Form, Function, and Phylogeny: Angiosperm Leaf Trait Evolution, with a Case Study in the Genus *Dioscorea*

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Abstract of the Dissertation

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Broad-scale correlations among leaf traits or between leaf traits and the environment support the hypothesis that natural selection in response to climate has played a major role in plant diversification. Yet species and leaf trait diversity is ultimately the result of divergences among closely related species. I examined trait relationships across these two scales to compare the roles of evolutionary history and natural selection in the diversification of leaf forms. This first chapter of this dissertation provides the first global-scale, phylogenetically based demonstration of relationships between leaf vein patterns and leaf functions in angiosperms. Minor vein density was significantly correlated with maximum photosynthetic rate, supporting the hypothesis of correlated evolution of leaf hydraulic capacity and photosynthetic ability. Evolutionary shifts in secondary vein type were accompanied by shifts in leaf life span, suggesting an adaptive relationship that is consistent with previously observed relationships between leaf form and climate. In contrast, the relationship between primary vein type and maximum photosynthetic rate appears to reflect the phylogenetic distribution of leaf traits, rather than adaptive co-evolution. The second chapter was at a narrower phylogenetic scale, but broad geographic scale. I examined relationships among leaf traits that are important for gas exchange and water delivery within the genus *Dioscorea*, and compared them to expectations from large-phylogenetic-scale studies. Some relationships within this genus were consistent with large-scale studies, while others were strikingly different and may indicate constraints among close relatives. This suggests that how species diversify along leaf trait co-variation axes will depend on the unique combinations of traits and ecological challenges present in different lineages. The third chapter examined a different set of 20 *Dioscorea* species collected from Mexico. I determined that species' values of many leaf functional traits were correlated with the climate in which they occur using standard correlation methods, but not using phylogenetically-based methods. The same set of traits, and climate parameters were phylogenetically conserved. These results suggest that while these leaf traits are important for adaptation to climate, their current association with climate is a result of earlier adaptation followed by niche conservatism, rather than repeated adaptive evolution. The traits that I expected to be under selection by micro-environmental factors were not significantly correlated with climate parameters using either method and were not phylogenetically conserved. The relationship between whether or not traits were correlated with climate and whether or not they were phylogenetically conserved supports the notion that niche conservatism is tightly linked to functional trait conservatism. The combination of conserved traits and niches at one scale with labile traits and niches at another be responsible for the high diversity of Mexican *Dioscorea* species. The research presented in this dissertation demonstrates how the complementary processes of change (adaptive evolution) and lack of change (phylogenetic conservatism) may act together to generate biodiversity.

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List of Symbols and Abbreviations

Term	Definition	Units
A_{area}	maximum photosynthetic rate on an area basis (Chapter 1 or 2)	μmol CO_2 m^{-2} sec^{-1}
A_{mass}	maximum photosynthetic rate on a mass basis (Chapter 1)	nmol CO_2 g^{-1} sec^{-1}
A_{max}	maximum photosynthetic rate on a mass basis (Chapter 2)	μmol CO_2 g^{-1} sec^{-1}
Capacitance	change in relative water content with change in water potential, a measure of leaf water holding capacity	MPa^{-1}
c_i	leaf internal CO2 concentration	not used in this document
GCL	guard cell length	mm
g_{min}	minimum conductance, also cuticular conductance. Loss of water per time of leaves with fully closed stomata	mmol H_2O m^{-2} sec^{-1}
g_s	stomatal conductance	mol H_2O m^{-2} sec^{-1}
K_{leaf}	leaf hydraulic conductance	Mpa-1 m-2 sec-1
$K_{t\text{-}mr}$	theoretical midrib conductivity (function of the number of midrib conduits and their size)	mmol m Mpa^{-1} sec^{-1} x 10^3
Lamina area	lamina area	cm^2
LLS	leaf life span	months
LMA	leaf mass per area	g m^{-2}
L:W	length to width ratio	unitless
MR TE area	average area of the tracheary elements in the midrib	mm
MR VB area	cross-sectional area of the midrib vascular bundle	mm
MVD	minor vein density	mm mm^{-2}
N content, %N	nitrogen content of dry leaves	unitless
N_{area}	N content on an area basis	g m^{-2}
N_{mass}	N content on a mass basis, same as N content or %N	unitless
Pet area	cross-sectional area of the petiole	mm^2
Pet VB area	cross-sectional area of all vascular bundles in the petiole	mm
PVD	primary vein density	cm cm^{-2}
RWC	Relative water content	unitless
SD	stomatal density	#stomata mm-2
SI	stomtal index	#stomata/#guard cells
SPI	stomatal pore index (SDxGLC^2)	unitless

WC	water content (mass of water/wet mass at full turgor)	unitless
WUE_{inst}	instantaneous water use efficiency	μmol CO_2 mol^{-1} H_20
Ψ_{min}	leaf minimum water potential	MPa
$\delta^{13}C$	carbon isotope discrimination	parts per thousand (unitless)

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INTRODUCTION

FORM, FUNCTION, AND PHYLOGENY: WHY ARE THERE SO MANY DIFFERENT LEAF FORMS?

Since the evolution of the first true leaves about 350 MYA, a great of diversity of leaf forms and functions have arisen. While some leaves serve highly specialized functions, like the brightly colored bracts of poinsettias or the insect-trapping leaves of pitcher plants, most leaves serve primarily to increase the surface area available for light absorption and gas exchange. Explaining the diversity of forms among these “normal” leaves presents an exciting challenge for evolutionary ecologists. Through my dissertation research, I tested two interacting explanations of how leaf trait variation in angiosperms arises and is maintained. The first is that variation in angiosperm leaf form is the result of natural selection acting on leaf function. This is based on the premise that there is a one-to-one link between leaf form and leaf function, and that variation in leaf function allows for optimal fitness under different environmental conditions. Taken to an extreme, this explanation suggests that all leaves that occur in the same environment should have the same form, and any variation in form at one location is due to small-scale differences in environmental conditions. However, unique events in the history of different lineages may also contribute to current diversity of leaf forms. If there are multiple combinations of traits leading to roughly equal fitness, then variation in one site may reflect the independent evolutionary trajectories of different lineages. This explanation is also based on natural selection and a link between leaf form and function, but it allows for multiple fitness optima, based the need for leaves to fulfill multiple functions (Niklas 1994; Gutschick 1999; Press 1999; Marks and Lechowicz 2006). In either case, variation may exist because species are not able to evolve the optimal combination of trait values for their environment, due to a plethora of possible constraints.

There is evidence that variation in leaf form is the result of natural selection acting on leaf functions in different environments. Convergence among distantly related species occupying similar habitats suggests that leaf form is under strong selection by the environment (Bailey and Sinnott 1916). For example, small, sclerophyllous leaves occur in Mediterranean ecosystems throughout the world, spines occur in desert plants in both the new world (Cactaceae) and old (Euphorbia), and cordate leaves are common in climbing plants (Givnish and Vermeij 1976; Goodwillie et al. 2004). Similar evidence comes from correlations between leaf traits and environmental factors that have been observed at large phylogenetic scales, such as the relationship between leaf size and precipitation or nutrient availability (Wolfe 1995; Wilf et al. 1998; Fonseca et al. 2000; Wright et al. 2001), the relationship between leaf hydraulic conductance and regeneration irradiance (Sack et al. 2005), or the relationship between stomatal or vein density and factors that contribute to vapor pressure deficit (Gutschick 1999; Uhl and Mosbrugger 1999). Global-scale relationships among leaf traits, such as the negative correlation between photosynthetic capacity and leaf life span (Reich et al. 1997; Reich et al. 1999; Wright et al. 2004b) suggest that certain evolutionary trade-offs exist for all land plants, and provide a context in which natural selection can lead to diversification along trade-off axes (Westoby et al. 2002; Westoby and Wright 2006).

At the same time, large-scale studies reveal significant variation in trait values within one site (Chaves et al. 2002; Wright et al. 2004b), and form-function relationships are not always consistent among taxa or environments (Woodward 2008). Theoretical work has shown that historical contingencies can lead to multiple alternative solutions to the same problem of how to maximize fitness in a particular habitat (Niklas 1994; Marks 2007). This supports the hypothesis that the variation in plant form within one site is related to the history of the species that occupy that site. There is a growing body of evidence that phylogenetic history can predict plant function, sometimes better than the environment in which the plants occur (Ackerly and Reich 1999; Cavender-Bares and Holbrook 2001; Edwards and Donoghue 2006; Bhaskar et al. 2007; Edwards and Still 2008; Hao et al. 2008). Resolving the conflict between the expectation of consistent form-function relationships and the reality of plant diversity calls for integrated knowledge of current plant function and historical data.

In this dissertation, I used a phylogenetic, comparative approach to bring together information on species' evolutionary history with information on their leaf form and function. Each of the chapters in the dissertation was designed to test some aspect of the hypothesis that variation in angiosperm leaf form is the result of natural selection acting on leaf function. Crucially, each study also considered the alternative hypothesis that historical events, as reflected in phylogenetic patterns, may be as important as selection by current environmental factors. I carried out three inter-related studies, each at different phylogenetic and spatial scales, each dealing with different aspect of leaf functional diversity.

In the first chapter, I examined the functional significance of different vein patterns across angiosperms. I compared major and minor vein patterns to leaf functional traits whose importance for fitness is well studied. In this way, I was able to link natural selection to macro-evolutionary patterns of trait variation. Despite being one of the most prominent aspects of leaf morphology, very little is known about how major vein patterns relate to variation in leaf function, and this is one of the first studies to examine these relationships (Roth et al. 1995; Roth-Nebelsick et al. 2001; Zwieniecki et al. 2004; Niinemets et al. 2007; Sack et al. 2008). Although several recent studies have examined relationships between minor vein density and leaf function (Uhl and Mosbrugger 1999; Sack and Frole 2006; Brodribb et al. 2007; Boyce et al. 2009), none has used phylogenetic methods to determine if the relationships they found are a result of repeated co-evolution or the product of a few divergences in minor vein patterns and leaf functions. While this distinction is not necessary for predicting leaf function from minor vein density, it is important for understanding the evolutionary processes that resulted in variation in minor vein density, and for predicting the evolutionary response of minor vein density to changes in environmental conditions.

