

LETTER

Phylogenetic structural equation modelling reveals no need for an 'origin' of the leaf economics spectrum

Chase M. Mason,^{1*} Eric W. Goolsby,^{1,2} Devon P. Humphreys³ and Lisa A. Donovan¹

Abstract

The leaf economics spectrum (LES) is a prominent ecophysiological paradigm that describes global variation in leaf physiology across plant ecological strategies using a handful of key traits. Nearly a decade ago, Shipley *et al.* (2006) used structural equation modelling to explore the causal functional relationships among LES traits that give rise to their strong global covariation. They concluded that an unmeasured trait drives LES covariation, sparking efforts to identify the latent physiological trait underlying the 'origin' of the LES. Here, we use newly developed phylogenetic structural equation modelling approaches to reassess these conclusions using both global LES data as well as data collected across scales in the genus *Helianthus*. For global LES data, accounting for phylogenetic non-independence indicates that no additional unmeasured traits are required to explain LES covariation. Across datasets in *Helianthus*, trait relationships are highly variable, indicating that global-scale models may poorly describe LES covariation at non-global scales.

Keywords

Leaf lifespan, leaf mass per area, nitrogen, photosynthesis, phylogenetic path analysis.

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INTRODUCTION

Leaves are the primary productive organs in plants, functioning as the basis of food webs and the primary entry point for solar energy into most terrestrial ecosystems. The structure and physiology of leaves varies greatly worldwide, both within and among biomes. The leaf economics spectrum (LES) describes global covariation among physiological traits that govern initial carbon and nutrient investment in leaves and the rate and length of photosynthetic return on that investment (Reich *et al.* 1997; Wright *et al.* 2004). The LES represents a synthesis of concepts in leaf ecophysiology that span decades (Grime 1977; Bloom *et al.* 1985) and reflects a major global-scale axis of variation in plant ecological strategies (Reich 2014). At one end of the spectrum, plants produce low-investment, high-productivity leaves supportive of fast growth, high resource use, high tissue turnover rates and general competitiveness, while at the other extreme, plants produce high-investment, low-productivity leaves supporting much slower growth, more conservative resource use, slower tissue turnover and general persistence under stress (Poorter *et al.* 1990; Hallik *et al.* 2009; Reich 2014). The LES has been defined primarily by four focal leaf traits: photosynthetic rate (A_{mass}), leaf nitrogen content (N_{mass}), leaf mass per area (LMA) and leaf lifespan (LL). The strong covariation of these four traits across global scales has been inferred to arise from leaf-level physiological trade-offs between productivity and persistence, shaped by natural selection into a spectrum of successful strategies (Reich *et al.* 1997; Wright *et al.* 2004; Donovan *et al.* 2011). While these traits tightly covary at global scales, the specific nature of the functional trait relationships generating this covariance is not well understood.

Nearly a decade ago, Shipley *et al.* (2006) conducted pioneering work that sought to untangle the origins of the global LES by examining causal functional relationships among LES traits through structural equation modelling. This study first identified and evaluated two 'intuitive' models based on physiological relationships identified from the literature. In brief, the first intuitive model (Fig. 1a) draws from Field & Mooney (1986) and Wright *et al.* (2004), where leaf nitrogen covaries with LMA, both leaf nitrogen and LMA drive photosynthetic rate, and both LMA and photosynthetic rate drive leaf lifespan (Shipley *et al.* 2006). In essence, this model specifies that there is a central trade-off between leaf carbon and nutrient investment, that both of these factors determine leaf productivity, and that both leaf productivity and initial carbon investment determine leaf lifespan. The second intuitive model (Fig. 1b) is a similar, but less complex hypothesis based on Meziane & Shipley (2001). This model lacks a direct relationship between photosynthetic rate and leaf lifespan, and the relationship between leaf nitrogen and LMA is unidirectional rather than a covariance. In essence, this model places leaf carbon investment in a central role, solely determining both leaf lifespan and leaf nitrogen investment, and along with leaf nitrogen investment determining leaf productivity. Using the GLOPNET dataset (Wright *et al.* 2004), both of these models were rejected by goodness-of-fit tests (Shipley *et al.* 2006). The GLOPNET dataset contains abundant missing data, and so was subsequently pruned down to complete cases (observations containing all four LES traits), and through exploratory path analysis, it was found that no models containing only the four LES traits fit the data (Shipley *et al.* 2006). These results suggested that one or more latent variables must be generating the correlations among

