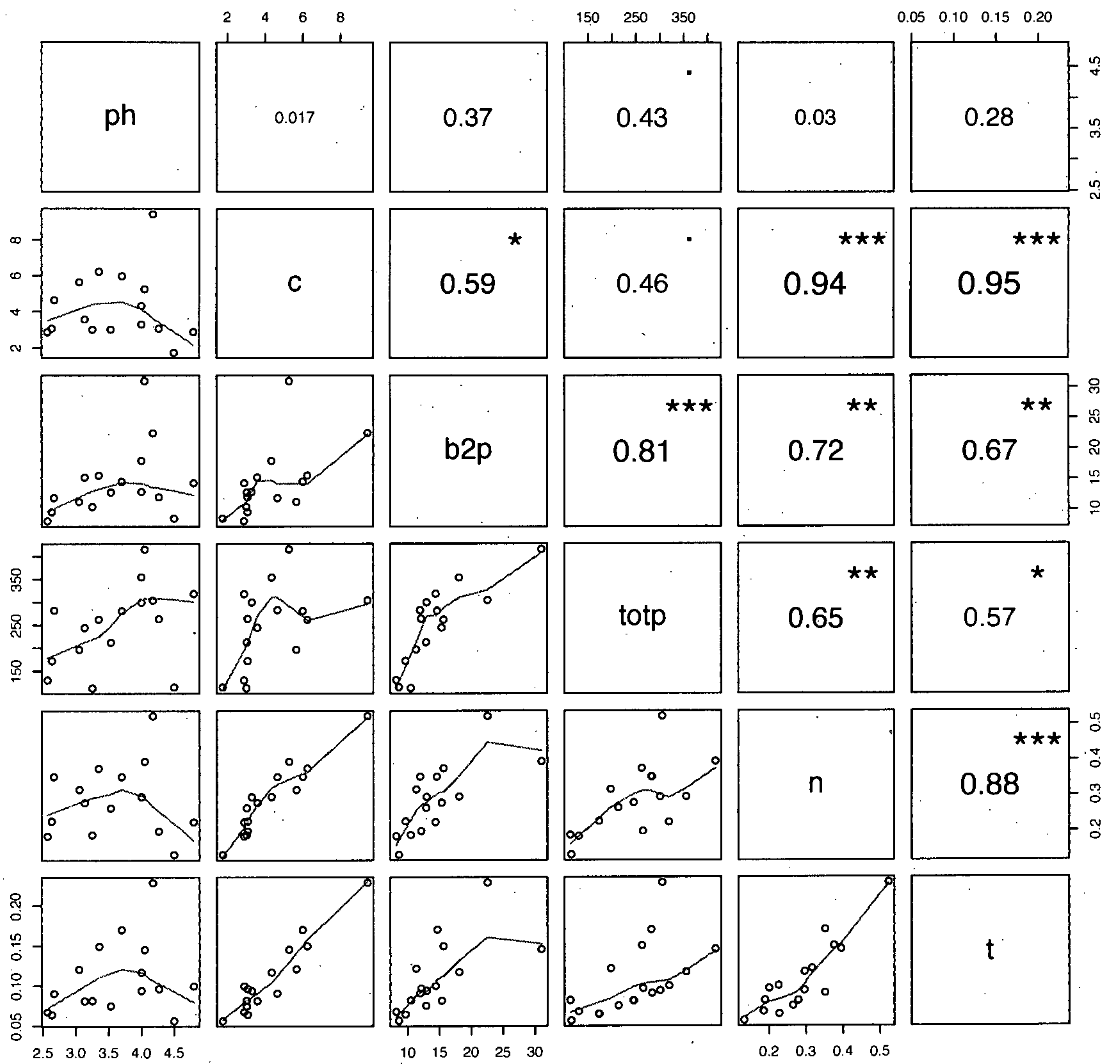

Figure 1. Correlations between soil nutrient variables

Lowess smoothers fitted; upper diagonals contain *r* values and asterisks indicate *P*.