In the second chapter, I examined correlations among twenty leaf functional traits in twelve species of the genus *Dioscorea* from throughout the world. I compared these relationships to what has been observed at larger phylogenetic scales. All *Dioscorea* are herbaceous vines, a growth form that is not well represented in leaf structure-function studies. My goal was to assess how constraints arising from the unique traits found in specific clades, such as one containing herbaceous monocot vines, might cause structure-function relationships at small phylogenetic scales to differ from those at larger scales. This is the first study, to my knowledge, to actually measure both leaf structural traits (midrib xylem conduit size, stomatal size and density, and nitrogen content) and physiological functions (hydraulic conductance, maximum photosynthetic rate, and leaf water potential) on a set of closely related species. Previous studies have assumed that structure-function relationships among close relatives are the same as in large-scale studies (e.g., Edwards 2006; Dunbar-Co et al. 2009), so I wanted to test the accuracy of this assumption. By measuring mutual correlations among a large set of interdependent traits, I was able to develop an integrated view of how evolutionary changes in leaf structures affect their functions, and to posit explanations for some of the unexpected relationships I found.

The third chapter was at the smallest spatial and phylogenetic scale. I examined the correlations between leaf functional traits, climate, and phylogeny in twenty Mexican *Dioscorea* species. Mexico has high diversity and endemism of *Dioscorea* and many other taxa, and also has high variation in elevation, temperature, and precipitation within a small area. I wanted to determine if natural selection on functional traits that are important for adaptation to climate played a role in the diversification of Mexican *Dioscorea*. I used a common garden study to measure variation in leaf functional traits among species, then analyzed correlations between functional traits and the climate in which each species occurs. I used both standard and phylogenetic correlation methods, and then assessed how phylogenetic conservatism of both traits and species' climate associations affected the correlations. Few researchers have examined the phylogenetic patterns of leaf physiological or functional traits, and the emphasis to date has been on C3/C4 photosynthesis (e.g., McKown and Dengler 2007; Edwards and Still 2008; Cerros-Tlatilpa and Columbus 2009). This study provides one of the first tests of phylogenetic conservatism in leaf functional traits.

Several themes run throughout my dissertation. First is the importance of incorporating phylogenetic information into studies of leaf structure-function relationships. Only by considering evolutionary relationships among species is it possible to determine whether a correlation between two traits arose through repeated co-evolution of the traits. While this information may not be important for understanding the current ecological significance of a trait (Westoby et al. 1995), it is crucial for determining how the ecology of different species and their traits contributes to diversification. With phylogenetic information, I was able explore multiple causes of why there are so many different leaf forms.

The second theme of my dissertation is the comparison of leaf trait relationships across different phylogenetic and spatial scales. Correlations among leaf traits may vary with sample size and spatial scale (Wright et al. 2004a), and different evolutionary patterns can occur at different scales (Cavender-Bares et al. 2004). Explicit comparisons of patterns at different scales can inform evolutionary studies, by providing insight into the potential constraints operating at different scales. Genetic constraints such as pleiotropy or epistasis are more likely to lead to trait correlations at smaller phylogenetic scales, since genetic correlation may be broken at larger scales (Armbruster et al. 2004). The same is true of certain functional constraints. For example, among closely related species, the size of the midrib vessel may be correlated with a leaf's hydraulic conductance (see Chapter 2), but across distantly related species, changes in major vein patterns may obscure this relationship (Sack and Frole 2006). Some leaf trait relationships may arise from convergence at larger scales (e.g., the relationships among maximum photosynthetic rate, leaf mass per area, and N content; Wright et al. 2004b), but be absent at smaller scales, due to constraints such as lack of variation or alternative selection pressures (Givnish et al. 2004; Dunbar-Co et al. 2009). I specifically address this issue in Chapter 2, but also find evidence of potential constraints in the other chapters, such as the lack of transitions among major vein types in Chapter 1 or phylogenetic niche conservatism in Chapter 3.

In the three chapters that follow, I provide evidence that leaf form diversity is related to leaf functional diversity (Chapters 1 and 2) and that functional diversity is related to environmental variation (directly in Chapter 3 and indirectly in Chapter 1). This evidence includes relationships that have never been described (such as major vein patterns and leaf economic traits) and relationships that have not been examined in closely related species (many of the relationships described in Chapters 2 and 3). I also provide new evidence that some aspects of leaf form and function are phylogenetically conserved. Neither the idea that leaf form variation is a result of natural selection on leaf function nor the idea that leaf traits are phylogenetically conserved is new, but research that combines structure-function relationships with phylogenetic patterns is rare. It is the combination of approaches that allowed me to gain a new perspective on leaf trait variation.

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CHAPTER 1

PHYLOGENY AND ADAPTATION IN THE EVOLUTION OF ANGIOSPERM VEIN PATTERNS

Abstract

This study provides the first global-scale, phylogenetically-based demonstration of relationships between leaf vein patterns and leaf functions. I examined relationships between angiosperm vein patterns and leaf economic traits, that is, the functional traits of the global leaf economic spectrum (LES; Wright et al. 2005, Nature 428:821-27). The leaf trait correlations of the LES describes a trade-off between plants' ability to construct leaves that live a long time versus leaves that have high physiological activity. Using standard ANOVA, I found highly significant relationships between primary vein type (pinnate, palmate, or parallel) and leaf economic traits [maximum photosynthetic rate (A_{max}), leaf N content, and leaf mass per area (LMA)]. These relationships appear to reflect the phylogenetic distribution of leaf traits, rather than adaptive co-evolution, since none of them were significant using phylogenetically controlled tests. In contrast, relationships between secondary vein type (open, intermediate, or closed at the leaf margin) and the LES were significant using both standard and phylogenetically controlled methods. The repeated co-evolution of open secondary veins with shorter leaf life span and higher N content suggests an adaptive trade-off between high physiological activity and the structural support provided by secondary vein tissue at the leaf margin. Across angiosperms, minor vein density was significantly related to A_{max}, using both standard and phylogenetic methods. This finding adds broad support to the notion that leaf hydraulic capacity and photosynthetic ability evolve in a coordinated fashion and demonstrates that photosynthetic capacity is a species-level property. The relationship between minor vein density and leaf N content was weak, since minor veins provide increased conductive capacity at the cost of increased leaf carbon content. The connections I found among vein patterns, the LES, and phylogeny reveal the importance of venation diversification in the diversifictation of angiosperms. The results have important implications for estimating functional attributes of both living and fossil plant species and communities.

Introduction

Veins provide leaves with support, water delivery, and carbohydrate export and are crucial for maintaining leaf water status and photosynthetic capacity (Roth-Nebelsick et al. 2001; Sack and Holbrook 2006). Vein size, density, and arrangement are associated with leaf functions such as maximum photosynthetic rate, leaf hydraulic conductance and resistance to leaf damage (Boyce et al. 2009; Brodribb et al. 2007; Niinemets 2007; Sack et al. 2008; Sack et al. 2005; Salleo et al. 2003), but as yet, no one has examined these patterns at the global scale. The leaf economic spectrum (LES) summarizes variation in leaf function at a global scale, by demonstrating that across thousands of species from a range of biomes, growth forms, and taxa, relationships among maximum photosynthetic rate (A_{max}), N content, respiration rate, leaf mass per area (LMA), and leaf life span

(LLS) fall along one multivariate axis (Reich et al. 1997; Reich et al. 2003; Wright et al. 2004). The LES describes a trade-off between plants' ability to construct leaves that live a long time versus leaves that have high physiological activity, that has lead to the evolution of a limited range of leaf trait values across all land plants. This work has made a significant contribution to our understanding of leaf diversity, but it conspicuously lacks a link to leaf structure or form. In this study, I create such a link, by testing global scale relationships between angiosperm leaf vein patterns and leaf economic traits, that is, the functional traits described by the LES. The Glopnet database published with the LES (Wright et al. 2004) provides a large enough sample size to test functional hypotheses about vein patterns that might otherwise be obscured by variation in other leaf traits.

Angiosperm leaf veins are arranged in a hierarchical fashion, with primary veins originating at the lamina/petiole junctions, secondary vein branching from the primaries, and so on (Leaf Architecture Working Group, 1999). Most leaves have 4-7 orders of veins, with the primary and secondary veins considered major veins and the 3rd order and higher considered minor veins (Leaf Architecture Working Group, 1999). Major vein are larger in diameter than minor veins, with more xylem conduits, and can be thought of as a rapid distribution network, while minor veins act as the sites of exchange between the mesophyll and the vascular system (Haritatos et al. 2000; Sack and Holbrook 2006). Major vein patterns are generally conserved within genera and families, and are useful as taxonomic characters (Doyle and Endress 2000; Taylor and Hickey 1996). Little is known about phylogenetic patterns of minor veins, but they also may be useful as taxonomic characters [(Roth 1996), unpublished data, R. Walls]. I combined new data on major and minor vein patterns in angiosperms with data on leaf economic traits from the Glopnet database to test two functional hypotheses related to major vein patterns and one related minor veins. Although relationships in the LES are robust to phylogenetic analysis (Ackerly and Reich 1999), I used phylogenetic methods for all tests. Phylogenetic signal in vein patterns or leaf economic traits could influence their relationships, and reveal whether or not relationships arise from correlated evolution of leaf traits.