¹Department of Plant Biology, University of Georgia, Athens, GA, USA

²Interdisciplinary Toxicology Program, University of Georgia, Athens, GA, USA

³Department of Integrative Biology, University of Texas, Austin, TX, USA

*Correspondence: E-mail: chasemason.evolution@gmail.com

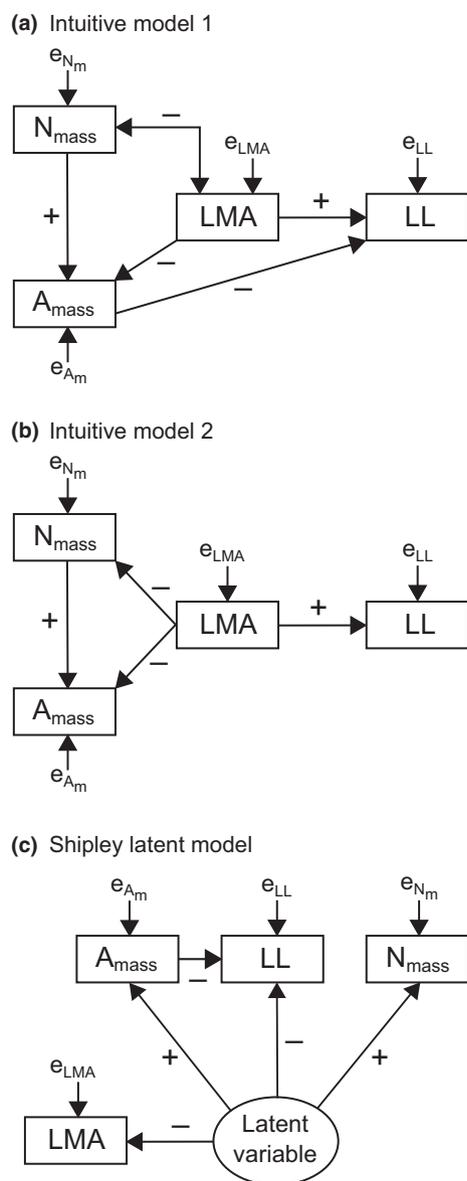


Figure 1 Conceptual diagrams of the two intuitive models (a, b) that were rejected by the non-phylogenetic analysis of Shipley *et al.* (2006), and (c) the latent variable model supported by the non-phylogenetic analysis of Shipley *et al.* (2006).

LES traits. Subsequent latent variable structural equation modelling on complete cases yielded a single supported model (Fig. 1c), with all LES trait variation passing through a single latent variable with the exception of the pairwise relationship between photosynthetic rate and leaf lifespan (Shipley *et al.* 2006). A major inference from this finding was that the latent variable likely represented a single unmeasured physiological trait, one that drives all of the LES traits and generates the observed covariation among them. Shipley *et al.* (2006) hypothesised that this latent variable was the ratio of leaf volume invested in intracellular space vs. in cell walls, approximated by leaf water content. This study has been cited well over a hundred times, has been highlighted in *Nature* (Whitfield 2006), and has sparked other efforts to identify the

latent trait underlying the ‘origin’ of the LES (e.g. leaf vein density, Blonder *et al.* 2011, 2013).

In comparative studies, it has long been recognised that multispecies datasets violate standard statistical assumptions of data independence, as such data are hierarchically autocorrelated due to underlying phylogenetic relationships (Felsenstein 1985; Freckleton 2009). Failing to account for phylogenetic non-independence in datasets amounts to pseudoreplication, which results in an increase in Type I error rates and can substantially bias estimates of trait covariance (Felsenstein 1985; Freckleton 2009; von Hardenberg & Gonzalez-Voyer 2013). With respect to path analysis and structural equation modelling, these issues are magnified given that many non-independent parameters are being simultaneously estimated (von Hardenberg & Gonzalez-Voyer 2013). However, at the time of the Shipley *et al.* (2006) study, methods accounting for phylogenetic non-independence in structural equation modelling were not well developed. Given the strong phylogenetic structure present across the wide array of land plants included in the GLOPNET dataset, this is potentially problematic. The recent development of methods to incorporate phylogenetic structure into structural equation modelling and path analysis (von Hardenberg & Gonzalez-Voyer 2013) now allows for more robust analysis of multispecies datasets. Here, we re-evaluate the conclusions of Shipley *et al.* (2006) applying these new phylogenetically conscious methods to the same dataset. In addition, we consider the applicability of global-scale models across multiple evolutionary and ecophysiological scales using the diverse genus *Helianthus*.

MATERIALS AND METHODS

Phylogenetic reanalysis of global LES covariation

For the phylogenetic re-evaluation of the analysis of Shipley *et al.* (2006), we proceeded in parallel to the original study while correcting for phylogenetic relatedness. Using the GLOPNET dataset (Wright *et al.* 2004), species names were converted to Phylomatic format using Plantminer (Carvalho *et al.* 2010). Using a 55 473-species molecular phylogeny of seed plants (Smith *et al.* 2011), a phylogeny of GLOPNET species was generated using the Phylomatic program in the Phylocom software bundle (Webb *et al.* 2008). Four taxa could not be placed: *Bactris trichophylla*, *Hedyosmum maximum*, and two unknown species of the genus *Nephrolepis*, and these were pruned from the GLOPNET dataset for our analyses. In order to determine whether phylogeny is, in fact, a problem for the Shipley *et al.* (2006) analysis, we assessed the extent of phylogenetic non-independence on trait distributions. Phylogenetic signal was estimated as Pagel’s λ for the phylogenetic residuals of each trait using the *fitContinuous* function in the R package *geiger* (Pagel 1999; Harmon *et al.* 2008; Revell 2010). All traits exhibited significant phylogenetic signal (Table 1), highlighting the importance of accounting for phylogenetic relatedness when conducting analyses with the GLOPNET dataset.

Because of the substantial presence of missing data in GLOPNET (e.g. 70.5% missing for leaf lifespan, 69.8% miss-

Table 1 Phylogenetic signal of trait values (Pagel's λ) and trait missingness (' a ' parameter) for photosynthetic rate (A_{mass}), nitrogen content (N_{mass}), leaf mass per area (LMA) and leaf lifespan (LL) in the GLOPNET database (Wright *et al.* 2004). Pagel's λ was calculated for trait phylogenetic residuals (Revell 2010), and significant values indicate that Pagel's λ is different from zero (i.e. phylogenetic signal is present in the data). The ' a ' parameter was estimated for each trait using phylogenetic logistic regression by treating trait missingness as a binary variable (Ives & Garland 2010; Ho & Ané 2014), resulting in a metric to quantify the extent to which trait data are missing non-randomly with respect to phylogeny. A significant ' a ' parameter greater than -4 indicates phylogenetic signal is present (Ives & Garland 2010).

