MACROEVOLUTION OF METABOLIC AND MORPHOLOGICAL TRAITS IN VASCULAR PLANTS

by

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Abstract

If we hope to mitigate the effects of the climate crisis, it is critical that we accurately represent the contributions of plants in global models and predictions. The need to link global cycles to biological processes that feed into these cycles involves making scaling assumptions. In the case of photosynthesis, most global models use parameters extrapolated from a handful of species. However, the variation in leaf form and function that we observe both within and across species makes one question the biological accuracy of this assumption. Despite advances in quantifying the differences between deeply divergent lineages, there is still a substantial gap in what we know about how C3 photosynthesis evolves both at very deep and very shallow evolutionary timescales. Filling this gap will allow us to better infer and incorporate photosynthetic behaviour in global models. Here we address two specific gaps: i) we assess the evolution of photosynthetic trait variation and ii) we evaluate the relationship between photosynthetic traits and a set of plant functional traits. These latter traits are often used as proxies for metabolic variation even though we don't know how metabolic and morphological features are coupled through their evolutionary trajectories. We collected data for a phylogenetically structured sample of 106 species growing in a common environment, sampling metabolic and functional traits from the same individuals. Using estimates of phylogenetic half-life, we find significant phylogenetic structure in both metabolic and morphological traits, but the evolution of metabolic traits is much more constrained than that of morphology. We also ask whether changes across traits are coupled, and find that even when there are present day trait correlations, most traits do not seem to share correlated evolutionary history. Our finding of substantial interspecific variation in photosynthetic traits is not captured in current global ecosystem models; further, using functional trait variation as a proxy does not

adequately represent this variation. Future work would benefit from using some element of evolutionary history or species identity in modeling photosynthetic behaviour.

Lay Summary

Vascular plants have been evolving and thus accumulating differences for over 400 Million years. Despite a good understanding of the evolution of leaf shape and size and the tradeoffs involved in plant forms, we do not yet understand how plants' metabolic behaviours have evolved and whether their evolution is correlated with the more-often-studied morphological features. Using morphological and metabolic measurements collected from plants growing in the UBC and VanDusen botanical gardens, we describe the variation and broad scale patterns of evolution of these traits. Metabolic traits are much more constrained than morphological traits in their long term evolutionary dynamics. We also find that metabolic and morphological traits are not evolving in a correlated fashion. Especially in the context of a changing environment, this work highlights a need to study metabolic traits directly and not through morphological proxies.

Preface

I discussed this project idea with M.W. Pennell, who had previously discussed these ideas with C.D. Muir. I collaborated with J. Whitton and M.W. Pennell to explore and elaborate the experimental design and methods involved. This also benefited from the helpful insights of A. Angert and S. Michaletz. I collected the data with the aid of undergraduate research assistant V. Pornsinsiriruk. I analyzed these data under the supervision of C.D. Muir, J. Whitton and M.W. Pennell. I wrote the manuscript and J. Whitton and M.W. Pennell edited it.

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Acknowledgements

I would like to acknowledge that this thesis was written on the traditional, ancestral and unceded territory of the Musqueam, Squamish and Tsleil-Waututh Nations.

I am extremely grateful for the guidance provided by my supervisors, Matthew Pennell and Jeannette Whitton. I feel very fortunate to have benefitted from your mentorship over the last few years and cannot thank you enough for your ongoing enthusiasm to chat through ideas with me.

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Finally, I would like to thank my parents, for nurturing my curiosity.

General Introduction

Overview

Comparative biology has the potential to provide powerful perspectives on trait ecology and evolution that might otherwise be missing from experimental or observational studies conducted within populations (Harvey and Pagel 1998; Weber and Agrawal 2012). Many traits of interest vary substantially more between species than they do within species, lending themselves to these comparative studies. Further, given that some processes (including constraints) require a fuller landscape of observations in order to be detected, comparative approaches allow us to notice these phenomena, which might otherwise be unnoticeable at microevolutionary scales (Hansen and Martins 1996; Pennell and Harmon 2013).

In order to ask and answer relevant questions, comparative biology needs to account for species' shared evolutionary history, as reflected through phylogeny. Taxa and their traits have shared evolutionary history, which is reflected in their underlying genetic architecture and presumably shapes much observed trait variation. This shared history must be incorporated into our statistical framework, as this non-independence between species as data points violates many of our standard statistical assumptions. The field of phylogenetic comparative methods has developed a series of approaches for dealing with this non-independence in macroevolutionary studies (recently reviewed in: (O'Meara 2012; Garamszegi 2014; Harmon 2018)).

Today's field of macroevolution is well equipped to ask and answer questions - we use macroevolutionary methods to impute trait values for data-deficient species (González-Del-Pliego et al. 2019), to document evidence of coevolution (Weiblen 2004), and to highlight the importance of specific traits in diversification (Weber and Agrawal 2014). Describing the dynamics of a single

trait's evolution, as well as evolutionary correlations between traits can shine a light on the constraints in trait evolution and clarify the directionality of causality.

In particular, plant leaf traits pose an interesting landscape in which to study macroevolutionary trends. For example, broad macroecological trends have been described for a suite of these traits and recognized as the leaf economics spectrum (LES) (Wright et al. 2004; Osnas et al. 2013). A small number of studies have used phylogenetic comparative methods to evaluate the evolutionary trends in this suite of traits (Ackerly 2009; Zanne et al. 2014; Pennell et al. 2015). While we have developed a macroecological and macroevolutionary perspective of leaf functional traits, we have not yet characterized the macroevolution of the metabolic traits they often stand in place for.

Objectives

In this thesis I use a macroevolutionary approach to explore the relationship between vascular plant metabolism and morphology. I describe the variation in a suite of metabolic and morphological traits, then use two complementary (semi-qualitative and quantitative) approaches to ask how these traits have evolved. I assess how well morpho-functional traits predict photosynthetic capacity. Finally, I evaluate whether any subset of the suite of metabolic and morphological traits share correlated macroevolutionary trajectories.

Introduction

Our ability to link changes in vegetation to changes in climate rests on models that link variation in plant traits with variation in carbon capture. The accuracy of Earth System Models (ESMs) that do so (reviewed in: (Fisher et al. 2018)) is particularly crucial in the context of forecasting and mitigating the effects of anthropogenic climate change. Plant metabolism provides this fundamental link in forecasting feedbacks between vegetation and climate (Yvon-Durocher et al. 2010)). Capturing photosynthetic variation therefore plays a crucial role in parameterizing the metabolic behaviour of vegetation in ESMs. However, a vast majority of our data on photosynthetic capacity used in ESMs is estimated from only a handful of sites and species (Friend and Kiang 2005; Wullschleger et al. 2014), making these models quite sensitive to the selected representation of metabolic diversity (Rogers 2014). Not only is it crucial to capture this variation for forecasting efforts in the context of a changing environment, it is also critical to understand the ecology and evolution of plant metabolism.

One major assumption embedded in both these ESMs and the Metabolic Theory of Ecology (MTE) (Brown et al. 2004) is that a plant is a plant when it comes to photosynthetic capacity. While metabolic models make use of functional trait variation, they assume that photosynthetic rates have a simple scaling relationship with these traits that is static through time and across taxa. For example, data from tobacco (*Nicotiana tabacum*, Solanaceae) was used to parameterize the activation energy for all plants' unimodal-shaped response to changing temperature and this estimate has since been used as a universal constant (Farquhar et al. 1980; Bernacchi et al. 2001; Allen et al. 2005). By using data from a single species to calibrate the link between a few key plant traits and whole community photosynthetic parameters, we implicitly assume that the relationship between the functional and photosynthetic traits is static across species i.e. it does not evolve.