Primary veins can be classified as pinnate (a single primary vein running the length of the leaf), palmate (multiple primary veins that radiate from the petiole) or parallel (multiple primary veins that run roughly parallel from the petiole to the leaf apex; fig. 1A). I hypothesize that leaves with palmate or parallel venation should have higher A_{max} and N content and lower LMA than leaves with pinnate venation, based on two known properties of pinnate versus palmate/parallel veined leaves: support allocation and vascular redundancy. Among 44 herbaceous and woody species from one region, Niinemets et al. (2007) showed that pinnate leaves had a lower primary vein density, but invested more in support tissue outside the mid-rib, with a higher density of second through fourth order veins and higher C content in their laminas. These differences in support allocation suggest that pinnate veined leaves should have higher LMA, as well as lower N content and lower A_{max}, than palmate or parallel veined leaves. Niinemets et al. found slightly higher LMA and lower N content in pinnate veined leaves, but the difference was not significant (Table 3, Niinemets et al. 2007). The higher primary vein density of palmate or parallel veined leaves also provides redundancy in the highest level of the leaf vascular system (Sack et al. 2008). In a comparison of seven species, Sack et al. (2008) showed that palmate veined leaves maintained higher leaf hydraulic conductance (K_{leaf}) and transpiration rates than pinnate veined leaves after their midribs were severed. This property has not been tested in parallel veined leaves, but their high primary vein density should provide the same or higher redundancy as palmate venation. Sack et al. (2008) predicted that the damage tolerance conferred by vascular redundancy should be most advantageous for thin leaves that lack the alternative form of protection offered by thick, tough laminas. Based on the relationship established by the LES (Reich et al. 1997; Wright et al. 2004), this leads to the prediction that leaves with palmate or parallel venation should have lower LMA, higher A_{max} and higher N content than leaves with pinnate venation.

Secondary veins come in many different patterns (Leaf Architecture Working Group, 1999), but can be divided into two main classes: open and closed (fig. 1B). Open secondary veins, such as craspedodromous, cladodromous, and reticulodromous vein patterns, end at or near the leaf margin and do not connect directly to other secondary veins. Closed secondary veins connect directly to other secondary veins, as in brochidodromous venation, or are connected by another secondary or primary vein, as in the intramarginal vein pattern. Intermediate forms include semi-craspedodromous, weak brochidodromous, or eucamptodromous vein patterns. There are other secondary vein patterns that do not fit well into this classification, particularly among monocots such as grasses or palms, but the majority of angiosperms can be classified as open, intermediate or closed. Closed vein patterns appear to be ancestral in angiosperms (Doyle 2008; Hickey and Doyle 1977) and offer several advantages. They reinforce the leaf edge, reducing the likelihood of leaf tearing (Niklas 1999; Roth-Nebelsick et al. 2001). They may also provide a more even distribution of water potentials across the leaf than branching vein patterns, a trait that should allow improved physiological functioning at marginal or apical areas of the lamina (Roth et al. 1995). Despite these advantages, open vein patterns have evolved multiple times. Theoretical studies have suggested that open vein patterns can supply a given leaf area using the least amount of vascular tissue, but with the cost of reduced safety and less even water distribution (Bohn and Magnasco 2007; Givnish 1978; Kull and Herbig 1995; Roth et al. 1995). Based on these alternative strategies, I hypothesize that different secondary vein patterns will be associated with different ends of the LES. I predict that open secondary vein patterns should be associated with species that have short LLS and use a strategy of reducing investment in safety, while increasing investment in physiologically-active tissue for fast returns (low LMA and high N). I predict that closed secondary veins should be associated with long-lived leaves that have high LMA and low N, because they are more likely to benefit from the increased support and safety that comes with closed venation. Leaves with intermediate vein patterns should have intermediate LLS, LMA and N.

The physiological effects of most minor vein arrangements remains an open questions, but the effects of minor vein density (MVD, length of veins per area) on leaf physiology have been examined by a number of researchers. MVD is positively correlated with stomatal density (Uhl and Mosbrugger 1999) and leaf thickness (Noblin et al. 2008), and thus represents a good proxy for the distance form the veins to the stomata. This distance was correlated with A_{max} and K_{leaf} across a range of land plants (Brodribb et al. 2007). MVD has been shown to correlate directly with K_{leaf} in tropical angiosperms (Sack and Frole 2006) and with transpiration rate in land plants (Boyce et al. 2009). The relationship between MVD and flux rates is thought to represent coordinated evolution of leaf hydraulic capacity and photosynthetic capacity (Brodribb et al. 2007; Sack and Holbrook 2006), but to date, no one has used phylogenetically-based tests to determine if there are correlated evolutionary shifts in these traits. A relationship between MVD and flux rates has important implications for estimating physiological traits of fossil taxa (Boyce 2005; Boyce et al. 2009), but the utility of such estimates depends on the ability to translate between contemporary and fossil leaves. Even when measured on the same leaves, factors such as leaf thickness, chemical composition and mesophyll structure may confound the relationship between MVD and flux rates. If the co-variation between A_{max} and K_{leaf} in contemporary taxa is a result of correlated evolutionary changes among species, then these functions should be species-level properties. Therefore, I hypothesize that MVD from one sample of leaves will be able to predict A_{max} from independent leaf sample of the same species. If hydraulic capacity and photosynthetic capacity are evolving in a coordinated fashion, then there should also be a positive relationship between mesophyll N content and MVD. However, when measured on a whole leaf basis, N content could be negatively associated with higher MVD, since vein tissue is low in N. Based on these conflicting forces, I predict a weak, but positive, relationship between N content and MVD across species.

Methods

Data Collection

To test the hypotheses I laid out above, I combined my own data on vein patterns with data from the Glopnet database (Wright et al. 2004). I collected data on major vein patterns for all species for which A_{max} and LLS data were available from Glopnet, and for which I could find images that clearly showed the major veins. This included 468 species for primary vein type and 361 species for secondary vein type. I scored leaves for two major vein patterns: primary vein type (pinnate, palmate or parallel) and secondary vein type (closed, intermediate and open; see Introduction for description of categories). To score major vein patterns, I examined photographs of fresh or dried leaves from online image collections of herbaria and botanical gardens. I collected data on MVD for 105 species from the National Cleared Leaf Collection at the Peabody Museum of Natural History. This included all species for which both cleared, stained leaves and photosynthesis data from Glopnet were available. I used a Leica MZ16 Microscope to record digital images of leaf veins at 40X magnification. Using ImageJ v. 1.41o (Rasband 2008), I cropped leaf images to an area of 0.25 mm^2, measured the length of all minor veins, and calculated MVD as total vein length/area. I measured MVD for four areas per leaf, all located near the midrib, approximately midway from the base to the tip of the leaf, and used the average of those four areas. If multiple leaves were available for a species, I measured MVD for each leaf, and calculated the species average.

Data on leaf economic traits came from the Glopnet database (Wright et al. 2004). For each of the species for which I had vein data, I compiled data on A_{max} and N content on a mass basis (A_{mass} and N_{mass}), LMA, and LLS. For comparison with MVD, I used A_{max} and N content on an area basis (A_{area} and N_{area}), since MVD is an area-based measurement, and area based flux rates were used in previous studies (Brodribb et al. 2007; Sack and Frole 2006). When there was more than one entry per species in the Glopnet database, I calculated the species average value of each trait.

Phylogenies

I created one phylogeny for each group of species unique to each data set (primary vein type, secondary vein type, and MVD). I first corrected species and genus names against the International Plant Names Index (http://www.ipni.org:80/ipni/plantnamesearchpage.do, accessed April 2009) or Tropicos Names database (http://www.tropicos.org/NameSearch.aspx, accessed April 2009) and added family names from the same sources. I then used the online software Phylomatic (Webb and Donoghue 2005) to construct phylogenetic trees. Phylomatic prunes a super tree to contain only the taxa of interest. As a basis for the trees, I used the conservative master tree based on the Angiosperm Phylogeny Group whole angiosperm study (Soltis et al. 2000), with additional resolution within some families provided by the authors of Phylomatic. Branches with bootstrap support of less than 80% are removed from this tree. I used the BladJ function in Phylomatic to determine branch lengths based on dated nodes from (Wikstrom et al. 2001). Despite the uncertainty, these branch lengths are more realistic than the alternative of using equal branch lengths. Equal branch lengths assume a punctuated model of evolution, which is highly sensitive to incomplete taxon sampling, such as occurs in the pruned trees generated by Phylomatic. I used Mesquite v. 2.6 (Maddison and Maddison 2009), to randomly resolve all polytomies to zero branch lengths and to prune trees as necessary to account for missing data in some traits.

Statistical Analysis

All statistical analyses were performed with R v. 2.8.1 GUI v. 1.27 Tiger for Macintosh (Urbanek and Iacus 2008). To analyze hypotheses relating to major vein patterns, I first conducted ANOVA on the raw data, using the R function “aov” with Tukey’s HSD test. I tested for differences in A_{mass}, N_{mass}, and LMA among primary vein types and for differences in LLS, LMA and N_{mass} among secondary vein types. To control for phylogenetic non-independence of the sample points, I used phylogenetically-controlled ANOVA (Garland et al. 1993), implemented with the “phy.anova” command in the R package Geiger (Harmon et al. 2008). This method uses simulations to assess how likely it is to arrive at a given distribution of traits by chance, assuming a Brownian motion model of evolution. To analyze the relationship between MVD and photosynthesis, I conducted major axis (MA or Model II) regression of A_{area} and N_{area} on MVD, using the R package smatr v. 2.1 (Warton and Ormerod 2007). I analyzed relationships between MVD and A_{mass} and N_{mass} for comparison. I used the R function “pic” in the CAIC package (Purvis and Rambaut 1995) to generate phylogenetic independent contrasts (PICs) of MVD, A_{area}, N_{area}, A_{mass}, and N_{mass}, then conducted MA regression analysis on the contrasts.