Trait	λ of trait	P	Missingness ' a '	P
A_{mass}	0.83	$< 1 \times 10^{-6}$	0.11	$< 1 \times 10^{-6}$
N_{mass}	0.88	$< 1 \times 10^{-6}$	-1.66	$< 1 \times 10^{-6}$
LMA	0.87	$< 1 \times 10^{-6}$	1.32	$< 1 \times 10^{-6}$
LL	0.95	$< 1 \times 10^{-6}$	-0.25	$< 1 \times 10^{-6}$

ing for photosynthesis, 79.9% missing for paired observations of leaf lifespan and photosynthesis), phylogenetic signal of missingness was estimated as the ' a ' parameter using phylogenetic logistic regression (Ives & Garland 2010) using the *phylglm* function in the R package *phylolm* (Ho & Ané 2014). Missingness of each trait exhibited significant phylogenetic signal (Table 1), indicating that trait data are not missing at random across the tree. Because of this, critical assumptions of methods that account for missing data are violated, rendering the results of analyses like structural equation modelling uninterpretable, as such analyses are particularly sensitive to covariance misspecification (Dray & Josse 2014). To account for this, the dataset was pruned to only include entries in which all four traits were present (complete cases), leaving 492 entries representing 447 species. These complete cases have good coverage of major plant groups, including 30 conifers, 15 magnoliids, 33 monocots, 109 asterids, 209 rosids and 45 other eudicots. It is important to note that this set of complete cases is identical to that used by Shipley *et al.* (2006) in their original exploratory path analysis. Because multiple observations were not available for the vast majority of species (90%), within-species variation could not be estimated. In order to perform phylogenetic correction, species trait means were calculated for the 45 species with multiple entries to generate a single set of LES trait values per species. Phylogenetic trait covariance was then estimated on complete cases using the R package *Rphylopars* (Goolsby *et al.* 2015), using restricted maximum likelihood (REML) and a Brownian motion model of evolution. The resulting phylogenetic covariance matrix is equivalent to the multivariate generalised least squares estimator $[(\mathbf{Y} - \mathbf{A})^t \mathbf{C}^{-1} (\mathbf{Y} - \mathbf{A})] / (N - 1)$, where $(\mathbf{Y} - \mathbf{A})$ is the difference between the matrix of species trait values \mathbf{Y} and the matrix of estimated ancestral trait values at the root of the tree \mathbf{A} . \mathbf{C} is the phylogenetic covariance matrix assuming Brownian motion, N is the number of species, and a superscripted t indicates the matrix transpose (Revell & Collar 2009). Using the estimated phylogenetic trait covariance matrix, structural equation modelling was performed in AMOS version 5 (AMOS, Spring House, PA, USA).

Additionally, we selected two large clades within the GLOPNET phylogeny to evaluate in comparison to the full set of 447 species and to determine if the same models would be supported and the same trait relationships found. These two clades were the rosids (209 species) and the asterids (109 species), and these two datasets were analysed alongside the full set of 447 species using the same methodology with pruned versions of the GLOPNET phylogeny for each of the two clades.

Structural equation modelling across scales in *Helianthus*

Five datasets from controlled environment common garden studies of the genus *Helianthus* were used to examine the applicability of the intuitive models to explain LES trait covariation across three evolutionary and ecophysiological scales: macroevolutionary, among 28 congeners adapted to diverse habitats across North America (Mason & Donovan 2015); microevolutionary, among eight populations of the homoploid hybrid sunflower *Helianthus anomalous* differentiated across a desert environmental gradient (Brouillette *et al.* 2014); and ontogenetic, across shifts with whole-plant development in *Helianthus annuus*, *Helianthus mollis* and *Helianthus radula* (Mason *et al.* 2013). Together examining the models of LES trait relationships in these five datasets allows for the evaluation of the applicability of global LES models across multiple scales of trait variation.

At the macroevolutionary scale across *Helianthus*, trait data were collected on multiple populations per species for a total of 83 populations across 28 species in a greenhouse common garden (Mason & Donovan 2015). A phylogenetic mixed model was used to include within-species variation and improve the estimation of species trait values (Felsenstein 2008), using a pruned version of the most recent and well-resolved molecular phylogeny of the genus (Stephens *et al.* 2015). Phylogenetic trait covariance (Felsenstein 2008) was estimated using the *varCompPhyloip* function in the R package *ape* (Paradis *et al.* 2004) to call the contrast program in *Phyloip* v. 3.68 (Felsenstein 2004). The phylogenetic trait covariance matrix was then used to perform structural equation modelling in AMOS.

A non-phylogenetic approach was used at the microevolutionary and ontogenetic scales. At the microevolutionary scale, trait data were collected on 97 genetic families grown in a greenhouse common garden, from seed collected across eight populations of *Helianthus anomalous* (Brouillette *et al.* 2014). $F_{\text{ST}}-Q_{\text{ST}}$ analysis of this data supported adaptive differentiation of leaf photosynthetic rate and nitrogen content across a desert environmental gradient (Brouillette *et al.* 2014). Family means were used to calculate the trait covariance matrix, which was then used to perform structural equation modelling in AMOS. At the ontogenetic scale, trait data were collected across four developmental stages on individuals of three sunflower species (*Helianthus annuus*, *Helianthus mollis*, and *Helianthus radula*) grown under controlled conditions in growth chambers (Mason *et al.* 2013). All species were found to undergo strong shifts in LES traits with ontogeny, especially photosynthetic rate and nitrogen content (Mason

et al. 2013). Data from all populations of each species were pooled to calculate the trait covariance matrix for each species, which were then used to perform structural equation modelling in AMOS.

Multigroup analyses of trait relationships

In order to statistically evaluate differences in path coefficients across systems and scales, we used multigroup analysis for both the GLOPNET and *Helianthus* datasets (Shipley 2002). Multigroup analyses were performed in AMOS by fitting a null model that constrained all path coefficients and intercept values to be equal across datasets, and then relaxing each path coefficient one at a time to compare changes in model χ^2 value (Shipley 2002). In this way, the null hypothesis that coefficients do not vary among datasets could be tested, with significant changes in χ^2 indicating that the relaxed path coefficient differs among datasets.