However, we know that evolutionary shifts in these scaling relationships do occur: for example, there are multiple shifts in metabolic scaling across the vertebrate phylogeny (Uyeda et al. 2017).

In the absence of metabolic data for many plant species, researchers have identified a small number of 'functional traits' that stand in to summarize plant performance. For example, the leaf economics spectrum (LES) is a suite of covarying leaf traits including specific leaf area (SLA), leaf nitrogen, maximum photosynthetic rates and leaf lifespan. When aggregating data across vascular plants, a great deal of the variation in these traits can be captured by one or two principal component axes (Wright et al. 2004). This trait covariation implicates a role for tradeoffs driven by the allocation of limited resources in shaping plant variation (Reich 2014; Shipley et al. 2016; Onoda et al. 2017). Because it is substantially easier to measure leaf traits over metabolic traits, a plant's position along the LES can then be used as a proxy for its metabolic behaviour.

While there is evidence that plant functional traits can perform as predictors of photosynthetic rates (Poorter and Bongers 2006), it is unclear across what range of metabolic traits and taxa these relationships hold. We still don't know whether plant metabolic and morphological trait covariation is linked on macroevolutionary timescales i.e., are metabolic and morphological traits evolving in a correlated fashion (Uyeda et al. 2017; Avaria-Llautureo et al. 2019)? We may expect coordinated evolution of these traits, as a trait for trait change (involved in scaling relationships (Brown et al. 2004)) would imply this coordination, but this has not yet been formally tested in plants.

There is compelling (albeit fairly limited) evidence, of substantial variation in photosynthetic rates both between major lineages and also among species. Studies highlight significant differences between the large evolutionary groups: Angiosperms, Gymnosperms and Pteridophytes (Lusk et al. 2003; Carriquí et al. 2015; Gago et al. 2019). There is also extensive

intraspecific variation, including evidence for local adaptation of photosynthetic traits, seen in population level differences in response to abiotic stress (Oakley et al. 2018). Taken together, these suggest that photosynthesis shows both plastic responses within populations, and genetic variation across a range of time scales. While some macroevolutionary studies have looked at the evolution of functional traits (Pennell et al. 2015), we lack a more directly descriptive understanding of how photosynthetic variation scales from shallow to deep evolutionary timescales.

Given what we know about the potential for variation in photosynthetic parameters, it is critically important that we gain a better understanding of the broadscale evolutionary signal in these traits. To address this need, we set out to quantify variation in two key photosynthetic parameters (J_{max} and V_{cmax}) and describe their evolutionary history. These parameters that are estimated from a biophysical model (Farquhar et al. 1980), are routinely used in ESMs and variation in these estimates is a substantial source of uncertainty in our ability to make predictions (Rogers 2014). We test whether we can predict photosynthetic capacity from functional traits and whether photosynthesis evolves in a correlated manner with other traits. Current metabolic theory and trait ecology assume that photosynthetic capacity is reasonably well predicted by functional traits, due to metabolic constraints. This further suggests that because of tradeoffs that occur during leaf construction (Reich 2014; Shipley et al. 2016; Onoda et al. 2017), photosynthetic capacity and functional traits should not only share correlations in their present states, but changes in photosynthetic capacity should be coupled with changes in functional traits, and therefore this suite of traits should show correlated rates of evolution.

While J_{max} and V_{cmax} have been previously measured in many taxa, meta-analysis of photosynthetic capacity poses some logistical challenges because data collected in different places, years and conditions may not be easily comparable (Walker et al. 2014). In fact, this

approach may result in real and measurable species differences being missed or misrepresented (Nakagawa and Santos 2012), due to the impacts that growing conditions are known to have. Collecting all of the data from one location in one field season allows us to mostly control for site, season and stress, and agglomerate data for multiple species that can more readily be compared. Here we accomplish this by using two botanical gardens located within the same urban region as *macroevolutionary common gardens*.

We gather a complete dataset for 106 species from two botanical gardens (University of British Columbia Botanical Garden and VanDusen Botanical Garden) in Vancouver, Canada taking repeated measures over a single growing season. We measure Rapid A/Ci Response (RACiR) curves in order to estimate 2 photosynthetic parameters (J_{max} and V_{cmax}). We also measure leaf area, mass, nitrogen content and carbon content. We then use the megaphylogeny from Zanne et al. (2014) to conduct our phylogenetic analyses, where we describe the accumulation of trait divergence through evolutionary time and estimate the half-life and Phylogenetic Independent Contrasts (PIC) of each trait.

Methods

Species selection

We sampled 138 species across the tree of vascular land plants. Working from the list of species growing at the UBC Botanical Garden (49.253841° N, 123.251101° W) and VanDusen Botanical Garden (49.2394° N, 123.1289° W), we randomly selected one species from each family represented in either garden for inclusion in our study. In addition, in order to quantify differences in traits among more recently diverged taxa, we also sampled more species from one family of ferns, conifers and angiosperms (Dryopteridaceae, Pinaceae and Ericaceae, respectively.) All of our measurements were taken between May 6th and July 18th 2019, from plants growing in these two botanical gardens. Although we expect that there are differences in light, temperature, water and nutrients both between and within each of the gardens, we suggest that the effects of this variation are likely to be greatly reduced relative to studies that might compile data from various sources and locations.

Metabolic data

We measured metabolic traits using a recently developed approach and new equipment and software that takes non-steady-state measurements. This allows us to measure a rapid A/Ci response (RACiR) (Stinziano et al. 2017) curve in much less than half the time taken for a traditional A/Ci curve. Instead of ramping CO2 to specific concentrations and giving the leaf time to acclimate (as is done for traditional A/Ci measurements), RACiR involves continuously ramping CO2 and taking measurements every two seconds, so that the instantaneous response to CO2 changes are measured in their non-steady-state form. Using this new method provides reliable estimates of J_{max} and V_{cmax}. However, other parameter estimates derived from this

approach may be less comparable to estimates from traditional methodology (Stinziano et al. 2017, 2019; Taylor and Long 2019).

We measured Rapid A/Ci Response (RACiR) curves, using a LICOR 6800 photosynthesis system (LICOR, Lincoln, Nebraska, USA) as described in Stinziano et al. (2017) on the newest fully open leaf of an individual plant (or, for plants with very small leaves, the youngest cluster of leaves.) We took these measurements on days with full sun, between 7:00 and 11:00 am Pacific Standard Time. The RACiR measurement comprises two separate measurements: i) a data curve - for which CO2 is ramped while a leaf is in the chamber and ii) an empty curve - for which CO2 is ramped with no leaf in the chamber. The empty chamber curve is used to differentiate between the rate of CO2 accumulation in the chamber and the real change in photosynthetic rate of the leaf. According to Stinziano et al. (2017) these empty curves should be collected periodically throughout the data collection - to account for possible environmental effects on the accumulation of CO2 throughout the day. We took at least two empty ramp measurements per morning, so that the longest period of time between an empty calibration curve and a data curve is less than 2 hours. We used the following settings for ramping: CO2 ramped from 10 to 1010 ppm, temperature leaf = 20 C, light = 1500 μmol m^{-2} s^{-1}. We wrote custom code (in line with the data manipulations described in (Stinziano et al. 2017)) to calibrate our data curves in order to correct for the true amount of leaf area in the chamber, as well as according to the empty CO2 ramp (all code used is available at https://github.com/bnetobradley/metamorphylo). Since some leaves are smaller than the total surface area in the clamped chamber, the LICOR machine generated estimates of photosynthetic rates must be recalculated based on the correct amount of leaf area (see following section). We used the *plantecophys* R package (Duursma 2015) to estimate the parameters (V_{cmax} and J_{max}) from our calibrated curves using the bilinear fitting method. Since the bilinear fitting

method always returns a parameter estimate, we manually checked our dataset to remove J_{max} and V_{cmax} estimates that were derived from insufficient data.