Results

Both major and minor vein patterns were significantly related to leaf economic traits, demonstrating general links between venation and leaf function across angiosperms. All predicted relationships were highly significant using raw data, and many relationships remained significant using phylogenetic correlations, indicating coordinated evolutionary shifts in leaf vein patterns and leaf functions (Table 1, figs. 2-

4). In contrast to the other tests, the relationships between primary vein type and leaf economic traits were strikingly different with and without consideration of phylogeny (Table 1, fig. 2). Using the raw data, primary vein type was highly significantly related to A_{mass}, LMA, and N_{mass}. A_{mass} did not differ between palmate and parallel veined leaves, and both had significantly higher A_{mass} than pinnate veined leaves (fig. 2A). Parallel and pinnate veined leaves both had higher N_{mass} and lower LMA than palmate veined leaves (figs. 2A and B). Using phylogenetic ANOVA, none of these relationships was significant (Table 1). Secondary vein type was significantly related to LLS, LMA and N_{mass} using the raw data (Table 1, fig. 3). Using phylogenetic correlations, LLS and N_{mass} were significantly related to secondary vein type, but the relationship between secondary vein type and LMA was no longer significant (Table 1). The difference between closed and intermediate secondary veins was not significant for any of the response variables (fig. 3). Both closed and intermediate secondary veins had significantly longer LLS and lower N_{mass} than open secondary veins (figs. 3A and C). The positive relationship between MVD and A_{area} was highly significant with or without the consideration of phylogeny (fig. 4A). N_{area} was significantly positively related to MVD using raw data, but not using PICs (fig. 4B). Regressions of A_{mass} and N_{mass} on MVD were weaker than for A_{area} and N_{area}, and both relationships became non-significant using PICs (Appendix 1.1).

Discussion

Leaf support, vascular redundancy, and the trade-off between leaf life span and photosynthesis.

One of the major findings of the LES was the trade-off between species' ability to construct leaves that live a long time (with high LMA) and the ability to construct leaves with high maximum photosynthetic rates (with high N content). Both of the major vein patterns I examined tie in to this trade-off, but at different ends of the spectrum. I predicted that leaves with palmate or parallel primary veins would fall at the high physiological activity end of the LES, with high A_{mass}, high N_{mass}, and low LMA. This implies that there is no trade-off between the high primary vein density and the ability to photosynthesize rapidly, but rather a trade-off between investment in primary veins and structural tissue outside the primary veins (Niinemets et al. 2007). My results lend little support to these predictions. Palmate and parallel veined leaves did have higher A_{mass}, but this relationship does not appear to be based on an evolutionary trade-off, since it was not significant using phylogenetic ANOVA. Furthermore, higher N_{mass} and lower LMA were associated only with palmate veined leaves, and parallel and pinnate veined leaves were similar for those traits. The fact that palmate and parallel veined leaves did not consistently group together indicates that factors other than primary vein density determine leaves' position on the leaf economic spectrum. The lack of significant results using phylogenetic methods demonstrates that the relationship between primary vein type and the LES is not based on correlated evolution of leaf traits.

I predicted that the putatively higher secondary vein density found in closed and intermediate veined leaves would be advantageous for leaves at the low physiological activity end of the LES, with long LLS, high LMA, and low N_{mass}. This prediction implies that, unlike for primary vein types, there is a trade-off between investing in secondary veins and investing in photosynthetic capacity. My results provide good evidence for this trade-off, since species with closed and intermediate secondary veins have longer average LLS and lower N_{mass} than those with open secondary veins. However, I did not directly measure secondary vein density or the proportion of leaf mass in secondary veins, so the trade-off may not be based directly on investment in secondary vein tissue. Higher LMA and low N_{mass} in closed or intermediate veined leaves suggests that characteristics such as smaller cells or a lower fresh mass/dry mass ratio may allow for long LLS in those species (Poorter et al. 2009; Shipley 1995; Shipley et al. 2006). The relationship between secondary vein type and LLS is also consistent with the climatic distribution of leaf margin types associated with those vein types. The majority of species with closed or intermediate veins had entire margins, while open vein patterns were much more common in toothed or lobed leaves (although I did find nearly every possible combination of open, intermediate, or closed secondary veins with lobed, toothed or entire leaf margins). The prominence of entire-margined leaves in wet tropical climates with many evergreen species and toothed or lobed leaves in temperate climates with many deciduous species (Bailey and Sinnott 1916; Wolfe 1995; Wiemann et al. 1998; Royer et al. 2005) suggests an association between leaf margin type and LLS. My results suggest that this relationship may be based in part on the vein patterns associated with those margin types.

Increased hydraulic safety due to redundancy of major veins could also be important for relationships between major vein patterns and the LES. Primary vein types are known to differ in their tolerance to mid-rib damage (Sack et al. 2008). Closed secondary veins may also provide tolerance to midrib damage (Roth-Nebelsick et al. 2001), but I am not aware of any empirical studies that test this. Sack et al. (2008) showed that loss of physiological functions after mid-rib damage decreased from open (*Quercus* and *Betula*), to intermediate (*Viburnum*) to closed (*Kalmia*) secondary veins (fig. 1, Sack et al. 2008), but they did not specifically address this question or test its significance. My data provide no evidence that the vascular redundancy is linked to the LES. Among primary vein types, palmate and parallel veined leaves should offer similar vascular redundancy, but they have different relationships with leaf economic traits (fig. 2). Among secondary vein patterns, intermediate veined leaves were statistically similar to closed veined leaves for LLS, LMA and N_{mass} (fig. 3). Intermediate veined leaves have no direct vascular connection between secondary veins, so they are unlikely to offer the same tolerance to mid-rib damage as closed veins. Instead, intermediate and closed secondary veins should provide similar support and reinforcement properties, since they both they both have extra vein tissue at the leaf margin. My results are consistent with the work of Sack et al. (2008), who found that severing second order or higher veins had no effect on leaf function, regardless of primary or secondary vein type, implying complete vascular redundancy of all second and higher order vein patterns.

Phylogenetic conservatism of major vein patterns and the leaf economic spectrum

Phylogenetic conservatism may have a significant effect on the relationships between primary vein type and leaf economic traits. Although there are some taxa for which major vein pattern may be relatively labile (Jones et al. 2009), primary vein type was invariable for many of the families surveyed in this study (Appendix 1.2). There were far fewer parallel and palmate veined species than pinnate veined species and relatively few transitions between primary vein types. Rather than repeated evolutionary convergence of structure and function, the relationships between leaf economic traits and primary vein type appear to arise from specific combinations of traits found within individual clades. For example, 33 of the 47 parallel veined species were in one clade (monocots), and 24 of those were grasses (Poaceae). C4 photosynthesis was present in a much larger proportion of parallel veined leaves (11% of the species for which C3/C4 status is known) than in pinnate (<1%) or palmate (3%) veined leaves, and all parallel veined species with C4 photosynthesis were in the Poaceae. C4 photosynthesis is known to have a phylogenetic signal (Edwards and Still 2008; McKown and Dengler 2007), and the C4 species used in this study had significantly higher A_{max} than the C3 species (1 tailed *T*-test, $P<0.001$, for both comparisons among all species and among only parallel veined species). Therefore, it appears that the relationship between in parallel veined leaves and A_{max} is based in part on the fact that C4 photosynthesis is common in the few clades I sampled that have parallel veined leaves.

Secondary vein type was more variable across the phylogeny than primary vein type, but while many families contained open and intermediate or closed and intermediate veins, there were few families with both open and closed secondary veins (Appendix 1.3). Genetic or developmental constraints preventing transitions of major vein types (Dengler and Kang 2001; Koizumi et al. 2004; Nelson and Dengler 1997) may make it difficult to evolve a different major vein pattern, even if it would be selectively advantageous. Phylogenetic conservatism of major vein patterns may explain much of the scatter in each of the categories in figs. 1 and 2, if leaves of particular taxa are “stuck” with a vein pattern, regardless of environmental selection pressures. For example, long LLS in some members of the Fagaceae suggests that closed vein patterns could be adaptive for those species, yet there are no species with closed secondary veins in the family (Appendix 1.3). Apparently, species can make durable leaves by altering other traits such as cell size, water content, or chemical composition, without changing secondary vein type. The phylogenetic conservatism of major vein patterns weakens the relationships between major vein type and the LES, making leaf functions harder to predict from vein patterns alone. However, phylogenetic conservatism also allows values of leaf functional traits to be inferred from phylogeny, providing a complementary source of information (Edwards et al. 2007).

Coordinated evolution of minor vein density and photosynthetic capacity

The use of independent contrasts to demonstrate correlated shifts in MVD and A_{max} across angiosperms adds broad support to the notion that leaf hydraulic capacity and photosynthetic ability evolve in a coordinated fashion (Boyce et al. 2009; Brodribb et al. 2007; Brodribb et al. 2005; Sack and Holbrook 2006). Although MVD only explained 12% of the variation in A_{area}, the relationship was highly significant (fig. 4A). Since the leaves used to measure A_{area} were collected from completely different sites than those used to measure MVD, a significant part of the unexplained variation must come from intraspecific variation, both genetic and plastic, with further variation due to differences in traits such as leaf thickness and N content. This result demonstrates that despite these sources of variation, MVD has a signal at the species level, and that species level values of MVD can be used to predict leaf level physiology across sites.

Despite the well-known link between N content and photosynthetic rate (Field and Mooney 1986; Reich et al. 1997), and the link between MVD and A_{area}, there was a weak relationship between N content and MVD. This highlights the dual effects of MVD on leaf physiology: higher MVD allows for faster water delivery and export of photosynthate, but also requires higher carbon content in leaf tissue to build the veins. The relationships between leaf economic traits and pinnate veined leaves reflect this conflict. Niinemets et al (2007) showed that pinnate veined leaves had higher MVD, which suggests that they might have higher A_{max}. They also found higher C content and marginally lower N content in pinnate veined leaves, suggesting that they might have lower A_{max}. I found lower A_{mass} in pinnate veined leaves, but this result was highly dependent on phylogeny (fig. 2A). Measurements of N content in isolated mesophyll tissue (without any minor vein tissue) would probably reveal a stronger relationship between N content and MVD.