RESULTS

Retesting Shipley's models with phylogenetic correction

While Shipley *et al.* (2006) unequivocally rejected both intuitive models for GLOPNET complete cases ($P < 0.0003$), we here fail to reject the first intuitive model when accounting for phylogeny ($P = 0.597$; Table 2). However, the second intuitive model is rejected ($P < 0.0001$; Table 2), indicating that the $A_{\text{mass}}\text{-LL}$ relationship and covariance between LMA and N_{mass} in the first intuitive model are necessary for good fit to the GLOPNET data. Furthermore, the latent variable model supported by Shipley *et al.* (2006) was likewise strongly rejected when accounting for phylogeny ($\chi^2 = 124.7$, $P < 0.0001$). This indicates that the support originally found for the latent variable model is likely an artefact of phylogenetic non-independence among species.

In parallel to the full set of GLOPNET complete cases, analysis of both the asterid and rosid clades failed to reject the first intuitive model, and rejected the second intuitive model (Table 2). Multigroup analysis of the first intuitive

Table 2 Summary statistics for both intuitive models (Fig. 1) across all datasets, including number of species included, number of observations, χ^2 and P -values. Models with P -values in bold are rejected by χ^2 goodness-of-fit test. Note that Intuitive Model 1 is not rejected for any dataset, and Intuitive Model 2 is rejected for all datasets except two.

Dataset	Spp.	Obs.	Intuitive Model 1		Intuitive Model 2	
			χ^2	P	χ^2	P
GLOPNET						
All complete cases	447	447	0.28	0.597	115.84	< 0.001
Rosids	213	213	3.78	0.052	110.17	< 0.001
Asterids	109	109	0.10	0.750	13.09	< 0.001
Genus <i>Helianthus</i>	28	83	0.96	0.327	18.71	< 0.001
<i>H. anomalus</i>	1	97	2.56	0.109	6.424	0.040
Ontogeny						
<i>H. annuus</i>	1	61	2.67	0.103	4.06	0.131
<i>H. mollis</i>	1	44	0.07	0.792	2.11	0.349
<i>H. radula</i>	1	51	2.28	0.131	6.37	0.041

Table 3 Multigroup analyses for (a) GLOPNET datasets and (b) *Helianthus* datasets. These analyses were performed by fitting a null model that constrained all path coefficients and intercept values to be equal across datasets, and then relaxing each parameter one at a time to identify which particular coefficients differed among datasets. χ^2 values were compared between the null and relaxed models to assess significant differences in path coefficients between datasets (Shipley 2002). The critical P -value (α) was adjusted to account for multiple comparisons ($\alpha = 0.01$), with path coefficients found to be significantly different among groups in bold.

Varying parameter	χ^2	$\Delta\chi^2$	d.f.	Δ d.f.	P
(a) GLOPNET datasets					
None (null)	131.239	–	21	–	–
LMA \rightarrow LL	116.397	14.842	19	2	0.001
$A_{\text{mass}} \rightarrow$ LL	128.173	3.066	19	2	0.216
LMA $\rightarrow A_{\text{mass}}$	106.359	24.880	19	2	0.000
$N_{\text{mass}} \rightarrow A_{\text{mass}}$	130.441	0.798	19	2	0.671
$N_{\text{mass}} \leftrightarrow$ LMA	122.301	8.938	19	2	0.011
(b) <i>Helianthus</i> datasets					
None (null)	3402.514	–	41	–	–
LMA \rightarrow LL	3400.593	1.921	37	4	0.750
$A_{\text{mass}} \rightarrow$ LL	3398.097	4.417	37	4	0.353
LMA $\rightarrow A_{\text{mass}}$	3390.066	12.448	37	4	0.014
$N_{\text{mass}} \rightarrow A_{\text{mass}}$	3367.071	35.443	37	4	0.000
$N_{\text{mass}} \leftrightarrow$ LMA	3273.525	128.989	37	4	0.000

model shows significant differences in path coefficients for the LMA \rightarrow LL and LMA $\rightarrow A_{\text{mass}}$ relationships (Table 3), indicating that the magnitudes of these trait relationships differ among the GLOPNET datasets. In particular, the LMA \rightarrow LL relationship appears much weaker in the asterids than in the rosids or complete cases, and the LMA $\rightarrow A_{\text{mass}}$ relationship appears much stronger in the asterids than in the rosids or complete cases (Fig. 2). Indeed the non-significance of the LMA $\rightarrow A_{\text{mass}}$ relationship in rosids and LMA \rightarrow LL relationship in asterids highlights that these different clades do not fully conform to predictions under the global LES (Fig. 2).

Testing the intuitive models across scales in *Helianthus*

Across the three datasets of *Helianthus*, the first intuitive model was never rejected (Table 2). Conversely, the second intuitive model was rejected at both macroevolutionary and microevolutionary scales, and at the ontogenetic scale in *H. radula*, though not in *H. annuus* or *H. mollis* (Table 2). Both the first and second intuitive models were equally well supported by the Bayesian Information Criteria in *H. annuus* and *H. mollis*, with models within 2 BIC units of one another (Fig. S1).