Morphological data

After measuring a RACiR curve, the leaf (or leaves) that was sampled was clipped from the plant and placed in a resealable bag to prevent desiccation and stored in an ice-filled cooler overnight. We scanned each leaf (at 300 DPI, using a Perfection V750 PRO Epson flatbed scanner) and calculated total leaf area as well as the leaf area inside the chamber during our RACiR measurement using ImageJ (Schneider et al. 2012). We measured leaf wet mass using a Mettler Toledo B154 analytical balance. After these measurements were taken, we placed the leaves in a drying oven at 60 degrees C for 48 hours, and then measured dry mass. Dried leaf tissue was sent to the Analytical Chemical Services Laboratory (at the BC Ministry of Environment and Climate Change Strategy) for combustion analysis to obtain leaf Nitrogen and Carbon content.

Phylogenetic and statistical analyses

We used the time calibrated megaphylogeny from Zanne et al. (2014) for all of the following phylogenetic analyses. We pruned the tree (available at: https://datadryad.org/stash/dataset/doi:10.5061/dryad.63q27) to include only the species present in our macroevolutionary common garden as tips on the tree. To facilitate cross-trait comparisons, our complete trait dataset is made up of 106 species, in which every species has a measurement for each trait.

i) Models of trait divergence through time

We used plots of trait divergence through time to assess broad patterns of trait divergence. We estimated the divergence in trait values between each species pair in our dataset (as seen in

(Uyeda et al. 2011)) for each of the traits we measured (J_{max}, V_{cmax}, leaf area, leaf mass, leaf nitrogen and leaf carbon), and plotted these divergence values against the divergence time between species (i.e., the age of their most recent common ancestor) as estimated from the node ages of the Zanne et al. phylogenetic tree (Zanne et al. 2014). We then used the 'fitContinuous' function in the R package geiger (Pennell et al. 2014) to fit two phylogenetic models of trait evolution: Brownian motion (BM; (Felsenstein 1973)) and Ornstein-Uhlenbeck (OU; (Hansen 1997)). The OU model fits a random walk with a central tendency whereas the Brownian motion model assumes a trait value correlation structure that is proportional to species shared evolutionary history (Pennell et al. 2014). In these model fits, measures of leaf area and mass were log transformed, and measurement error for all traits was incorporated by using the variance of our species sample of 3 leaves. From these fits, we estimated the half-life of each trait, and assessed the support for these alternative models of evolution.

ii) Rate of trait evolution (PICs)

For each trait we estimate phylogenetic independent contrasts (as described in Felsenstein (1985)) using the R package 'ape' (Paradis et al. 2004) for our traits (J_{max}, V_{cmax}, SLA, Leaf Nitrogen and Leaf Carbon). The log of the absolute value of these contrasts can be seen as the rate of evolution of that trait along the phylogeny. We then test whether different traits' rates of evolution (as measured by the log of the absolute value of their phylogenetic independent contrast) are correlated with each other. We contrast this to the trait value correlations, as a way of assessing whether trait correlations have indeed been driven by correlated evolution in this suite of traits.

Results

The plants in our macroevolutionary common garden exhibit substantial variation in photosynthetic capacity. Consistent with previous studies, this variation in J_{max} and V_{cmax} is not spread evenly among major taxonomic groups ($F(2,103) = 9.3991$, $p < 0.001$; $F(2,103) = 11.791$, $p < 0.001$). Angiosperms span 22.34 to 239.71 for estimates of J_{max} and 16.55 to 139.20 for estimates of V_{cmax}, this range is broader than that of Pteridophytes ($17.05 < J_{max} < 84.71$; $11.50 < V_{cmax} < 45.76$) or Gymnosperms ($51.50 < J_{max} < 237.89$; $28.58 < V_{cmax} < 124.89$) (Figure 1). This is further illustrated when we plot J_{max} and V_{cmax} values on a phylogeny (Figure S1 & S2). Pteridophytes exhibit less variation in trait values and these values are lower than those seen in angiosperms and gymnosperms (Figure 2 & Figure S2).

By fitting models of continuous trait evolution to our dataset we quantitatively describe the relationships between trait values and evolutionary time. We estimate the maximum likelihood estimate for phylogenetic half-life of leaf nitrogen (9 MY), J_{max} (59 MY), V_{cmax} (89 MY), leaf area ($3.64 \times 10_{14}$ MY), leaf mass (194 MY) and leaf carbon (151 MY) (Figure 3). Estimates of phylogenetic half-life that are far greater than the earliest split included in the tree (here equal to 390 MY) indicate strong support for a model of brownian motion, whereas estimates very close to 0 indicate a white noise process (a scenario with no-phylogenetic structure in trait values), and estimates between these indicate varying degrees of support for a more bounded Ornstein-Uhlenbeck process.