Implications of results

This study provides the first global-scale demonstration of relationships between vein patterns and leaf functions. Including phylogenetic information allowed me to distinguish which relationships represent correlated evolution of structures and functions (secondary vein type and LLS, MVD and A_{area}) and which appear to be based on particular combinations of traits arising in just a few clades (primary vein type and A_{mass}). To date, there have been few analyses of the phylogenetic patterns of vein traits at scales lower than the family (Boyce 2005; Boyce et al. 2009; Dimichele and Gastaldo 2008; Hickey and Doyle 1977), and phylogenetic studies of leaf physiological traits are even more rare (Edwards et al. 2007; Feild et al. 2003; Feild et al. 2004). My use of species-level trait values for all of the comparisons in this study demonstrates that that leaf economic traits have a signal at the species level, while the reduction in *P* values of most of the phylogenetically-based tests suggests large-scale phylogenetic signal in leaf economic traits. By highlighting both the functional significance of vein patterns and the potential for phylogenetic signal in leaf economic traits, I hope to encourage more in depth analyses of their evolutionary history.

Linking the LES to leaf structural traits such as vein patterns provides a basis for analyzing the functional significance of leaf form diversity (Sack et al. 2008), and complements studies that have examined the chemical or genetic determinants of leaf economic traits (Beaulieu et al. 2007; Mediavilla et al. 2008; Poorter et al. 2009). The phylogenetic approach I used in this study, combined with targeted sampling of species for physiological measurements, could allow for a broad-scale analysis of the relationships between the LES and other vein traits such as Kranz anatomy (Muhaidat et al. 2007) or xylem element types (Sperry et al. 2007). Other physiological functions could also be incorporated. For example, patchy stomatal conductance is associated with vein traits such as bundle sheath extensions and higher MVD (McKown and Dengler 2007) and appears to be related to the LES (Liakoura et al. 2009). Relationships between vein patterns and leaf functions could be useful in large-scale studies that examine the distribution of vein patterns across communities or ecosystems in the context of the LES. This would be particularly useful for major vein patterns, which are easy to score. The link between vein patterns and the LES may be most useful for analysis of plant fossil data. Major vein patterns are highly visible and do not require preservation of the entire leaf. In conjunction with other methods such as herbivory damage (Royer et al. 2007), vein characteristics could be used to estimate leaf economic traits for individual fossil taxa or assemblages of leaves. More detailed analyses of the phylogenetic patterns of both leaf economic traits and vein traits could clarify how their relationships evolve and allow for increased predictive power based on phylogenetic relationships.

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Tables

Table 1. Statistical output for standard and phylogenetically controlled ANOVA, testing the relationship between leaf economic traits and major vein patterns. Bold numbers represent $P<0.05$.

Predictor	Response	*F*	d.f.	*P* (raw data)	*P* (phylogenetic)
Primary Vein Type	A_{mass}	15.982	2, 464	**<<0.001**	0.272
	LMA	21.123	2, 464	**<<0.001**	0.205
	N_{mass}	15.516	2, 428	**<<0.001**	0.241
Secondary Vein Type	LLS	51.334	2, 358	**<<0.001**	**< 0.001**
	LMA	6.1549	2, 312	**0.002**	0.136
	N_{mass}	13.716	2, 330	**<<0.001**	**0.008**

Figure Legends

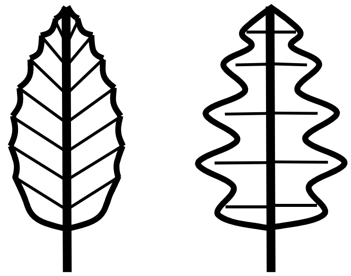
Fig. 1. Schematic representation of **A.** primary and **B.** secondary vein patterns. These illustrations represent possible leaf shape/vein type combinations, but there were different shapes within each category.

Fig. 2. Box plots of primary vein type versus **A.** A_{mass}, **B.** N_{mass}, and **C.** LMA. Lower case letters indicate statistically similar values among categories, using standard ANOVA. Upper case letters indicate statistically similar values among categories using phylogenetic ANOVA.

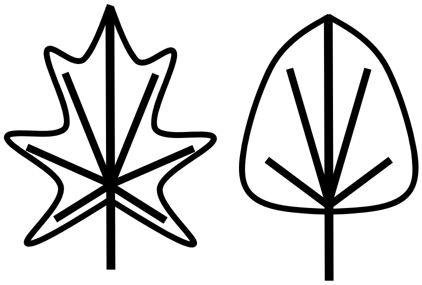
Fig. 3. Box plots of secondary vein type versus **A.** LLS, **B.** LMA, and **C.** N_{mass}. Letters as per fig. 2.

Fig. 4. Scatter plots and major axis regression coefficients of independent contrast of minor vein density versus independent contrasts of **A.** A_{area} and **B.** N_{area}. Insets: scatter plots and major axis regression coefficients of raw data.

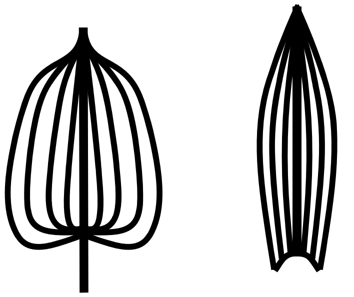
A.