Despite being supported across all scales in *Helianthus*, multigroup analysis of the first intuitive model shows that the $N_{\text{mass}} \rightarrow A_{\text{mass}}$ and $N_{\text{mass}} \leftrightarrow$ LMA relationships significantly differ among datasets (Table 3). In particular, the $N_{\text{mass}} \rightarrow A_{\text{mass}}$ relationship appears much stronger at the macroevolutionary scale than the microevolutionary scale (Fig. 3), and both the $N_{\text{mass}} \rightarrow A_{\text{mass}}$ and $N_{\text{mass}} \leftrightarrow$ LMA relationships appear highly variable among the three ontogenetic datasets (Fig. S1). Differences among *Helianthus* datasets in the significance of trait relationships also mirror that seen between the GLOPNET rosid and asterid datasets, with the

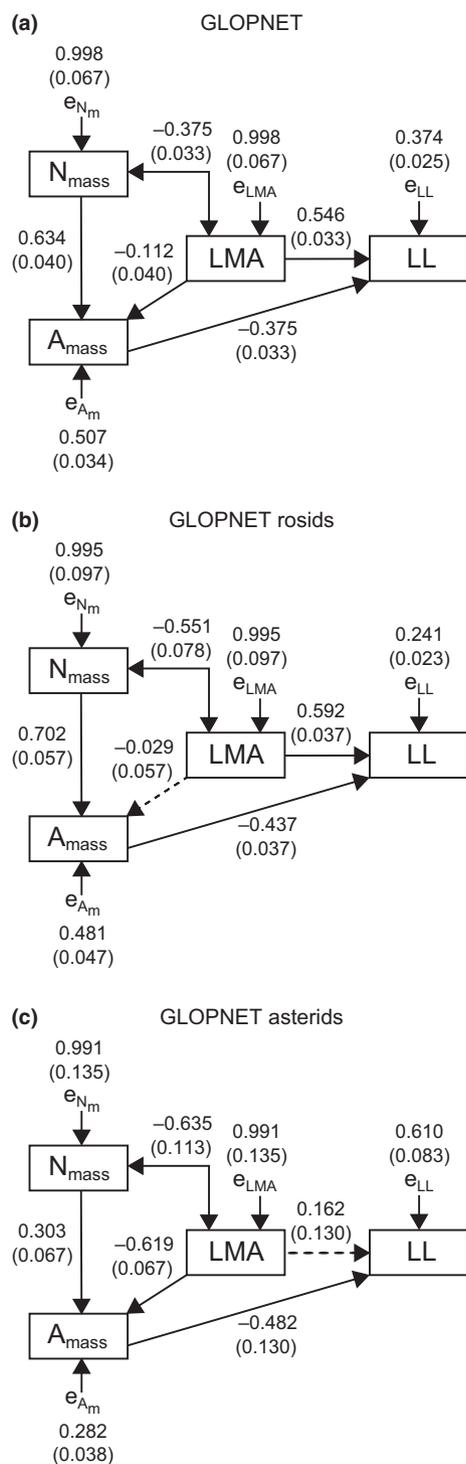


Figure 2 Phylogenetic structural equation modelling on GLOPNET data (Wright *et al.* 2004): (a) GLOPNET complete cases, (b) GLOPNET rosids, and (c) GLOPNET asterids. Standardised path coefficients and errors are presented, with standard errors in parentheses. All solid paths are significant and broken paths are non-significant at $P < 0.05$.

LMA \rightarrow A_{mass} relationship significant at the microevolutionary scale, but not at the macroevolutionary scale, and the reverse pattern seen for the LMA \rightarrow LL relationship (Fig. 3). Among the three ontogenetic datasets, there are also differ-

ences in the significance of both the LMA \rightarrow A_{mass} and $A_{mass} \rightarrow$ LL relationships (Fig. S1). Furthermore, in three of the five *Helianthus* datasets, the directionality of at least one trait–trait relationship differs from that predicted by the first intuitive model as applied to the global LES (Fig. 1). Together these results highlight that many systems do not fully conform to predicted trait relationships under the global LES.

DISCUSSION

Phylogenetic analysis refutes the need for a latent ‘origin’ of the LES

Comparing the original results of Shipley *et al.* (2006) to the phylogenetic reanalysis presented here reveals a strong difference in conclusions when accounting for phylogeny. Given the strong phylogenetic signal in both trait values and missingness of data, non-phylogenetic analysis of the GLOPNET dataset is statistically inappropriate, and conclusions drawn from non-phylogenetic analyses should be treated with considerable caution (Felsenstein 1985; Freckleton 2009; von Hardenberg & Gonzalez-Voyer 2013). While it may be tempting to interpret the original rejection of the first intuitive model in the non-phylogenetic analysis of Shipley *et al.* (2006) as indicating that the latent variable represents phylogenetic signal or some deep evolutionary event, drawing such conclusions based on comparisons of phylogenetic and non-phylogenetic models is statistically unsound. When hierarchical autocorrelation is ignored, non-phylogenetic path analysis exhibits extremely high Type-I error leading to high rejection rates for the true model, and the differences between the results of phylogenetic and non-phylogenetic analysis are uninterpretable (von Hardenberg & Gonzalez-Voyer 2013). Latent variable analysis requires that the covariances of observed variables are estimated accurately, such that a latent variable model cannot be fitted with confidence to datasets with unaccounted for phylogenetic signal. Accordingly, the latent variable of Shipley *et al.* (2006) cannot be interpreted as representing the contribution of phylogenetic non-independence.