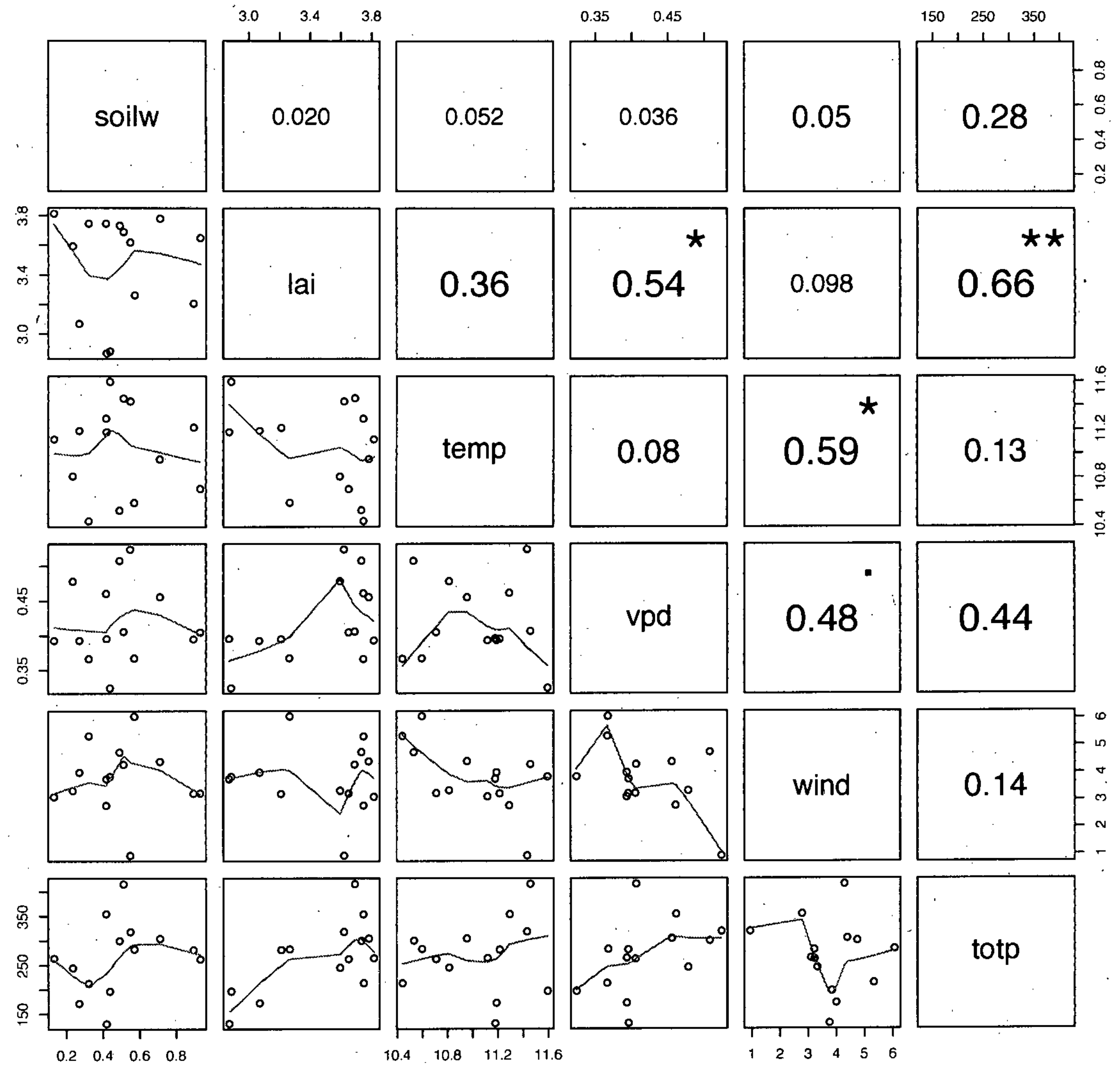

Figure 2. Correlations between habitat variables

Lowess smoothers fitted; upper diagonals contain *r* values and asterisks indicate *P*.