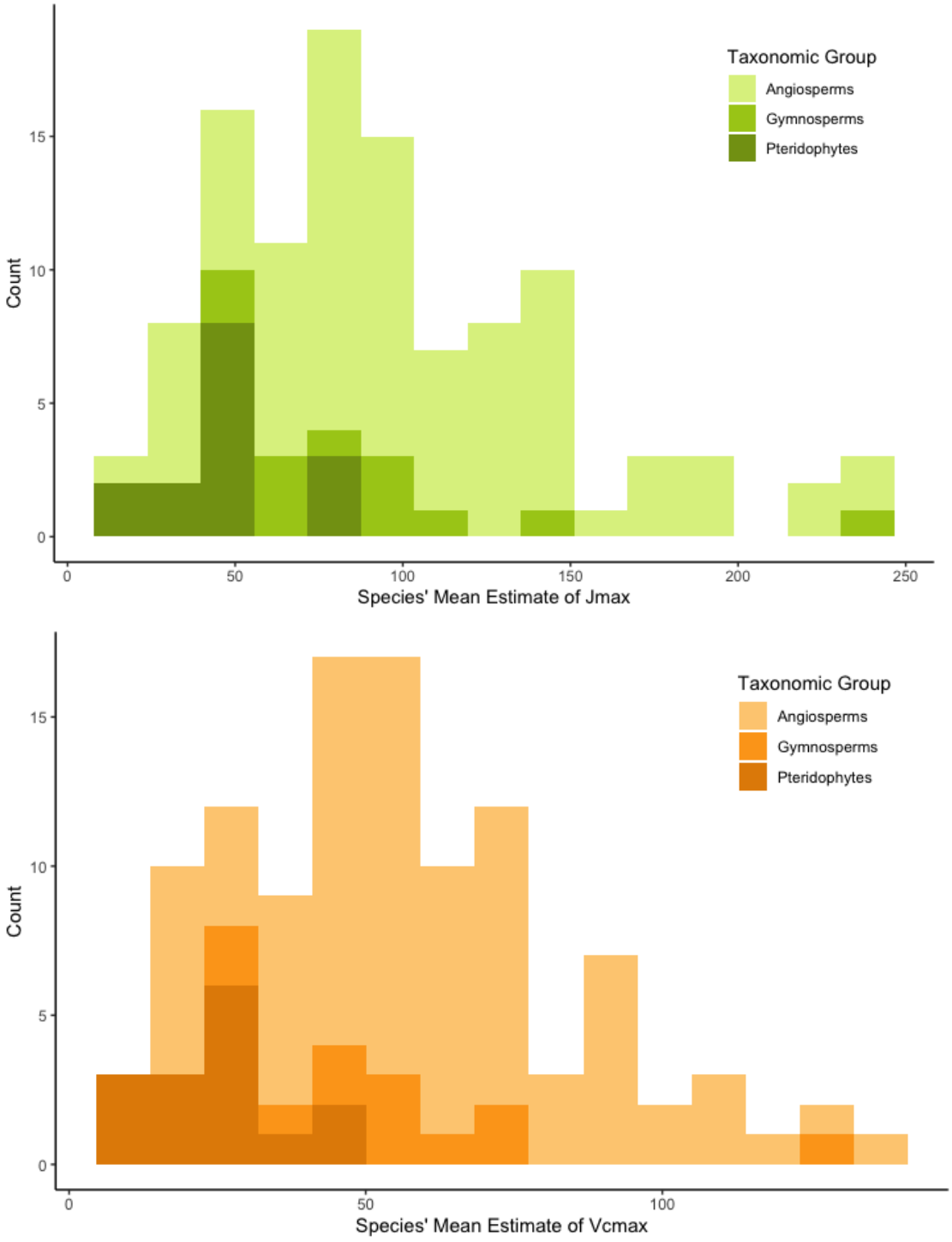

Figure 1. Histogram of the J_{max} and V_{cmax} estimates from our macroevolutionary common garden. Angiosperm taxa are shaded in the lightest colour, followed by Gymnosperm taxa shaded in a darker colour and Pteridophyte taxa, shaded in the darkest colour.

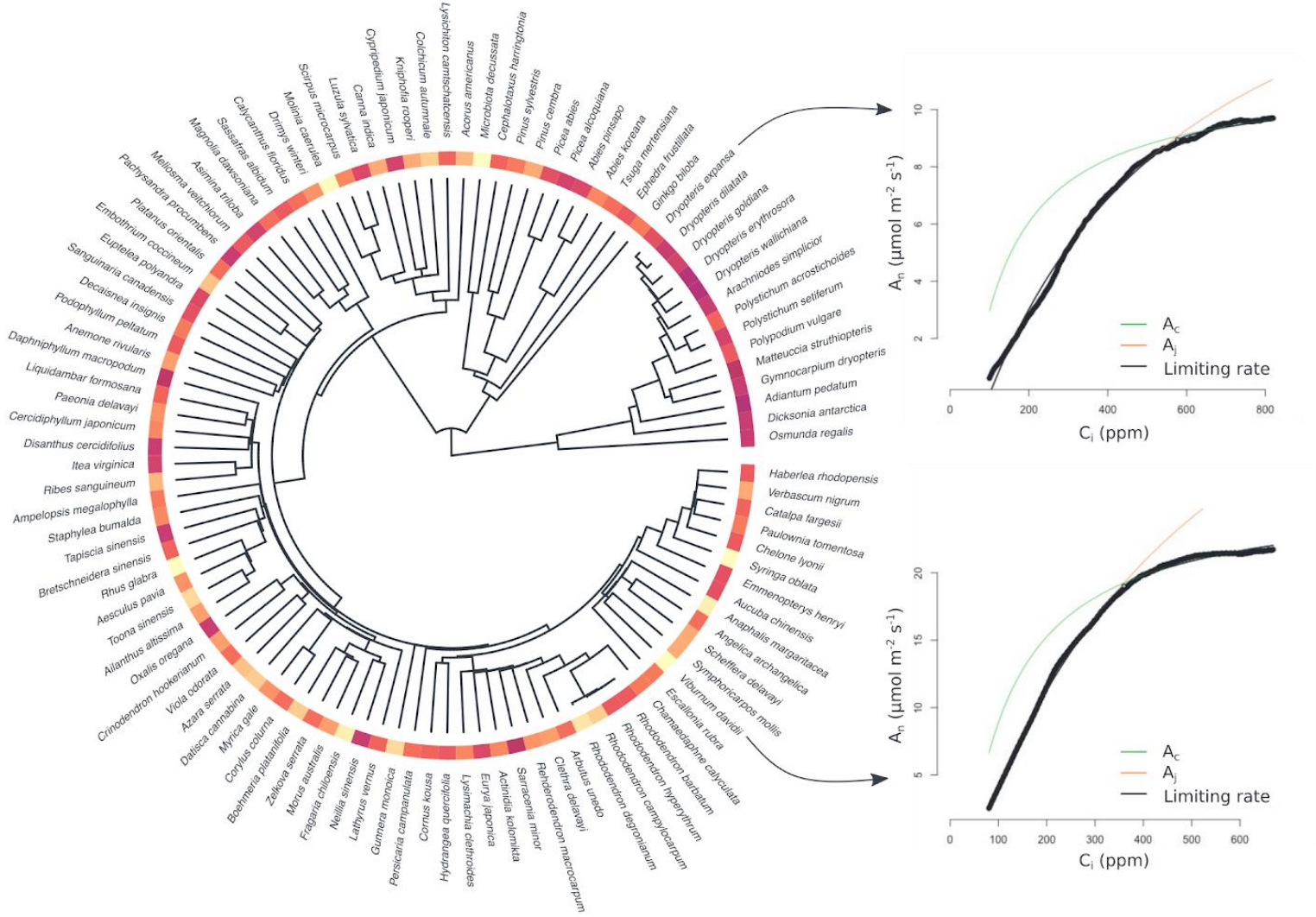

Figure 2. Pruned vascular plant phylogeny of 106 species, depicting the structure of species' mean V_{cmax} estimates from our macroevolutionary common garden. Low V_{cmax} estimates are shown in dark shades and high values in light shades. Two examples of the RACiR curves these estimates are derived from are shown on the right; for Dryopteris expansa's low V_{cmax} estimate (top panel) and Viburnum davidii's high estimate (bottom panel).

We can infer that V_{cmax} and J_{max} are more tightly constrained in their evolution than leaf area, mass and carbon, and that leaf nitrogen has little phylogenetic structure. All traits share an increase in divergence at shallow macroevolutionary timescales, while at deeper timescales the divergence of J_{max}, V_{cmax} and Nitrogen is bounded. (Figure 3). J_{max} and V_{cmax} follow similarly shaped regimes, levelling off in their trait divergence beyond divergence times of ~100 MY (Figure 3). This shows support for a model of bounded trait evolution. In contrast, patterns of trait divergence in leaf area, mass and carbon seem to follow a Brownian motion-like process, as they do not reach a bound and these traits share higher estimates of phylogenetic half-life. Leaf nitrogen's bounded trait divergence and negligible phylogenetic half-life suggests that it is a trait with little phylogenetic structure.

The differences in phylogenetic structure are insensitive to the few very deep splits included in the dataset. In order to evaluate whether our results may be driven by the smaller number of comparisons between species in deeply divergent lineages (e.g., between Angiosperms and Gymnosperms), we fit the same models as used above to Angiosperm only and Angiosperm plus Gymnosperm subsets of our dataset (Figure S3). While the exact estimates of half-life fluctuate in doing so, the overall trend of distinct half-life for photosynthetic traits compared to functional ones remains, allowing us to reject the possibility that these are driven by substantial differences between deeply divergent groups.