From the phylogenetic analysis of GLOPNET data, it can be concluded that the tight correlations observed among LES traits at global scales can be explained solely by causal interactions among the LES traits themselves, and do not require an unmeasured latent physiological trait to explain their covariation. This suggests that a single phenotypic trait linking the LES traits may not exist; at best, there is no evidence to support its existence in the original Shipley *et al.* (2006) data when corrected for phylogeny. However, it should be noted that phylogenetic path analysis, while a useful tool, is based on a number of key assumptions (discussed below). Nonetheless, additional lines of evidence support our conclusions that an unmeasured latent trait may not exist. For example, the empirical evidence does not support either cell-wall-to-cell-volume-ratio (Shipley *et al.* 2006) or leaf vein density (Blonder *et al.* 2011; Sack *et al.* 2013) as being the missing latent trait. Furthermore, a recent structural equation modelling study of trait variation across diverse accessions of *Arabidopsis* found results consistent with those found here, including failing to reject the first intuitive model while

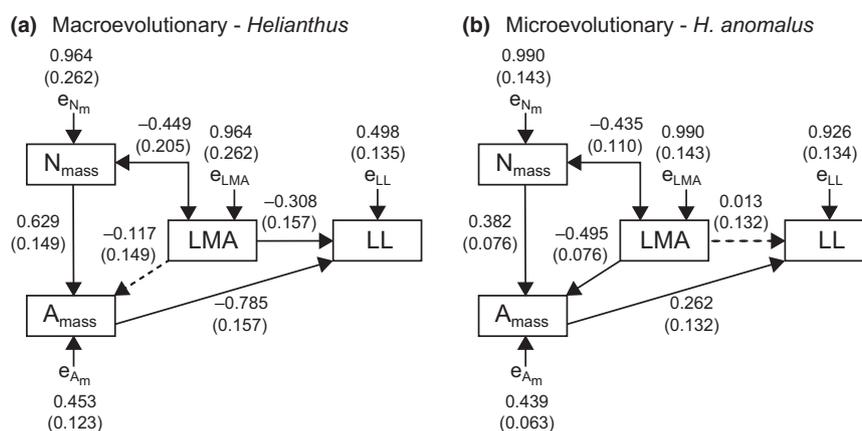


Figure 3 Phylogenetic structural equation modelling on (a) 28 diverse species of the genus *Helianthus* (Mason & Donovan 2015), and (b) 97 genetic families of *H. anomalus* from populations distributed across a desert environmental gradient (Brouillette *et al.* 2014). Standardised path coefficients and errors are presented, with standard errors in parentheses. All solid paths are significant and broken paths are non-significant at $P < 0.05$.

rejecting the second, while additionally rejecting the cell-wall-to-cell-volume-ratio latent model of Shipley *et al.* (2006), and vein density as the latent trait in that model (Blonder *et al.* 2015). Only a less parsimonious latent model with no pairwise relationships among any measured traits could be supported by the data in that study (Blonder *et al.* 2015), and the only other major study of leaf vein density in a structural equation modelling context (in *Populus tremuloides*; Blonder *et al.* 2013) included only two leaf economic traits and could not directly evaluate the hypotheses put forward in Shipley *et al.* (2006).

While the phylogenetic reanalysis performed here appears to eliminate the need for a latent physiological trait to explain LES covariation, support for the first intuitive model across scales is consistent with the more general concept of fundamental evolutionary trade-offs between productivity and persistence at the leaf level (Wright *et al.* 2004; Shipley *et al.* 2006; Reich 2014). Furthermore, it is important to note that the lack of a need for a latent ‘origin’ trait should not be interpreted to mean that there are no general biological mechanisms that give rise to the LES, for instance selection or genetic constraints (Donovan *et al.* 2011). Additionally, because phylogenetic structural equation modelling inherits the assumptions and potential limitations common to all phylogenetic comparative methods (e.g. sensitivity to misspecification of the evolutionary model, reliance on accurate inference of tree topology and branch lengths), our results are of course dependent on these general assumptions. Likewise, the general assumptions and limitations inherent to conventional path analysis also apply (e.g. linearity of trait relationships, the absence of multicollinearity, contrasting methods for model selection), and our results are dependent on these general assumptions as well. However, it should be noted that all of the limitations of conventional path analysis also apply to the original results of Shipley *et al.* (2006).

Models derived from global datasets are not universal

While the first intuitive model is supported across all the datasets examined in this study, the strong variation in

directionality, magnitude and significance of pairwise LES relationships among scales indicates that the LES causal trade-offs identified with the GLOPNET database are not universal. Thus, while serving as useful initial hypotheses, these trade-offs do not always well represent trait covariation among closely related species or populations within species. This is increasingly understood to be the case, as investigations of LES evolution within genera have repeatedly failed to find many of the key pairwise trait relationships predicted under the global LES (e.g. Givnish *et al.* 2004; Dunbar-Co *et al.* 2009; Edwards *et al.* 2014; Mason & Donovan 2015). It is quite likely that the shape of phenotypic space into which specific groups of species diversify results in a strengthening of some LES relationships and an elimination of others, with a strong dependence on other aspects of the biology of these systems (Pigliucci 2007; Funk & Cornwell 2013; Edwards *et al.* 2014). As such, global LES trade-offs are likely not particularly useful for predicting causal trait relationships at many microevolutionary scales to which the LES framework has increasingly been applied, for instance adaptation across populations (Grady *et al.* 2013; Brouillette *et al.* 2014), indirect selection on traits under artificial selection (Donovan *et al.* 2011; Cornwell & Cornelissen 2013); or plant responses to climate change (Gornish & Prather 2014).