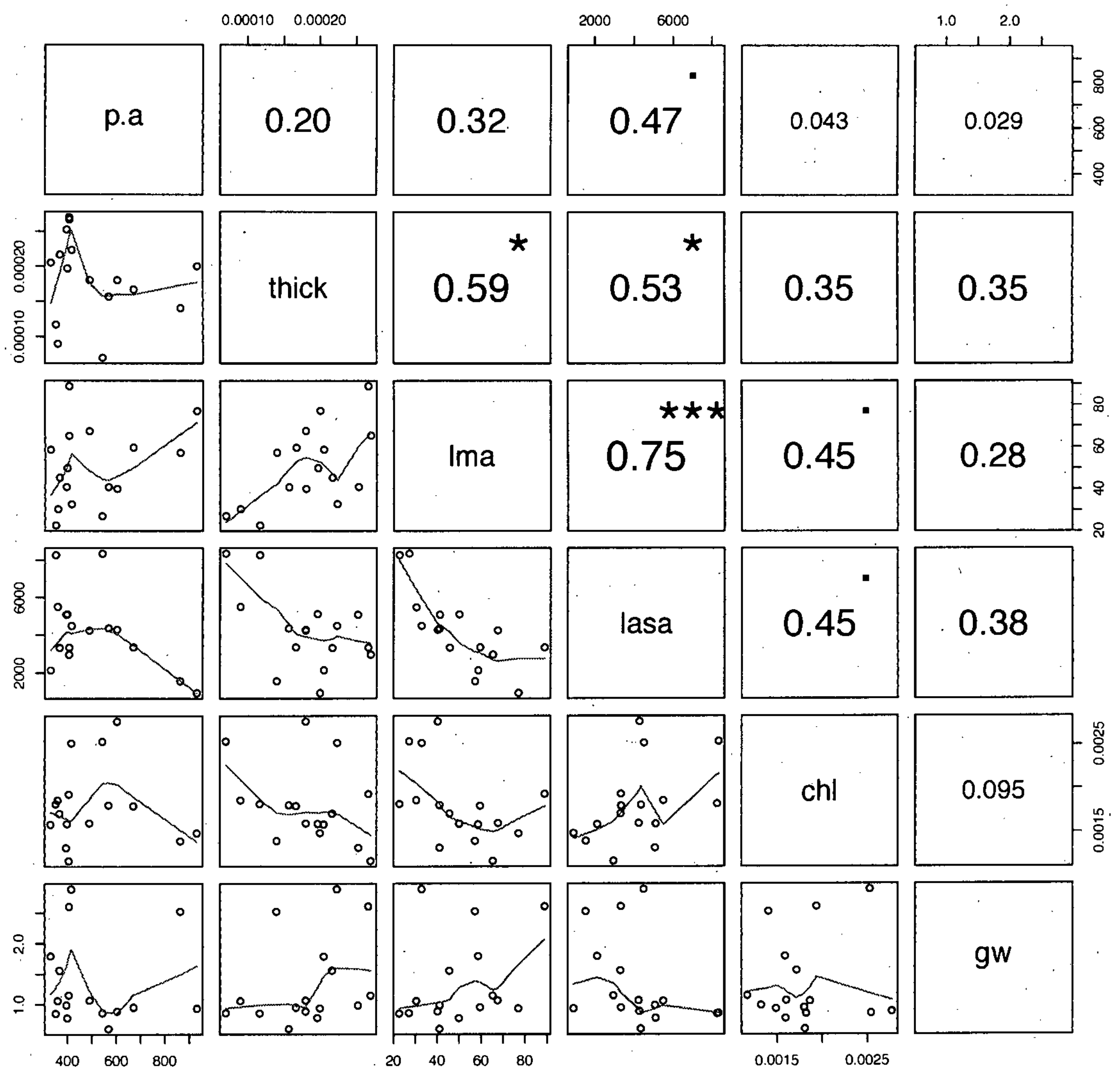

Figure 3. Correlations between leaf traits

Lowess smoothers fitted; upper diagonals contain *r* values and asterisks indicate *P*.

what are your variable codes?