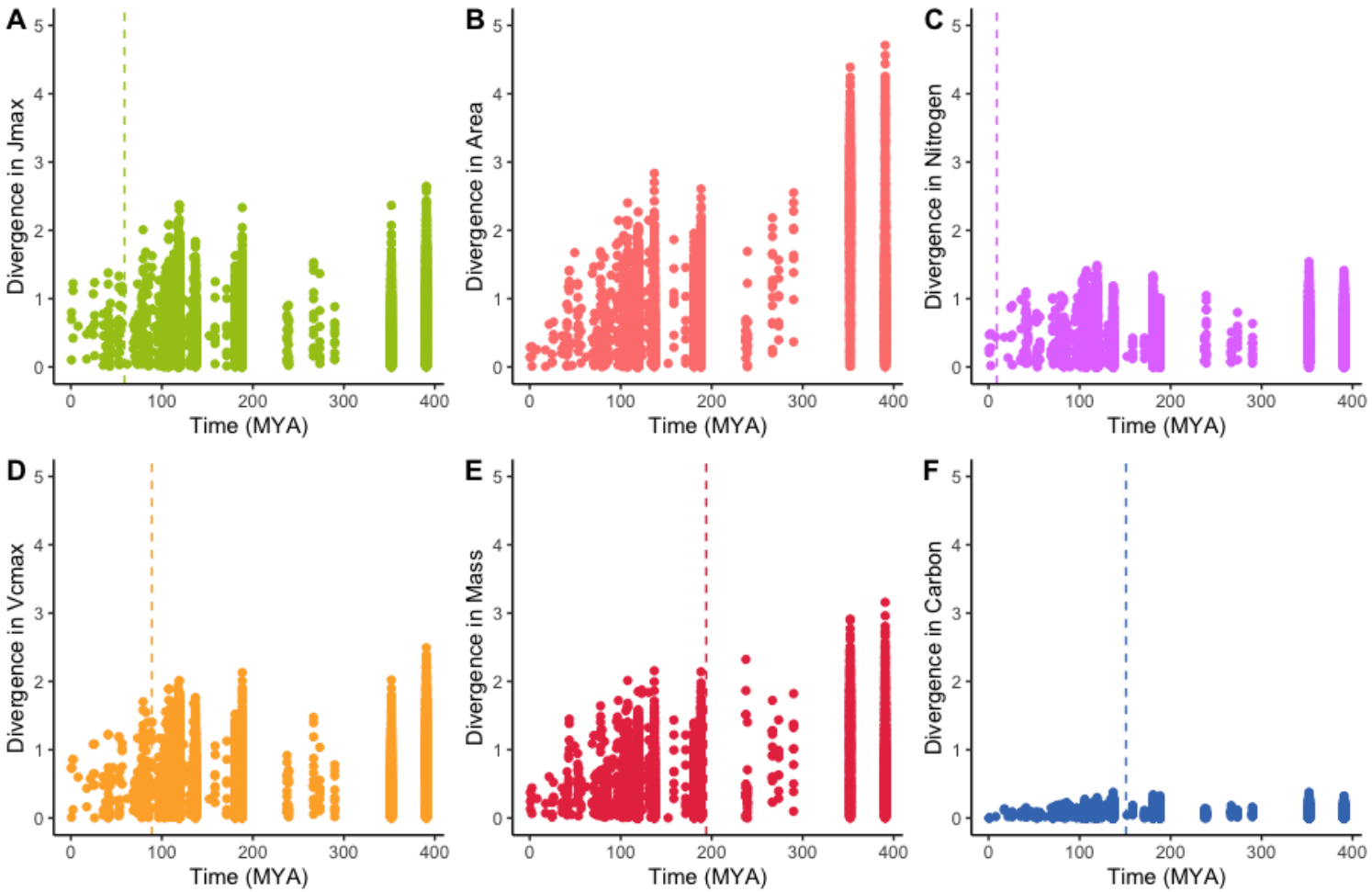

Figure 3. Divergence through time plots of J_{max} (A), Leaf area (B), Leaf Nitrogen (C), V_{cmax} (D), Leaf mass (E) and Leaf carbon (F). Divergence is the log difference between two trait values, corrected by their dimensionality (ie. whether these are mass, area or linear based measurements). The absolute values of Divergence (a unitless measure) are plotted along the y axis and time, measured in millions of years (MYA) is plotted on the x axis. Using our novel 'macroevolutionary common garden' dataset, containing trait data on 106 species; each point depicts the absolute value of the estimated trait divergence between two taxa drawn from our dataset. Each divergence estimate is plotted against the time since two species shared a common ancestor (i.e. time since MRCA) in millions of years. The dashed line in each panel depicts the estimated phylogenetic half-life for that trait. The half-life estimate for area (estimated as 3.64×1014 MYA) is omitted from panel B for visual ease.

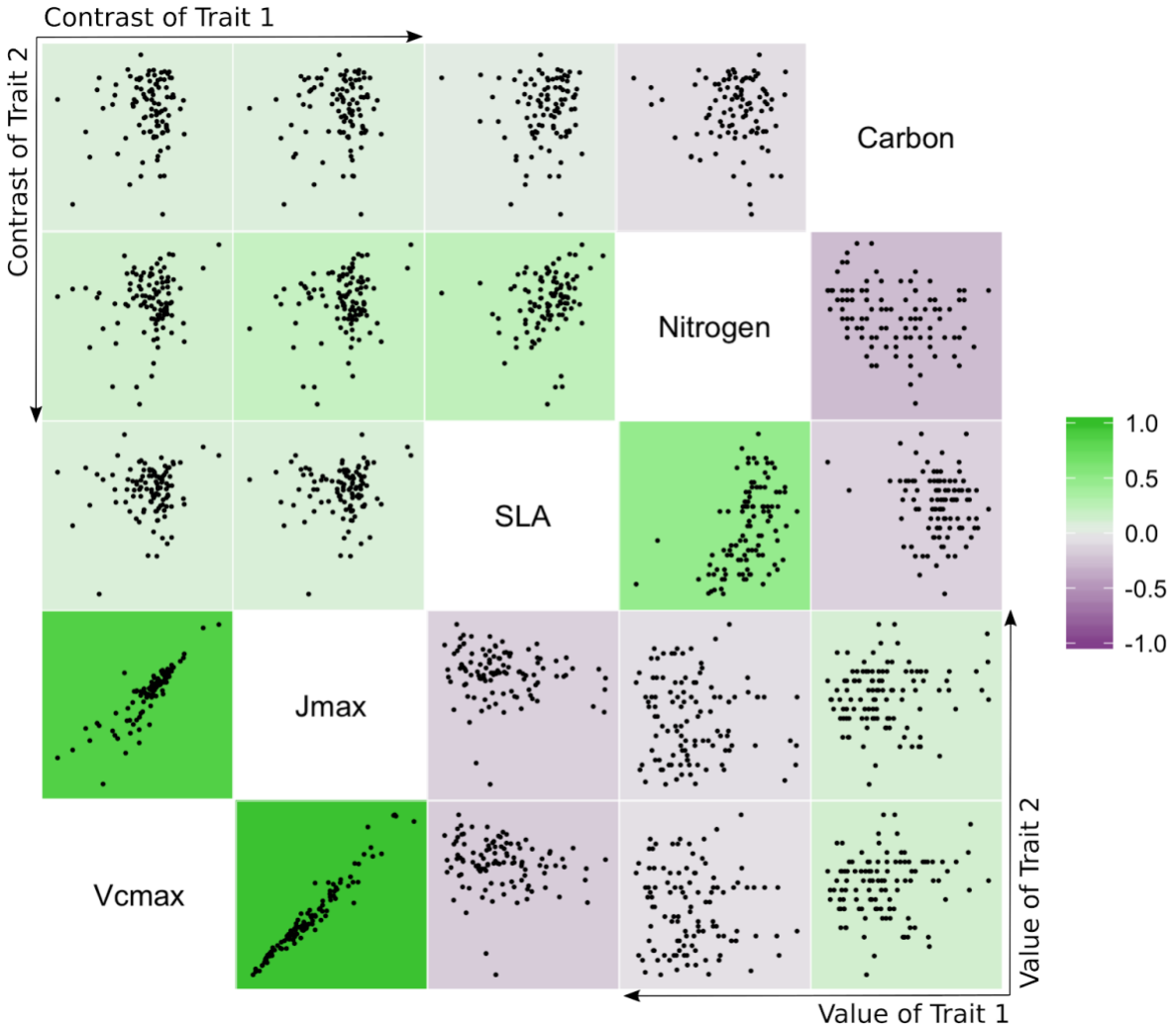

Figure 4. Scatter plots of all pairwise combinations of the phylogenetic independent contrasts (PICs) for J_{max}, V_{cmax}, Specific Leaf Area (SLA), Leaf Nitrogen and Leaf Carbon. The background of each scatter plot is coloured corresponding to the strength of the correlation between the two PICs, with green tones depicting positive correlations and purple tones depicting negative correlations.