Better approaches to understanding LES covariation

It has been suggested by multiple sources that better insights into LES covariation can be derived by investigating large suites of physiological traits simultaneously in order to better understand the importance of the LES as part of integrated leaf and whole-plant trait networks (e.g. Sack *et al.* 2013; Poorter *et al.* 2014). In particular, this approach is likely to be particularly powerful when coupled with evolutionarily explicit phylogenetic comparative analyses within diverse groups of closely related taxa (like genera) sampled to minimise environmentally induced variation. Such approaches allow for the careful examination of trait–trait relationships during diversification in systems with well-understood biology, and are now compatible with informative statistical approaches like structural equation modelling. This approach

has been well employed recently in both *Viburnum* (Edwards *et al.* 2014) and *Helianthus* (Mason & Donovan 2015). Combining inferences from many individual well-controlled case studies of trait evolution through a confluence of evidence approach (Pigliucci 2007) is likely to be more informative than the big-data macroecological approaches that have dominated most work on the leaf economics spectrum. Big-data approaches focus on sparse data collection of small numbers of traits across large numbers of taxa, where a strong biological context for trait evolution is lacking and unrecognised sources of error (e.g. phylogenetic, environmental, etc.) may bias results in unexpected ways. This is especially true given how highly plastic leaf economic traits have been shown to be (Donovan *et al.* 2014) and how variable pairwise LES relationships have been found to be among scales and systems, as demonstrated here. Physiological trade-offs that hold in one group of species may not in another (e.g. Funk & Cornwell 2013; Edwards *et al.* 2014; Mason & Donovan 2015), diminishing the interpretability of results from big-data analyses that pool sparse data collection from a wide range of taxa. In addition, an emerging and highly complementary approach to understanding LES covariation lies in identifying the genetic basis of LES traits. Work in *Arabidopsis* and *Populus* has identified a small handful of genes and genetic regions with strong associations with multiple LES traits (Vasseur *et al.* 2012; McKown *et al.* 2014), and such work expanded to an ever-increasing number of species is likely to provide improved understanding of the role of genetic constraints in the generation of observed LES trade-offs (Donovan *et al.* 2011).

In summary, analyses that account for phylogenetic non-independence provide no support for a latent physiological trait driving global LES covariation, and further modelling indicates that global-scale models are insufficient to describe LES covariation across evolutionary and ecophysiological scales.

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AUTHOR CONTRIBUTIONS

CMM and EWG conceived and designed the study. EWG and DPH devised and performed the analyses. CMM and EWG made the figures. CMM wrote the article along with EWG, DPH and LAD.

REFERENCES

Blonder, B., Violle, C., Bentley, L.P. & Enquist, B.J. (2011). Venation networks and the origin of the leaf economics spectrum. *Ecol. Lett.*, **14**, 91–100.

- Blonder, B., Violle, C. & Enquist, B.J. (2013). Assessing the causes and scales of the leaf economics spectrum using venation networks in *Populus tremuloides*. *J. Ecol.*, **101**, 981–989.
- Blonder, B., Vasseur, F., Violle, C., Shipley, B., Enquist, B.J. & Vile, D. (2015). Testing models for the leaf economics spectrum with leaf and whole-plant traits in *Arabidopsis thaliana*. *AoB Plants*, **7**, plv049.
- Bloom, A.J., Chapin, F.S. III & Mooney, H.A. (1985). Resource limitation in plants—an economic analogy. *Annu. Rev. Ecol. Syst.*, **16**, 363–392.
- Brouillette, L.C., Mason, C.M., Shirk, R.Y. & Donovan, L.A. (2014). Adaptive differentiation of traits related to resource use in a desert annual along a resource gradient. *New Phytol.*, **201**, 1316–1327.
- Carvalho, G.H., Cianciaruso, M.V. & Batalha, M.A. (2010). Plantminer: a web tool for checking and gathering plant species taxonomic information. *Environ. Modell. Softw.*, **25**, 815–816.
- Cornwell, W.K. & Cornelissen, J.H.C. (2013). A broader perspective on plant domestication and nutrient and carbon cycling. *New Phytol.*, **198**, 331–333.
- Donovan, L.A., Maherali, H., Caruso, C.M., Huber, H. & de Kroon, H. (2011). The evolution of the worldwide leaf economics spectrum. *Trends Ecol. Evol.*, **26**, 88–95.
- Donovan, L.A., Mason, C.M., Bowsher, A.W., Goolsby, E.W. & Ishibashi, C.D.A. (2014). Ecological and evolutionary lability of plant traits affecting carbon and nutrient cycling. *J. Ecol.*, **102**, 302–314.
- Dray, S. & Josse, J. (2014). Principal component analysis with missing values: a comparative survey of methods. *Plant Ecol.*, **216**, 657–667.
- Dunbar-Co, S., Sporck, Margaret.J. & Sack, L. (2009). Leaf trait diversification and design in seven rare taxa of the Hawaiian *Plantago* radiation. *Int. J. Plant Sci.*, **170**, 61–75.
- Edwards, E.J., Chatelet, D.S., Sack, L. & Donoghue, M.J. (2014). Leaf life span and the leaf economic spectrum in the context of whole plant architecture. *J. Ecol.*, **102**, 328–336.
- Felsenstein, J. (1985). Phylogenies and the comparative method. *Am. Nat.*, **125**, 1–15.
- Felsenstein, J. (2004). *Phylyp (Phylogeny Inference Package) Version 3.68*. Department of Genetics, University of Washington, Seattle, Washington D.C.
- Felsenstein, J. (2008). Comparative methods with sampling error and within-species variation: contrasts revisited and revised. *Am. Nat.*, **171**, 713–725.
- Field, C.H. & Mooney, H.A. (1986). The photosynthesis-nitrogen relationship in wild plants. In: *On the Economy of Plant Form and Function* (ed Givnish, T.J.). Cambridge University Press, London, UK, pp. 25–55.
- Freckleton, R.P. (2009). The seven deadly sins of comparative analysis. *J. Evol. Biol.*, **22**, 1367–1375.
- Funk, J.L. & Cornwell, W.K. (2013). Leaf traits within communities: context may affect the mapping of traits to function. *Ecology*, **94**, 1893–1897.
- Givnish, T.J., Montgomery, R.A. & Goldstein, G. (2004). Adaptive radiation of photosynthetic physiology in the Hawaiian lobeliads: light regimes, static light responses, and whole-plant compensation points. *Am. J. Bot.*, **91**, 228–246.
- Goolsby, E.W., Bruggeman, J. & Ané, C. (2015). *Rphylopar: phylogenetic comparative tools for missing data and within-species variation*. R package version 0.1.1.
- Gornish, E.S. & Prather, C.M. (2014). Foliar functional traits that predict plant biomass response to warming. *J. Veg. Sci.*, **25**, 919–927.
- Grady, K.C., Laughlin, D.C., Ferrier, S.M., Kolb, T.E., Hart, S.C., Allan, G.J. *et al.* (2013). Conservative leaf economic traits correlate with fast growth of genotypes of a foundation riparian species near the thermal maximum extent of its geographic range. *Funct. Ecol.*, **27**, 428–438.
- Grime, J.P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am. Nat.*, **111**, 1169–1194.