Pinnate

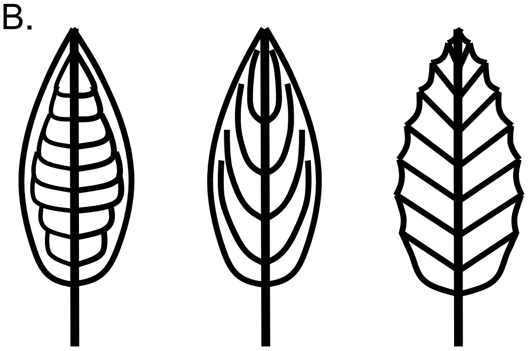


Palmate

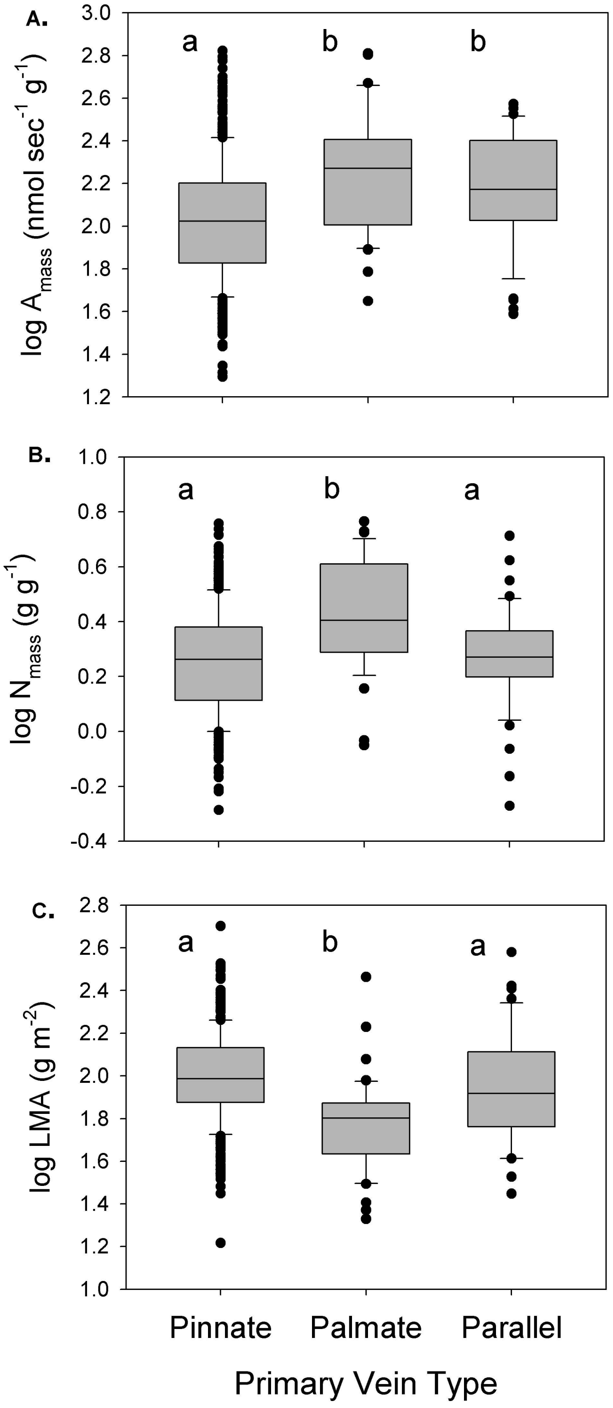


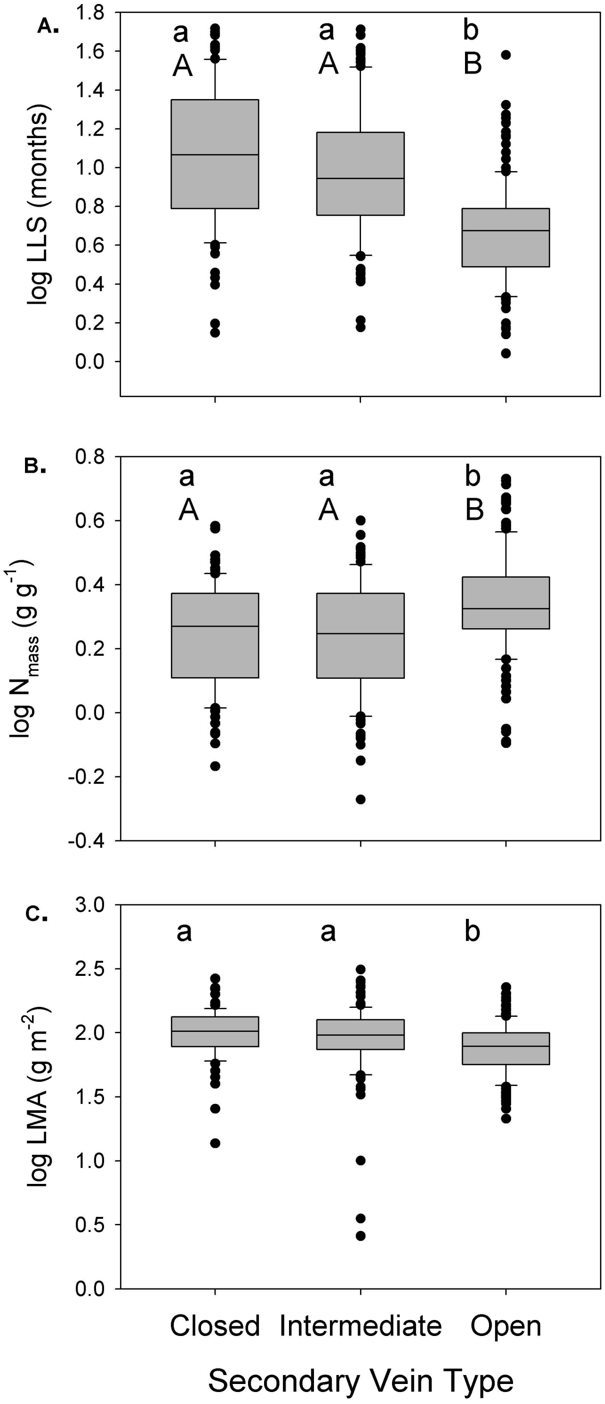
Parallel

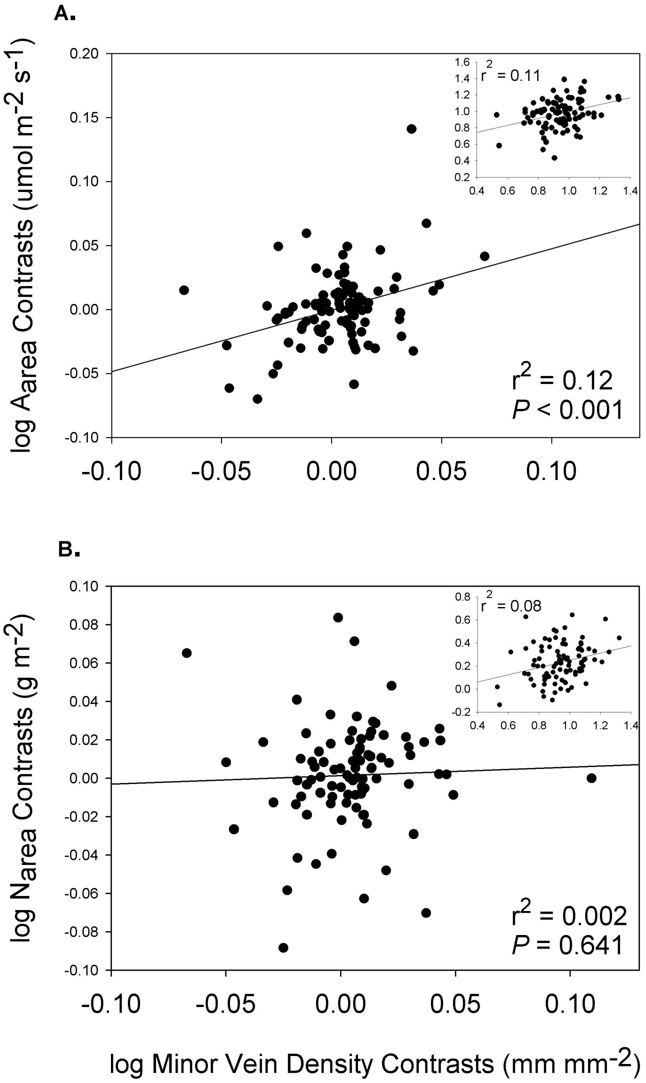
B.



Closed Intermediate Open


A.
log A_{mass} (nmol sec^{-1} g^{-1})
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
a
b
b
B.
log N_{mass} (g g^{-1})
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
a
b
a
C.
log LMA (g m^{-2})
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
a
b
a
Pinnate
Palmate
Parallel
Primary Vein Type


A.
log LLS (months)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
A
a
A
b
B
B.
log N_{mass} (g g^{-1})
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
a
A
a
A
b
B
C.
log LMA (g m^{-2})
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
a
b
Closed
Intermediate
Open
Secondary Vein Type


A.
log Aarea Contrasts (umol m-2 s-1)
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.10
-0.05
0.00
0.05
0.10
r2 = 0.12
P < 0.001
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
r2 = 0.11
0.4
0.6
0.8
1.0
1.2
1.4
B.
log Narea Contrasts (g m-2)
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
-0.10
-0.05
0.00
0.05
0.10
r2 = 0.002
P = 0.641
0.8
0.6
0.4
0.2
0.0
-0.2
r2 = 0.08
0.4
0.6
0.8
1.0
1.2
1.4
log Minor Vein Density Contrasts (mm mm-2)

CHAPTER 2

TRAIT CORRELATIONS ACROSS PHYLOGENETIC SCALES: STOMATAL TRAITS AND LEAF SIZE AFFECT LEAF FUNCTION IN UNEXPECTED WAYS IN THE GENUS *DIOSCOREA*

Abstract

Correlations among leaf traits at large phylogenetic scales are thought to represent optimal combination of trait values based on evolutionary trade-offs or physical limitations. If evolutionary diversification is optimizing species trait values' in response to their ecological conditions, then close relatives should evolve along the axes described by large-scale relationships. Differences in trait correlations across phylogenetic scales may indicate constraints among more closely related species. I examined relationships among leaf traits that are important for gas exchange and water delivery, to determine how relationships within a genus compare to large-scale relationships. I studied twelve species of *Dioscorea*, a genus of herbaceous, perennial, monocot vines, with a worldwide distribution. The growth form and evolutionary history of this genus may lead to constraints in the ways it can respond to the challenge of balancing carbon gain with water loss. I found that some relationships were consistent with large-scale studies, such as the correlations among maximum photosynthetic rate (A_{max}), stomatal conductance (g_s), N content, and leaf mass area (LMA). There were positive correlations among some of the structures that control the flux of water through leaves, but, surprisingly, fluxes (A_{max} and g_s) were negatively correlated with those structures. Leaf hydraulic conductance was independent of other fluxes and most leaf structures. I found two main suites of leaf traits: those associated with carbon isotope discrimination ($\delta^{13}C$) and those associated with minimum leaf water potential. The first suite appears to be based on the relationship between stomatal density and g_s, while the second appears to be based on cell size. Leaf size was linked to both suites of traits, as shown by the significant relationships among lamina area, stomatal density, and guard cell length. These correlations may arise from selection for heightened stomatal sensitivity, due to the larger xylem elements in large-leaved *Dioscorea* species and their monocot vine form. My results suggest that how species diversify along leaf trait co-variation axes depends on the unique combinations of traits and ecological challenges present in different lineages.

Introduction

The need to balance carbon gain with water loss has played a central role in leaf trait evolution (Givnish 1986b). Research at large phylogenetic scales has revealed general principles of how leaf traits co-vary to meet this balance. The high cost of vascular tissue is thought to select for coordination between the capacity of the vascular system to supply water and the demand placed on it by transpiration or photosynthesis (Sack and Holbrook 2006). This selection is revealed by a correlation between maximum photosynthetic rate (A_{max}) and leaf hydraulic conductance (K_{leaf}) across a broad range of species (Sack et al. 2003; Brodribb et al. 2005; Brodribb et al. 2007). As part of this balance, many of the physical structures that control leaf water supply and demand, such as the size and number of xylem conduits in midrib, minor vein density (MVD), stomatal pore index [SPI = stomatal density (SD) x guard cell length (GCL)], and the thickness of the palisade mesophyll, are positively correlated with each other and with leaf level flux rates (Wylie 1939; Uhl and Mosbrugger 1999; Sack et al. 2003; Cochard et al. 2004; Sack et al. 2004; Nardini and Salleo 2005; Sack and Frole 2006; Sack and Holbrook 2006). Perhaps the best-known set of correlations among leaf traits is the "leaf economic spectrum" or LES. The LES represents a trade-off between the ability to photosynthesize rapidly and the ability to maintain leaves for a long time (Wright et al. 2004). A_{max}, dark respiration, leaf N content, and g_s Were all positively correlated with each other and negatively correlated with leaf mass per area (LMA) and leaf lifespan (LLS), across a broad taxonomic and geographic range (Reich et al. 1997; Reich et al. 1999; Wright et al. 2004). Fig. 1 summarizes the relationships among leaf traits that are predicted by large-scale studies. These relationships represent leaf-level strategies that allow plants to optimize water loss and carbon gain over their lifetime.

Large-scale studies of leaf trait co-variation describe patterns of evolutionary convergence but ultimately arise from divergences of sister species. If large-scale correlations represent optimal combinations of trait values, then close relatives should diverge along the same axes. However, these patterns may not represent an optimal solution for a particular taxon. How different lineages evolve in response to environmental shifts will depend on the combinations of traits present in each lineage, that is, the phenotypic and genetic material they have to work with (Edwards 2006; Marks and Lechowicz 2006). Furthermore, at smaller phylogenetic scales, constraints due to pleitropy, genetic linkage, or other mechanisms, may obscure the patterns described in fig. 1. A handful of studies among close relatives have begun to reveal how leaf traits evolve within the general principles outlined above (Givnish et al. 2004; Edwards 2006; Coomes et al. 2008; Dunbar-Co et al. 2009), but I are unaware of any comparative studies that actually measured both leaf structural traits and leaf-level fluxes among close relatives, to verify the predicted correlations among structures and functions. Variable results among studies, and variable results for different species within large-scale studies (Galmes et al. 2007; Milla and Reich 2007), suggest that different taxa have evolved idiosyncratic solutions to the adaptive challenges they face.