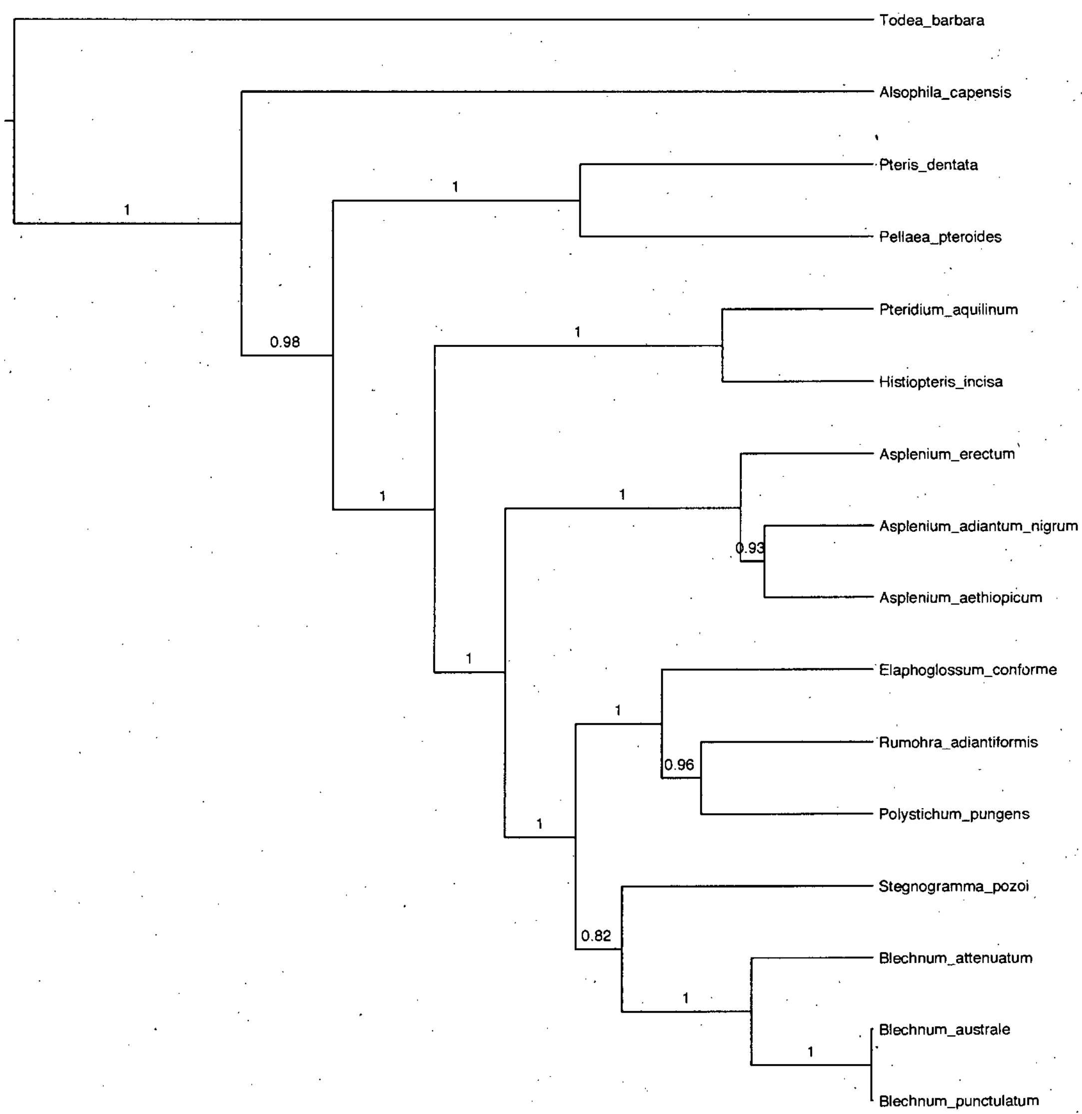

Figure 4. Bayesian phylogeny estimation for the taxa in this study

Values above branches indicate posterior probabilities as a measure of node support.