- Hallik, L., Niinemets, Ü. & Wright, I.J. (2009). Are species shade and drought tolerance reflected in leaf-level structural and functional differentiation in Northern Hemisphere temperate woody flora? *New Phytol.*, 184, 257–274.
- von Hardenberg, A. & Gonzalez-Voyer, A. (2013). Disentangling evolutionary cause-effect relationships with phylogenetic confirmatory path analysis. *Evolution*, 67, 378–387.
- Harmon, L.J., Weir, J.T., Brock, C.D., Glor, R.E. & Challenger, W. (2008). GEIGER: Investigating evolutionary radiations. *Bioinformatics*, 24, 129–131.
- Ho, L.S.T. & Ané, C. (2014). A linear-time algorithm for Gaussian and non-Gaussian trait evolution models. *Syst. Biol.*, 63, 397–408.
- Ives, A.R. & Garland, T. (2010). Phylogenetic logistic regression for binary dependent variables. *Syst. Biol.*, 59, 9–26.
- Mason, C.M. & Donovan, L.A. (2015). Evolution of the leaf economics spectrum in herbs: evidence from environmental divergences in leaf physiology across *Helianthus* (Asteraceae). *Evolution*, 69, 2705–2720.
- Mason, C.M., McGaughey, S.E. & Donovan, L.A. (2013). Ontogeny strongly and differentially alters leaf economic and other key traits in three diverse *Helianthus* species. *J. Exp. Bot.*, 64, 4089–4099.
- McKown, A.D., Klapste, J., Guy, R.D., Gerald, A., Porth, I., Hannemann, J. *et al.* (2014). Genome-wide association implicates numerous genes underlying ecological trait variation in natural populations of *Populus trichocarpa*. *New Phytol.*, 203, 535–553.
- Meziane, D. & Shipley, B. (2001). Direct and indirect relationships between specific leaf area, leaf nitrogen and leaf gas exchange. Effects of irradiance and nutrient supply. *Ann. Bot.*, 88, 915–927.
- Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature*, 401, 877–884.
- Paradis, E., Claude, J. & Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20, 289–290.
- Pigliucci, M. (2007). Finding the way in phenotypic space: the origin and maintenance of constraints on organismal form. *Ann. Bot.*, 100, 433–438.
- Poorter, H., Remkes, C. & Lambers, H. (1990). Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiol.*, 94, 621–627.
- Poorter, H., Lambers, H. & Evans, J.R. (2014). Trait correlation networks: a whole-plant perspective on the recently criticized leaf economic spectrum. *New Phytol.*, 201, 378–382.
- Reich, P.B. (2014). The world-wide ‘fast–slow’ plant economics spectrum: a traits manifesto. *J. Ecol.*, 102, 275–301.
- Reich, P.B., Walters, M.B. & Ellsworth, D.S. (1997). From tropics to tundra: global convergence in plant functioning. *Proc. Natl Acad. Sci. USA*, 94, 13730–13734.
- Revell, L.J. (2010). Phylogenetic signal and linear regression on species data. *Methods Ecol. Evol.*, 1, 319–329.
- Revell, L.J. & Collar, D.C. (2009). Phylogenetic analysis of the evolutionary correlation using likelihood. *Evolution*, 63, 1090–1100.
- Sack, L., Scoffoni, C., John, G.P., Poorter, H., Mason, C.M., Mendez-Alonzo, R. *et al.* (2013). How do leaf veins influence the worldwide leaf economic spectrum? Review and synthesis. *J. Exp. Bot.*, 64, 4053–4080.
- Shipley, B. (2002). *Cause and Correlation in Biology: A User’s Guide to Path Analysis, Structural Equations and Causal Inference*. Cambridge University Press, Cambridge, UK.
- Shipley, B., Lechowicz, M.J., Wright, I. & Reich, P.B. (2006). Fundamental trade-offs generating the worldwide leaf economics spectrum. *Ecology*, 87, 535–541.
- Smith, S.A., Beaulieu, J.M., Stamatakis, A. & Donoghue, M.J. (2011). Understanding angiosperm diversification using small and large phylogenetic trees. *Am. J. Bot.*, 98, 404–414.
- Stephens, J.D., Rogers, W.L., Mason, C.M., Donovan, L.A. & Malmberg, R.L. (2015). Species tree estimation of diploid *Helianthus* (Asteraceae) using target enrichment. *Am. J. Bot.*, 102, 910–920.
- Vasseur, F., Violle, C., Enquist, B.J., Granier, C. & Vile, D. (2012). A common genetic basis to the origin of the leaf economics spectrum and metabolic scaling allometry. *Ecol. Lett.*, 15, 1149–1157.
- Webb, C.O., Ackerly, D.D. & Kembel, S.W. (2008). Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics*, 24, 2098–2100.
- Whitfield, J. (2006). The cost of leafing. *Nature*, 444, 539–541.
- Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F. *et al.* (2004). The worldwide leaf economics spectrum. *Nature*, 428, 821–827.

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