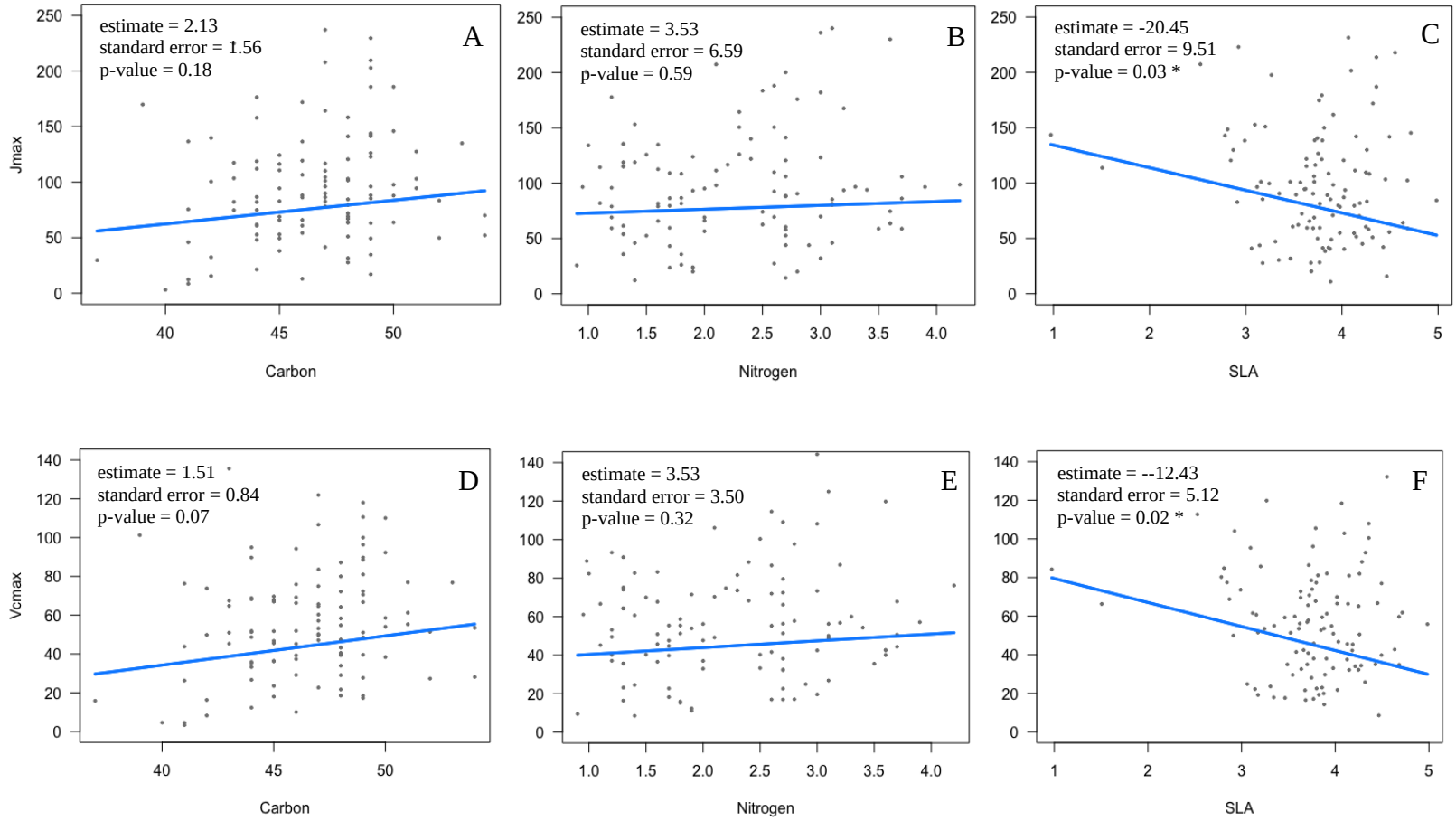

Figure 5. Partial-residual plots showing the relationships between J_{max} (panels A, B & C) or V_{cmax} (panels D, E & F) and Carbon, Nitrogen and SLA. Summaries of the phylogenetically corrected model fits are presented in the top left corner of each subplot.

Functional traits are poor predictors of a species' J_{max} and V_{cmax} values. When we use phylogenetic linear models to predict either J_{max} or V_{cmax} using SLA, leaf nitrogen and leaf carbon and species' identity, only SLA shows a significant relationship with either J_{max} or V_{cmax} and this relationship appears to be driven by two data points (Figure 5).

Only J_{max} and V_{cmax} show strong evidence of both correlated states and correlated rates of evolution, despite evidence of pairwise correlations in trait values of many trait combinations (Figure 4). This indicates that while the present states of these traits show more inter-relatedness than we might expect by chance, this does not seem to be the result of coupled evolution of these traits.

Conclusion

We found substantial variation in photosynthetic strategies, and this variation is phylogenetically structured. Photosynthetic capacity shows a more bounded evolutionary trajectory than morphological or functional traits. We also show that functional traits are poor predictors of photosynthetic capacity and that correlations between traits do not necessarily reflect correlated evolution.

The substantial and phylogenetically structured variation in photosynthetic capacity that we measure extends previous broad scale comparisons of photosynthetic traits (Gago et al. 2019) and importantly, reduces the impact of sampling across environmental differences. We also expand sampling of relatively underrepresented non-angiosperm taxa. The generality of our claim is of course derived from the taxa present in our common gardens - and by default this requires that plants be able to grow in the coastal temperate climate of Vancouver. We expect that a similar study conducted in a different climatic zone may differ in its specific findings (for example: in the exact breadth of measured variation). Nevertheless, we predict that the general finding of unmeasured variation, and low predictability of photosynthetic traits from functional traits will hold true across sampling efforts. We reason that it is unlikely that unmeasured variation would be clustered in this coastal temperate climate and not also present in others.

Our results highlight a fundamental mismatch; photosynthetic capacity and functional traits do not share the same evolutionary dynamics. That is, while we often use functional traits as proxies for metabolic ones, our results show that i) functional traits are poor predictors of photosynthetic capacity and ii) they hardly follow the same evolutionary trajectory: each suite of traits has a different range of estimates of phylogenetic half-life. For example, while there is phylogenetic signal in leaf area (throughout the phylogeny) there is little phylogenetic signal in

photosynthetic traits (J_{max} and V_{cmax}) on timescales deeper than 50 - 80 MY. This means that while it might be reasonable to use family-level means for studying certain functional traits because there is phylogenetic signal throughout taxonomic ranks, this is not the case with photosynthetic traits, for which there is a more imminent bound to the useful phylogenetic information.