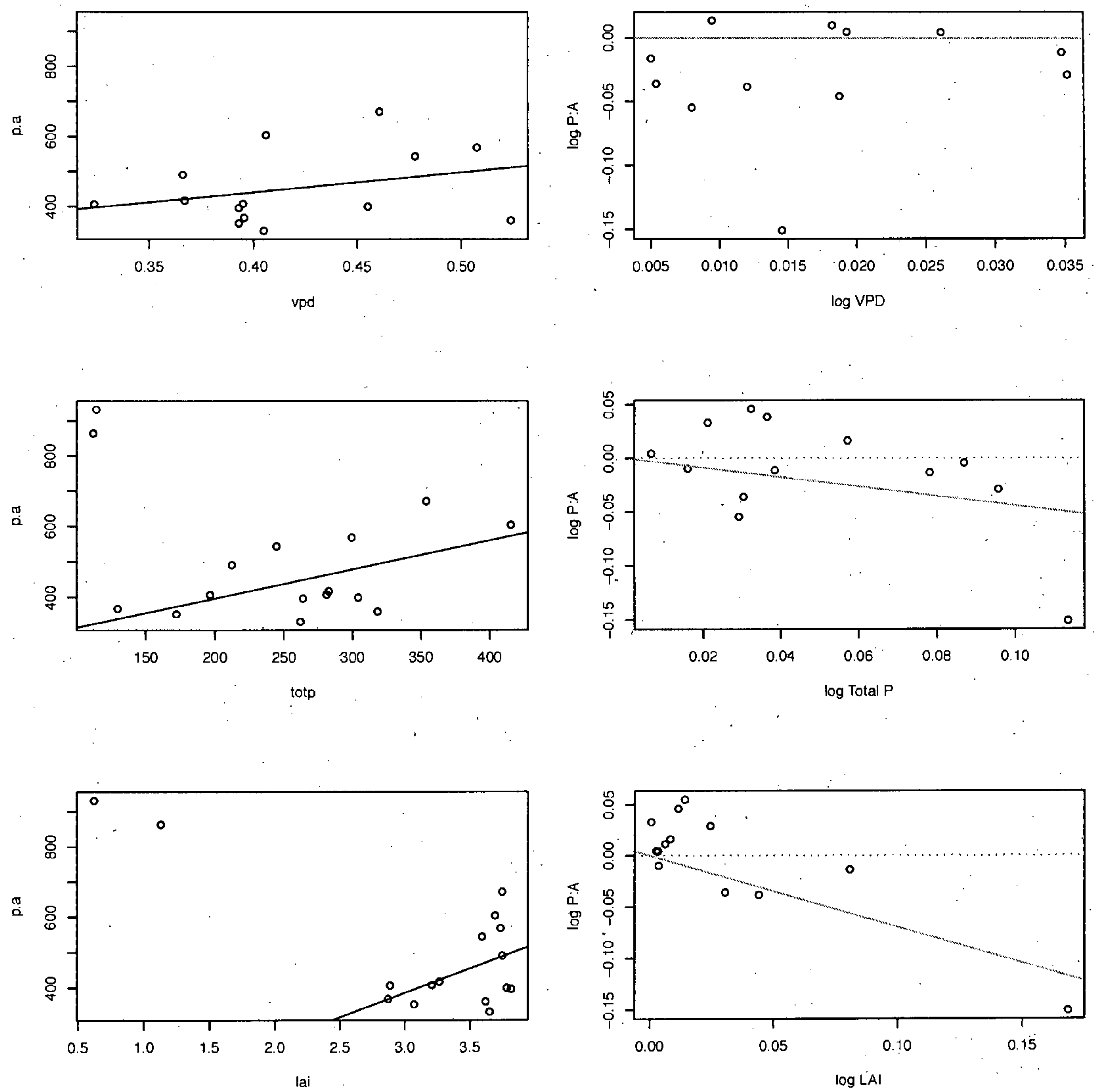

Figure 5. Regressions of P:A on VPD, Total P, and LAI

left: tip values, right: PICs

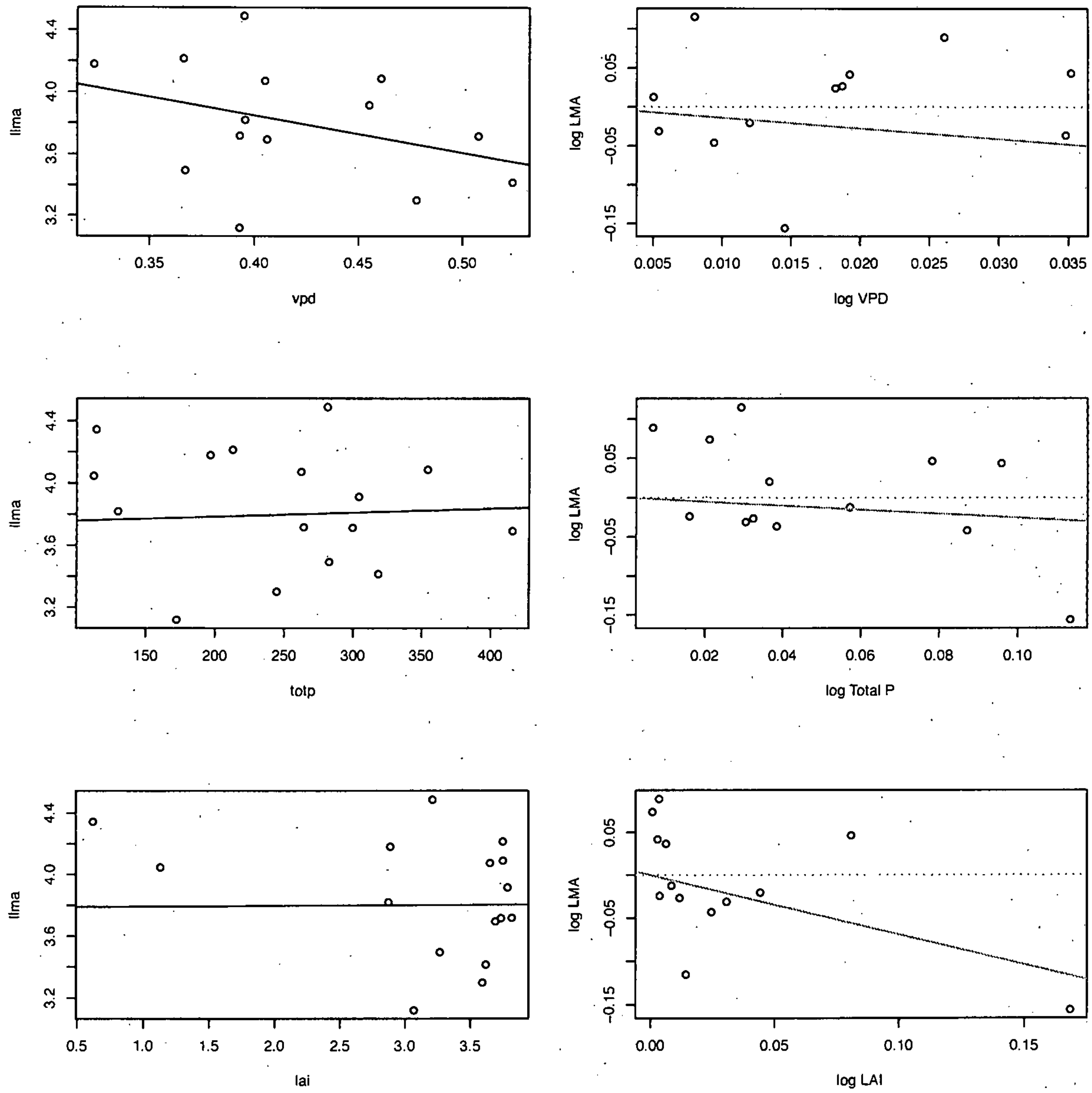

Figure 6. Regressions of LMA on VPD, Total P, and LAI

left: tip values, right: PICs

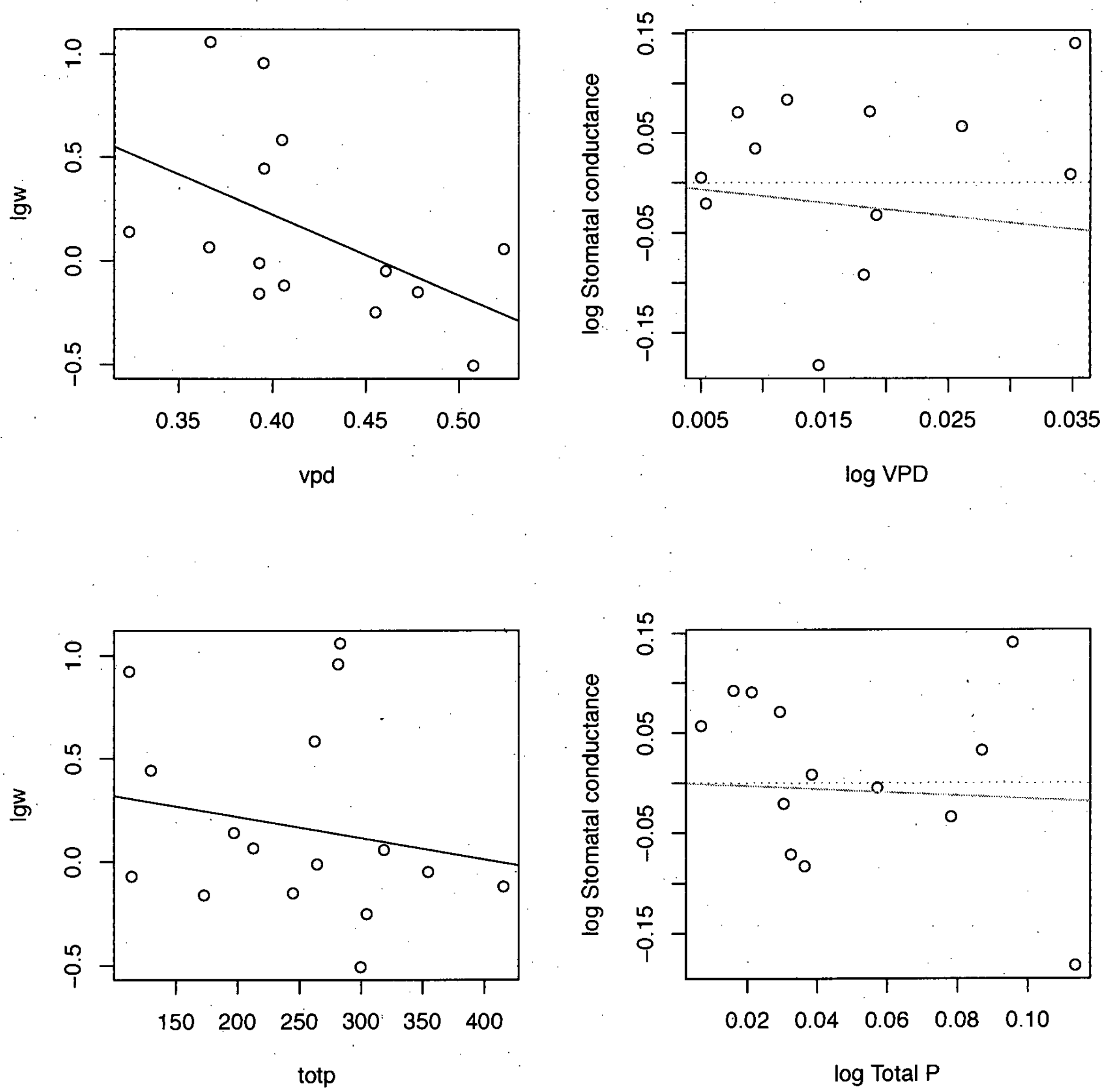

Figure 7. Regressions of log $g_{w\ max}$ on VPD and Total P

left: tip values, right: PICs

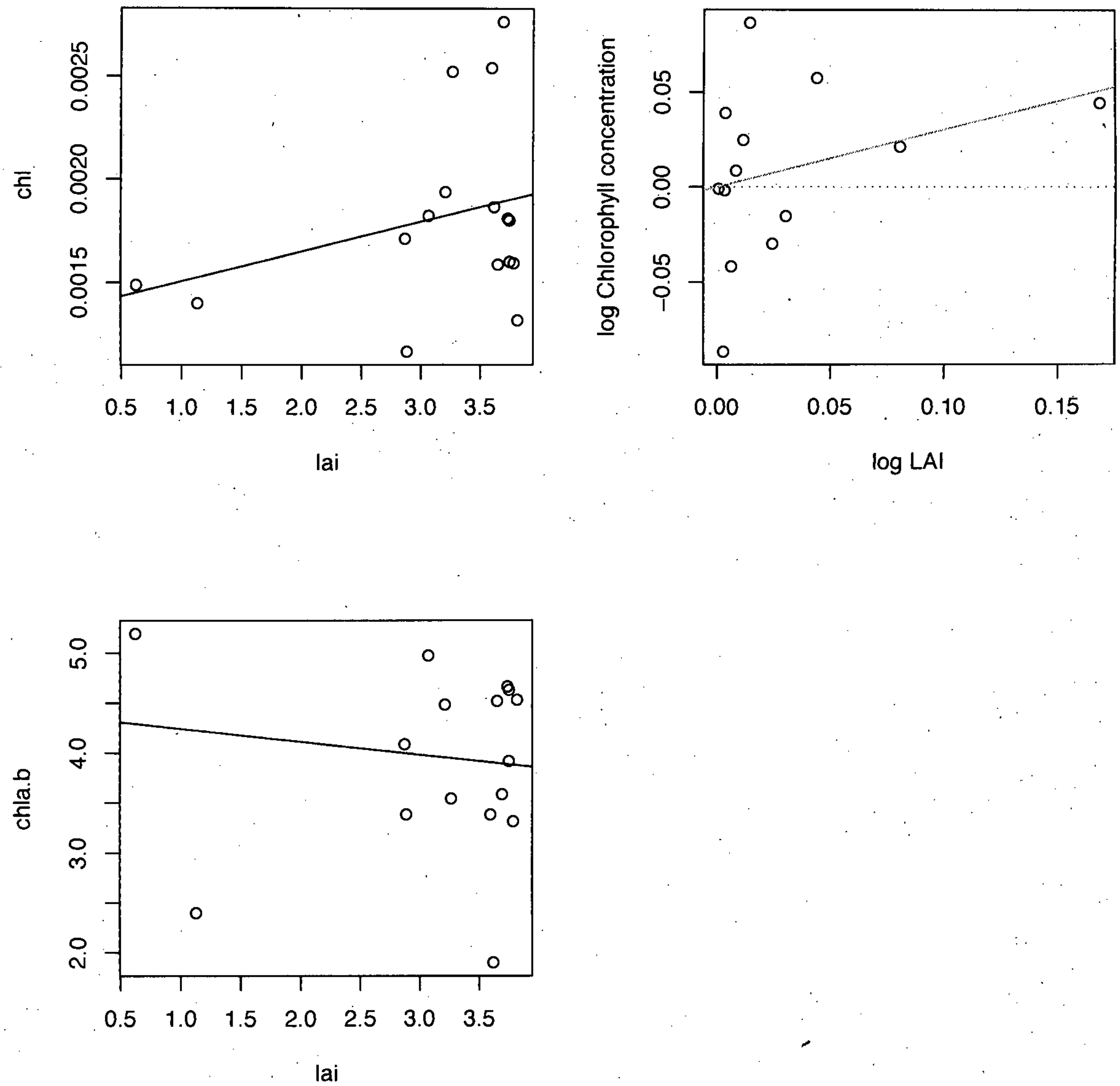

Figure 8. Regressions of Chlorophyll content on LAI

left: tip values, right: PICs

Table 1. Summaries of regression results (tip values)

```
p.a ~ vpd
            Estimate Std. Error t value Pr(>|t|)
vpd            567.5      506.0   1.122    0.284
Adjusted R-squared: 0.01944

p.a ~ totp
            Estimate Std. Error t value Pr(>|t|)
totp          0.8166     0.3377   2.418   0.0324 *
Adjusted R-squared: 0.2716

p.a ~ lai