I used a common garden study to examine correlations among leaf traits in twelve species of the monocot genus *Dioscorea*. My goal was to determine whether trait correlations within one genus are consistent with the expectations from studies at larger phylogenetic scales, as summarized in fig. 1. These relationships have only been examined in a few genera and growth forms, and never in a group of closely related monocots or vines. Vines are known to have larger stem xylem elements with higher stem conductivity than other growth forms, but may have higher risk of stem embolisms as a result (Ewers and Fisher 1991; Ewers et al. 1991; Hacke et al. 2006). Because they are monocots, *Dioscorea* have no secondary growth and cannot increase the conductive capacity of their stems in response to changes in water availability or total leaf area. Water availability may be the main factor controlling the global distribution of *Dioscorea* (Burkill 1960), so traits related to water use should be important for adaptive differentiation within this genus. If the inter-specific trait correlations within this genus are similar to large-scale patterns, it would suggest that those patterns represent optimal solutions for *Dioscorea*, and that there are few genetic constraints preventing their evolution. A lack of significant correlations among traits could indicate genetic or other constraints, while unexpected correlations (among different traits or in different directions from large-scale studies) would suggest that *Dioscorea* is using alternative strategies to cope with environmental variation.

There are many traits that are important for leaf water use and gas exchange (Sack and Holbrook 2006), but I focus on a limited set of traits that summarizes different parts of the leaf hydraulic pathway (fig. 1C). I measured leaf level fluxes (A_{max}, g_s, and K_{leaf}), as well as traits from the LES (LMA and N content, plus A_{max} and g_s). Because all *Dioscorea* species share a similar growth form, variation in leaf size is one of the primary morphological differences among species. Therefore, I measured leaf size, to determine if the relationships between leaf size and other traits reflected the unique ecological challenges faced by monocot vines such as *Dioscorea*. To gain more insight into how leaf traits affect integrated water and carbon use, I examined carbon isotope discrimination ($\delta^{13}C$) and leaf minimum water potential (Ψ_{min}). $\delta^{13}C$ indicates the internal CO_2 concentration (c_i) experienced by a leaf during its lifetime and is a common surrogate for lifetime water use efficiency (WUE; Farquhar et al. 1989; Lambers et al. 1998; Dawson et al. 2002). Within and among species, $\delta^{13}C$ is linked to drought tolerance or native water availability (Korner et al. 1991; Ehleringer and Monson 1993; Anderson et al. 1996; Ehleringer et al. 1997; Schulze et al. 1998; McKay et al. 2003). Ψ_{min} is the water potential experienced by leaves during the period of highest evaporative demand, usually at mid-day under full sun. Across species, Ψ_{min} has been shown to correlate negatively with drought experienced in the field and vulnerability to cavitation (Pockman and Sperry 2000; Bhaskar et al. 2007; Jacobsen et al. 2007; Kursar et al. 2009). Since photosynthetic rate depends on leaf water status (Boyer 1976), Ψ_{min} potentially indicates leaf photosynthetic function. Despite numerous studies of the relationships between Ψ_{min} and stem or whole-plant traits, there is little information on which leaf traits correlates with Ψ_{min}.

To answer my larger questions about how leaf traits evolve among close relatives, I tested predictions from three specific hypotheses:

Hypothesis 1. Correlations among leaf traits that have been observed at larger phylogenetic scales will be present in *Dioscorea*. (see fig. 1). I predict that (a.) measures of the flux of water or CO_2 through the leaf (A_{max}, g_s, and K_{leaf}) will be correlated with each other across species, (b.) leaf structural traits that determine the flow of water or CO_2 through a leaf ($K_{t\text{-}mr}$, MVD, palisade thickness, and SPI) will be positively correlated with each other, (c.) fluxes will be positively correlated with leaf structural traits that determine the flow of water or CO_2 through a leaf, and (d.) leaf economic traits (LMA, A_{max}, g_s, and N) will be correlated across species, as predicted by the LES.

Hypothesis 2. As integrated measures of leaf water or CO_2 status, $\delta^{13}C$ and Ψ_{min} will depend on the same set of traits that describe gas exchange and hydraulic capacity. I predict that (a.) $\delta^{13}C$ and Ψ_{min} will be correlated with A_{max}, g_s, and K_{leaf}, and (b.) $\delta^{13}C$ and Ψ_{min} will be correlated with $K_{t\text{-}mr}$, MVD, palisade thickness, and SPI.

Hypothesis 3. Leaf size will indicate leaf function in *Dioscorea*, but in ways that reflect the ecological challenges faced by monocot vines. I predict that (a.) leaf size will be correlated with the traits that describe gas exchange and hydraulic capacity (A_{max}, g_s, and K_{leaf}; $K_{t\text{-}mr}$, MVD, palisade thickness, and SPI), (b.) investment in leaf support (LMA and petiole dimensions) will increase with increase with leaf size, and (c.) correlations between leaf size and other leaf traits in *Dioscorea* will differ from correlations found in studies of other genera.

Methods

Study system

The monocot genus *Dioscorea* is a large group of tuberous or rhizomatous, herbaceous vines. It contains approximately 500 species, most of which are tropical (Burkill 1960; World Checklist of Plant Families 2009). Most *Dioscorea* species grow at forest edges in a range of well-drained soils and rainfall conditions, and total annual rainfall may be a major factor controlling the distribution of the genus (Burkill 1960). *Dioscorea* species are widely cultivated as food crops, and many species, both wild and cultivated, are harvested for their pharmaceutical properties (Ayensu and Cmysey 1972; Chu and Figueiredoribeiro 1991). For this study, I used 12 tropical and temperate species from 8 sections, from both the New and Old World (Table 1). I acquired most plants through the nursery trade, except for *D. alata*, which was purchased as tubers from a produce market, and *D. villosa*, which was collected from wild populations on Long Island, New York. All plants were raised in a greenhouse for a minimum of two years before the beginning of this study.

Growth conditions

I grew plants in Sunshine Mix #1 growing medium (Sun Gro Horticultural, Bellevue, Washington), fertilized with Osmacote slow release fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, Ohio) and watered the pots whenever the growing medium dried out. At the beginning of the summer, I transplanted tubers to large pots, ranging in volume from 4 to 20 L depending on the species, to reduce the likelihood of bound roots. Temperature in the greenhouse during the growing season ranged from 21°C to 38°C during the day and 16°C to 25°C during the night. For the leaves I measured, light levels on a sunny day ranged between 100 μmoles photons m^{-2} s^{-1} and 1800 μmoles photons m^{-2} s^{-1}, with leaves experiencing 3-4 hours per day of full light. I measured morphological, anatomical and physiological traits on one to three leaves per species (two leaves for most traits) and used the species average value. I used leaves from different plants for all species except *D. sylvatica*, for which I only had one individual.

Morphological and anatomical traits

I scanned fresh leaves on a Canoscan 8000F flatbed scanner (Cannon USA Inc., Lake Success, NY) at 300 dots per inch resolution, and measured lamina area and petiole length using ImageJ v.1.36b (Rasband 2008). *D. dumetorum* and *D. pentaphylla* have compound leaves, so for these two species, I also measured the area of the central leaflet. I dried the laminas in an oven at 60°C and measured the dry mass. I divided lamina area by dry mass to determine LMA. To measure primary vein density (PVD), I used ImageJ to trace all primary veins on the scanned leaf images and measure their length, then divided the total vein length by the lamina area. I included all large veins that originated from the lamina/petiole junction (true primary veins), as well as large veins that branched from the first true primary near the base of the leaf (fig. 2). Although these veins may be considered secondary veins, structurally and functionally they behave as primary veins, and they are morphologically distinct from other secondary veins.

I used hand-cut cross sections of ethanol preserved leaves to measure petiole and mid-rib vascular dimensions as well as thickness of the different components of the lamina. I cut petiole cross-sections from the mid point of the petiole, and mid-rib cross-sections one third of the distance from the petiole/lamina junction to the leaf tip. I stained all sections with phloroglucinol, photographed them under 40x to 400x magnification, and used ImageJ to calculate areas. I measured the cross-sectional area of the petiole (Pet area), the total cross sectional area of the vascular bundles in the petiole (Pet VB area), and the cross-sectional area of the mid-rib vascular bundle (MR VB area). I calculated the average mid-rib tracheary element diameter (MR TE diameter) based on the longest axes of each mid-rib tracheary element. Assuming that tracheary elements were ellipses, I calculated the theoretic hydraulic conductivity of the midrib ($K_{t\text{-}mr}$) as: $\Sigma[\pi a^3 b^3/\eta(a^2+b^2)]$, where a and b are the long and short axes of an ellipse, and η is the viscosity of water at 25°C (Lewis and Boose 1995; Cochard et al. 2004; Sack and Frole 2006). The volume of water in the numerator was transformed to mass, so $K_{t\text{-}mr}$ is reported as mmol m s^{-1} MPa^{-1}. I used sections of leaf tissue adjacent to the mid-rib to measure the thickness of the palisade and spongy mesophyll and total lamina thickness

I boiled fresh leaves (whole, or pieces for larger leaves) in 70% ethanol for 1 hour and transferred these leaves to 5% NaOH for one to seven days until clear. I stained cleared leaves with 1% w/v safranin dye in 95% ethanol. I photographed minor veins at 40x magnification and calculated the vein length per area using ImageJ. For each leaf, I photographed and measured three separate regions and used the average value per leaf. I took all photographs between the second and third primary veins, approximately one third of the way from the base of the leaf to the tip. In all *Dioscorea* species I have examined to date, first through third order veins are major veins (they protrude above the abaxial surface of the leaf and are covered with elongated epidermal cells), so I measured fourth order and higher veins as minor veins.

I used nail polish peels of fresh leaves to measure stomatal characteristics (Sally Hansen Dries Instantly nail polish). I photographed epidermal impressions at 400x magnification and used ImageJ to measure SD (# stomata per area), stomatal index (SI, #stomata per epidermal cell), GCL, and SPI. SI provides and measure of the rate of initiation of stomata per epidermal cell, and SPI provides a measure of the total stomatal area available for gas exchange per leaf area (Sack et al. 2003; Sack and Tyree 2005). For each character, I used the average of three photographs per leaf, taken from the same area of the leaf as for MVD. For GCL, I measured the length of ten guard cell pairs per photograph and used the average of all thirty measurements.