We also detect a second element to this mismatch: correlated traits need not share correlated evolution. In particular, we infer trait tradeoffs in leaf construction from negative trait correlations, but we find that these correlations do not hold true over macroevolutionary timescales. If these tradeoffs are indeed driven by functional constraints, we would expect trait-for-trait change that would yield strong correlations in traits' rates of evolution. We do see this trait-for-trait constraint in the rates of evolution of J_{max} and V_{cmax}: and given that we know J_{max} and V_{cmax} are truely and tightly linked, this can serve as a null model for how interdependent traits co-evolve. This trait-for-trait change is missing from functional traits' tradeoffs. An example of this is that carbon and nitrogen display a negative correlation in trait space, but a much weaker correlation in their rates of evolution. Our results suggest that caution is warranted when making causal inferences from trait correlations. However, our approach also provides a way forward for more directly testing correlation and better assessing causation on these evolutionary timescales.

It would be fruitful to more directly incorporate variation in vegetative forms into ecosystem models. Different ecosystems are hosts to an array of different taxonomic compositions - our results suggest that this likely reflects substantial variation in metabolic behaviour that is unlikely to be well predicted from morphological or functional traits. If our aim is to account for variation in metabolism, we should measure it. Using already measured species or genus level differences (which still remain within the bound of useful phylogenetic information) likely

incorporates a more realistic range of variation than assuming data from one species can be scaled across environments and evolutionary structure.

While functional traits have been suggested as a way to incorporate more of plants' metabolic diversity into earth system models, we show that these traits are not the proxies we hoped they would be and using them as such will likely incorporate more error. Implementing and incorporating metabolic variation will require both i) more direct measures of plant metabolism and ii) more comprehensive knowledge of the species composition of specific ecosystems into the models used. This is not to say that functional traits are useless - however, it does mean that we should more carefully consider what information may or may not be extrapolated from them.

MTE and trait ecology will benefit from better integration with macroevolutionary theory. If we wish to explain and better describe variation, an evolutionary perspective can inform us of the causality underlying trait correlations. The mismatch between trait correlations and the correlations in traits' rates of evolution that we present demonstrate the usefulness of this approach.

At the beginning of this thesis I proposed that there is a gap in our knowledge of photosynthetic variation and its evolution. I showed that there is in fact substantial, phylogenetically stratified variation and that it does not share the same evolutionary trajectory as functional traits. I suggest that this creates a potential for misrepresenting ecosystem function and vegetations' metabolism in broader efforts to model ecosystem behaviour. I show that functional traits act as poor predictors of photosynthesis and therefore, are not good proxies for metabolic variation. I also show that trait correlations do not translate into evolutionary correlations. This observation is crucial to better understanding, explaining and predicting metabolic variation from a subset of taxa moving forward.

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Appendices

Supplementary Materials

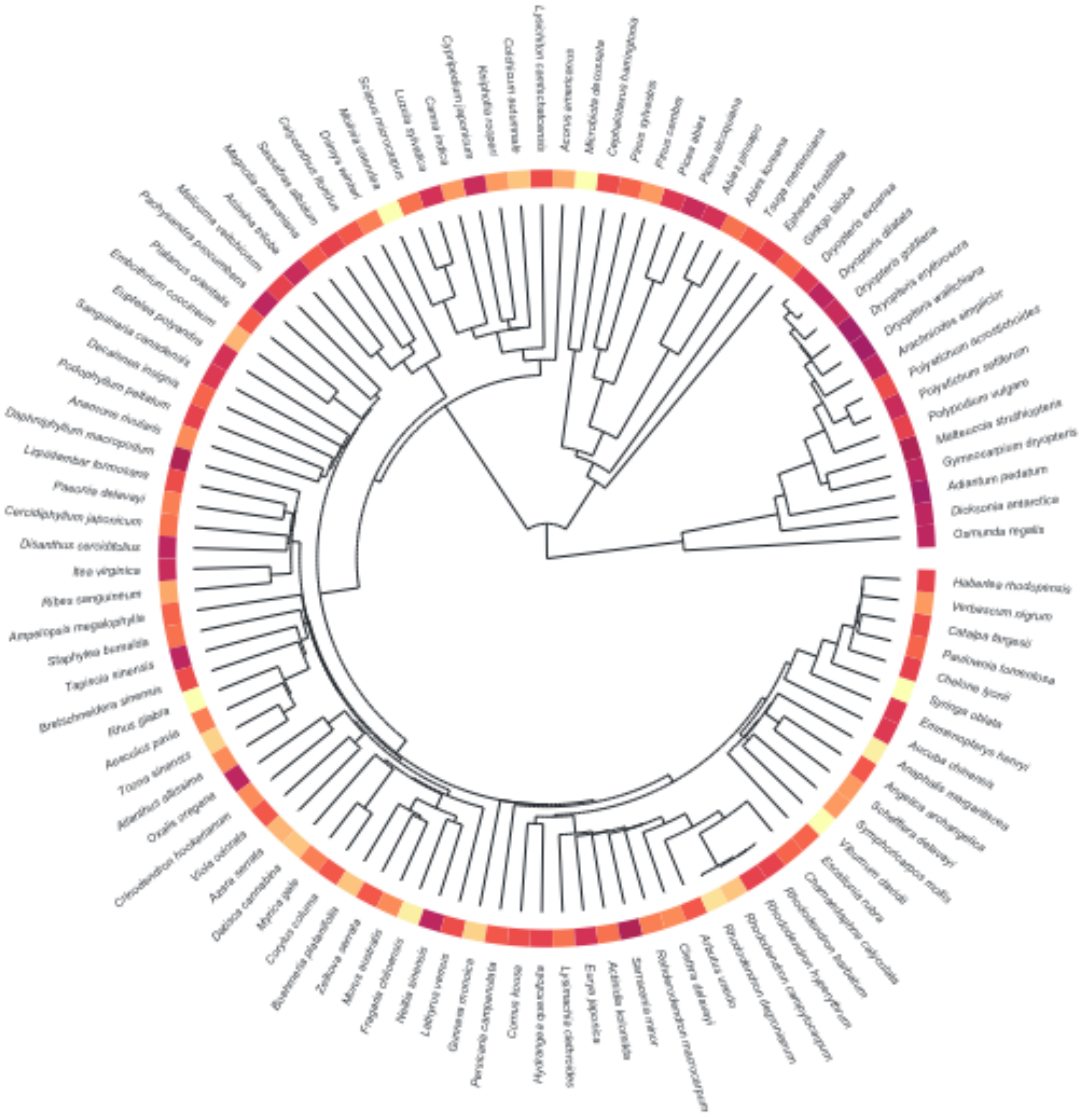

Figure S 1. Pruned vascular plant phylogeny of 106 species, depicting the structure of species' mean V_{cmax} estimates from our macroevolutionary common garden. Low V_{cmax} estimates are shown in dark shades and high values in light shades.