            Estimate Std. Error t value Pr(>|t|)
lai           138.23      79.65   1.735    0.108
Adjusted R-squared: 0.134

log lma ~ vpd
            Estimate Std. Error t value Pr(>|t|)
vpd          -2.4097     1.8153  -1.327    0.209
Adjusted R-squared: 0.05537

log lma ~ totp
             Estimate Std. Error t value Pr(>|t|)
totp        0.0002569 0.0015034   0.171    0.867
Adjusted R-squared: -0.0807

log lma ~ lai
            Estimate Std. Error t value Pr(>|t|)
lai         0.004367   0.325629   0.013    0.990
Adjusted R-squared: -0.08332

log gw ~ vpd
            Estimate Std. Error t value Pr(>|t|)
vpd          -3.8934     1.9881  -1.958   0.0738 .
Adjusted R-squared: 0.179

log gw ~ totp
             Estimate Std. Error t value Pr(>|t|)
totp        -0.001025  0.001743  -0.588    0.568
Adjusted R-squared: -0.05302

chl ~ lai
             Estimate Std. Error t value Pr(>|t|)
lai         0.0001431 0.0003858   0.371    0.717
Adjusted R-squared: -0.07106

chla.b ~ lai
            Estimate Std. Error t value Pr(>|t|)
lai          -0.1305     0.6816  -0.191    0.851
Adjusted R-squared: -0.08003
```

Table?

Table 2. Summaries of regression results (PICs)

```
pa ~ vpd
     Estimate Std. Error t value Pr(>|t|)
vpd -0.006557   0.456130  -0.014    0.989
Adjusted R-squared: -0.09089

pa ~ totp
     Estimate Std. Error t value Pr(>|t|)
totp  -0.4433     0.2115  -2.095    0.058 .
Adjusted R-squared: 0.2069

pa ~ lai
    Estimate Std. Error t value Pr(>|t|)
lai  -0.6964     0.1761  -3.954  0.00192 **
Adjusted R-squared: 0.5295

lma ~ vpd
   Estimate Std. Error t value Pr(>|t|)
vpd  -1.3819     0.8089  -1.708    0.116
Adjusted R-squared: 0.1378

lma ~ totp
     Estimate Std. Error t value Pr(>|t|)
totp  -0.2564     0.3263  -0.786    0.447