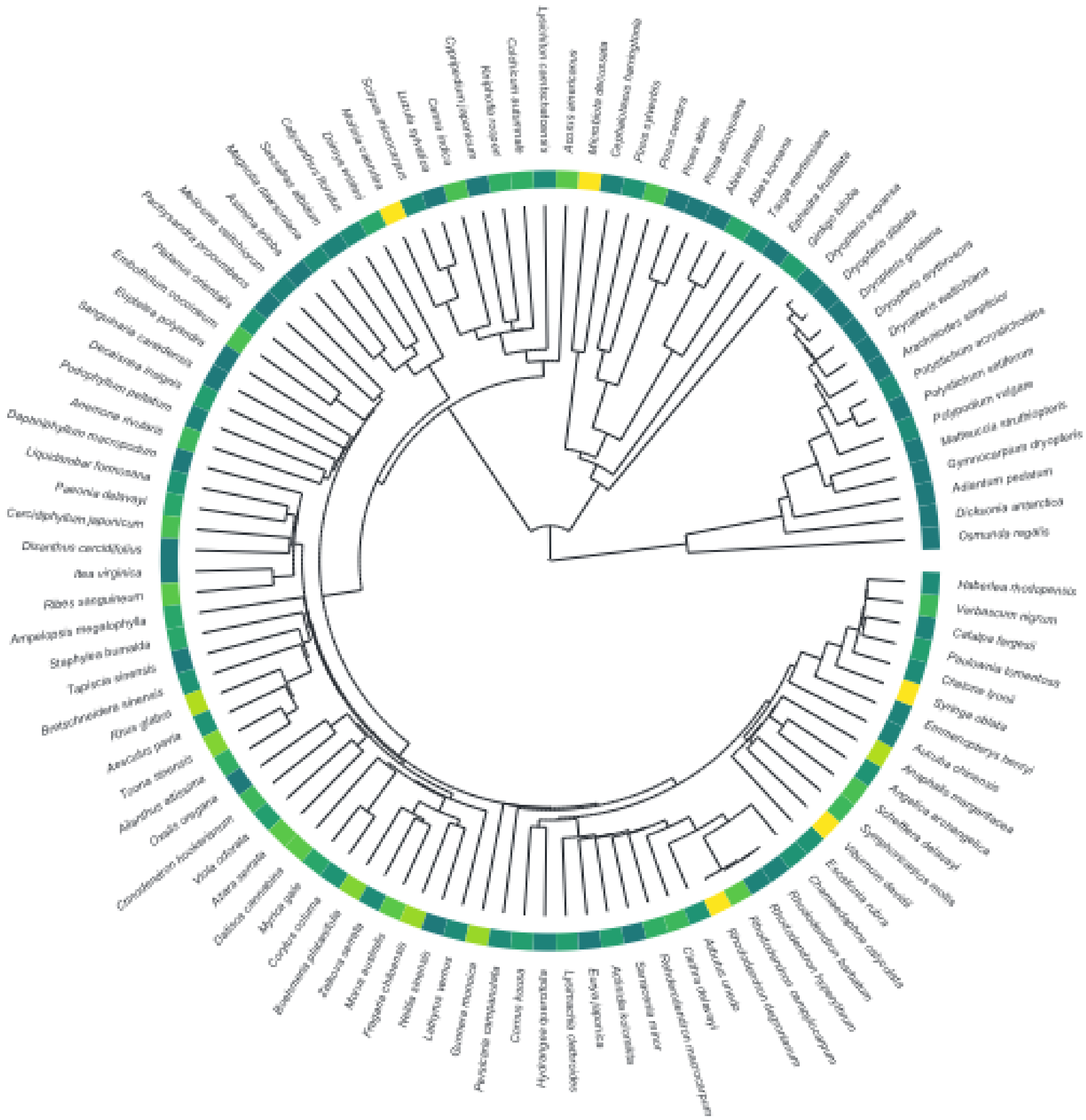

Figure S 2. Pruned vascular plant phylogeny of 106 species, depicting the structure of species' mean J_{max} estimates from our macroevolutionary common garden. Low J_{max} estimates are shown in dark shades and high values in light shades.

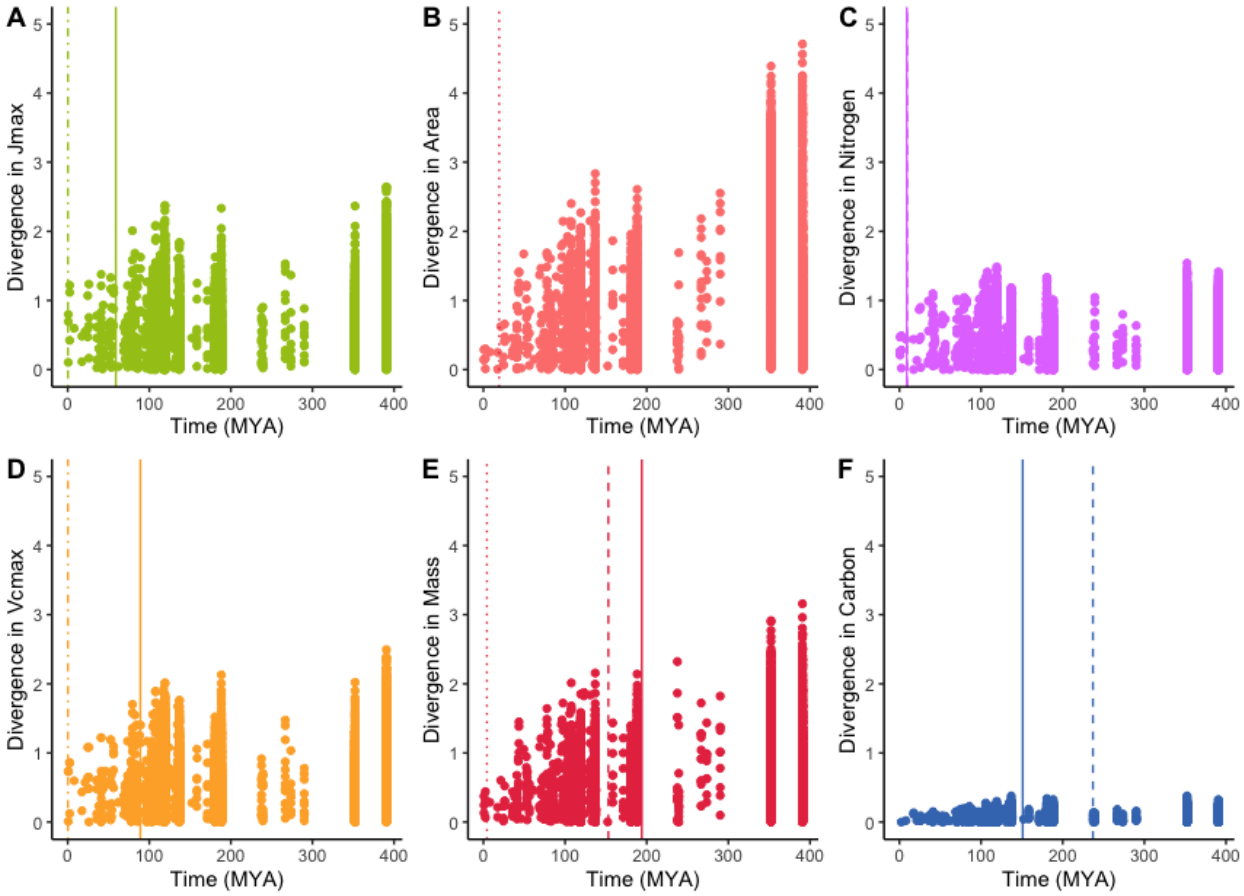

Figure S 3. Divergence through time plots of J_{max} (A), Leaf area (B), Leaf Nitrogen (C), V_{cmax} (D), Leaf mass (E) and Leaf carbon (F) (as in Figure 3). The solid line in each panel depicts the whole-dataset estimate for phylogenetic half-life. The dashed line in each panel depicts the phylogenetic half-life for that trait, estimated from only Angiosperm and Gymnosperm data. The dotted line in each panel depicts the phylogenetic half-life for that trait, estimated from only Angiosperm data. Mixed dashed and dotted lines depict consensus between the Angiosperm only and Angiosperm + Gymnosperm estimate. For visual ease, the whole dataset half-life estimate for area (estimated as 3.64e+14 MY), and Angiosperm plus Gymnosperm estimate (estimated as 1289 MY) are omitted from panel B, and the Angiosperm only half-life estimate for carbon (estimated as 724 MY) is omitted from panel F.