Adjusted R-squared: -0.03029

lma ~ lai
    Estimate Std. Error t value Pr(>|t|)
lai  -0.6929     0.3013    -2.3   0.0402 *
Adjusted R-squared: 0.2481

gw ~ vpd
    Estimate Std. Error t value Pr(>|t|)
vpd   -1.330      1.005  -1.324    0.212
Adjusted R-squared: 0.05905

gw ~ totp
     Estimate Std. Error t value Pr(>|t|)
totp  -0.1585     0.4103  -0.386    0.706
Adjusted R-squared: -0.07002

chl ~ lai
    Estimate Std. Error t value Pr(>|t|)
lai   0.3025     0.2189   1.382    0.192
Adjusted R-squared: 0.0654
```

Table 3. Degree of phylogenetic conservatism of each leaf or habitat trait

	K	*P*
soil moisture	0.04	0.54
LAI	0.04	0.76
Temp	0.02	0.71
VPD	0.07	0.55
wind	0.17	0.25
total P	**0.44**	**0.06**
Leaf thickness	0.14	0.49
$g_{w\ max}$	0.18	0.44
P:A	**0.44**	**0.06**
LD	0.41	0.17
LMA	0.19	0.35
Tissue density	0.36	0.10
LASA	0.04	0.62
[chlorophyll]	0.07	0.48