Physiological traits

I measured A_{max} with an LI6400 infra-red gas analyzer (Licor Inc., Lincoln, Nebraska). I maintained chamber conditions between 30 and 33 °C, 48-65% relative humidity, adjusting incoming CO_2 concentration to maintain c_i between 190 and 240 ppm. I began by illuminating leaves at a level close to ambient conditions, usually between 300 and 700 μmoles photons m^{-2} s^{-1}, since measurements were begun in the morning. I allowed the leaf to equilibrate to chamber conditions then raised the light level in steps of 200 μmoles photons m^{-2} s^{-1}, allowing the leaf to equilibrate at each new light level. All species reached a maximum net photosynthetic rate between 1000-1200 μmoles of photons m^{-2} sec^{-1}. I recorded stomatal conductance (g_s) and instantaneous water use efficiency (WUE_{inst} = photosynthesis/transpiration) at A_{max}. I measured minimum water potential (Ψ_{min}) with a pressure chamber (PMS Instruments, Corvallis, Oregon), using only leaves that were in full sun, between 1PM and 3PM, on well-watered plants.

I calculated leaf hydraulic conductance (K_{leaf}) based on steady state water loss by evaporation from attached leaves (Melcher et al. 1998). The evening before measurements, I covered one leaf in aluminum foil and a plastic bag, to halt transpiration. On the day of measurement, I measured conductance on the adjacent leaf (opposite, in the case of opposite-leaved plants, or one node above in the case of alternate leaf plants), between 10 AM and noon. First I used an LI1600 porometer (Licor Inc., Lincoln, Nebraska) to measure leaf temperature and steady state transpiration (E_{LI1600}) at four points on the exposed leaf, using the average values. I then covered the leaf with a plastic bag, cut it from the plant, and immediately measured its water potential (Ψ_{leaf}) with the pressure chamber. Next I measured the water potential of the covered leaf, assuming that it was in equilibrium with the stem water potential (Ψ_{stem}). Using E_{LI1600} as the rate of flux of water through the leaf, and the difference between stem and leaf water potential as the driving force, I calculated the leaf hydraulic conductance as: $K_{leaf} = E_{LI1600}/(\Psi_{leaf} - \Psi_{stem})$, correcting for difference in the viscosity of water at different temperatures (Korson et al. 1969; Yang and Tyree 1994; Sack et al. 2002). This method assumes that water flux through leaves is at a steady state, so I verified that transpiration was stable for fifteen minutes leading up to K_{leaf} measurements.

To measure nitrogen content (%N) and carbon content (%C), I collected fresh leaves, dried them at 60°C, and ground them in liquid N. I used a Thermo Quest Flash 1112 elemental analyzer (CE Instruments, Hindley Green, Wigan, UK) at the Functional Ecology Research and Training Laboratory, Stony Brook University, to determine the %N and %C in leaf samples. I used sub-samples of the same leaf tissue prepared for C and N analysis to analyze $\delta^{13}C$, using stable isotope mass spectrometry (Stable Isotope Laboratory, Institute of Ecology, University of Georgia).

Phylogenetic analyses

I used sequences from four regions of the chloroplast for the phylogenetic analysis. Sequences of rbcL and matK were from Wilkin et al. 2005 (downloaded from TreeBase, www.treebase.org/). Sequences of a portion of ndhF and the spacer between rbcL and atpB come from an ongoing study, and were collected, aligned and analyzed by R. Geeta. DNA was extracted from1-2 g fresh leaves, following (Asemota 1995). Amplification was done using slightly modified standard primers for ndhF (Olmstead and Sweere 1994) and rbcL_atpB spacer (Chiang et al. 1998). Primer sequences are listed in Appendix 2.1. Direct sequencing was done on an ABI 3730 at Davis Sequencing, Inc. (Davis, CA). Initial alignment of nucleotide sequences was performed using ClustalW (Thompson et al. 1994) and subsequent manual adjustments using MacClade 4.08 OSX (Maddison and Maddison 2005). In the case of ndhF, alignment was done so as to match nucleotides with the amino acids, thus placing indels in a biologically appropriate manner. Phylogenetic analyses were conducted under maximum parsimony (MP) using PAUP* (Swofford 2002). *Tacca* and *Trichopus* were assigned as outgroups. For MP analyses, unordered traits were optimized using ACCTRAN, and treating multistate characters and gaps as uncertainties. A complete search of all trees was done using the branch and bound option. Robustness of support was assessed by bootstrap analyses using heuristic searches on starting trees obtained by simple step-wise addition and branch swapping by tree-bisection and reconnection for 1000 replicates. I used a robust bootstrap consensus tree, with branch support of >70%, as the basis for the comparative analysis, randomly resolving unresolved nodes to obtain 100 trees. Maximum-likelihood (ML) values for branch-lengths for these 100 trees were obtained using PAUP*.

Statistical Analyses

I used JMP 5.1 (SAS Institute, Cary, NC) for all data manipulation and non-phylogenetic analyses. In order to make my results comparable to earlier work, and because many leaf traits are expected to be log-normally distributed (Niklas 1994; Wright et al. 2004), I log10 transformed the data for all variables except Ψ_{min} and $\delta^{13}C$. Because lamina area and lamina mass were highly correlated (r=0.94), I use only lamina area in my correlation analyses. I analyzed correlations between lamina area and other traits in two ways: first using the total lamina area, and second substituting the area of the central leaflet for the two compound-leaved species. Results were similar both ways (no changes in the direction or significance of correlations), so I report only the results for total lamina area. I was unable to collect data on K_{leaf} for *D. pentaphylla*, so this species is omitted from pair-wise correlations involving that trait.

I used phylogenetic comparative methods for all analyses, since my interest was in correlated evolutionary changes among traits. I used the phylogenetic generalized least squares (PGLS) method (Martins and Hansen 1997), as implemented in Compare v. 4.6 (Martins 2004) to examine correlations among pair-wise combinations of traits for each of my 100 trees. I assumed within species variation was zero, and used the ML value of α estimated simultaneously with the analysis. α estimates the strength of phylogenetic constraint, and an α value close zero yields results similar to independent contrasts (Felsenstein 1985). I examined the 95% confidence intervals of the regression slope generated by Compare to determine if regressions, and therefore correlation coefficients, were significant (Martins 2004). I report the average correlation coefficient (PGLS r) for the 100 trees. I also report non-phylogenetically-controlled, product-moment correlations (standard r) among traits, calculated in JMP 5.1, for comparison with the phylogenetically based results.

To determine how investment in leaf tissues varied with leaf size, I used linear regression of log-transformed variables. I examined the effects of lamina area on LMA, lamina mass and PVD, as well as the relationship between lamina mass and petiole length and cross-sectional area. To determine how conductive capacity of mid-ribs and petioles changed with leaf size, I examined the relationship between lamina area and petiole VB area, MR VB area, MR TE diameter and $K_{t\text{-}mr}$. I used PGLS regression in Compare v.4.6 (Martins 2004) to determine if the slopes of the relationships between selected leaf traits and lamina area or lamina mass Were significantly different from 0 or 1. I report the average regression coefficients (r^2), slopes, and confidence intervals for 100 trees.

Results

Fig. 3 shows the bootstrap consensus tree from MP and ML analyses. *D. villosa* and *D. nipponica* were sister species, and this clade was sister to the remaining species. *D. alata* and *D. batatas* were sisters, but their relationship to other *Dioscorea* species was not well resolved. This phylogenetic result is consistent with previous results for this genus (Wilkin et al. 2005), as well as with taxonomic classifications, although the relationship between *D. bulbifera* and *D. cotinifolia*, which are in the same section, is not well supported (Table 1).

Appendix 2.2 lists the mean and standard error of all traits for each species. All pair-wise phylogenetically controlled (PGLS r) and non-phylogenetic (standard r) correlations among leaf traits are listed in Appendix 2.3. I analyzed a limited set of relationships, but list all correlations for future studies that may wish to use them generate new hypotheses. Most correlations among traits were similar whether or not phylogeny was taken into consideration. There were no cases in which correlations changed directions with different methods, other than very weak correlations. For all of my PGLS analyses, the ML values of α were at or close to the program's maximum of 15.5, suggesting an exponential increase in between-taxon divergence with phylogenetic distance (Martins and Hansen 1997; Martins 2004). However, correlations calculated using independent contrasts were very similar to those from PGLS analysis, for all pairs of traits.

Do leaf trait correlations match the predictions from large-scale studies?

Relationships among leaf economic traits within *Dioscorea* were similar to those predicted by the LES. A_{max}, g_s, and %N were all positively correlated with each other, and negatively correlated with LMA, although the g_s-%N relationship was marginally significant (figs. 4A, 5). Values of leaf economic traits for *Dioscorea* fell within the range of values of Wright et al. (2004), but were toward the high end for A_{max} and %N and the low end for LMA (Appendix 2.2 and Wright et al. 2004).

Support for coordination between hydraulic supply (K_{leaf}) and demand (A_{max} or g_s) was weak. A_{max} and gs were positively correlated, but K_{leaf} was unrelated to A_{max} or g_s, despite a trend in the right direction (figs. 4A, 6). Among structural traits, some correlations were positive, as predicted (figs. 4A, 7). SPI, MVD and $K_{t\text{-}mr}$ formed a module, with mutual positive correlations, although only the SPI-MVD correlation was significant. Palisade thickness was not correlated with any of the other structures. The most surprising deviation from my predictions was that fluxes were negatively correlated with the leaf structural trait module (figs. 4A, 8). A_{max} was significantly negatively correlated with SPI, marginally negatively correlated with $K_{t\text{-}mr}$, and weakly negatively correlated with MVD. g_s was significantly negatively correlated with $K_{t\text{-}mr}$ and weakly negatively correlated with SPI, but unrelated to MVD. K_{leaf} was unrelated to the structural trait module, but was positively correlated with palisade thickness (figs. 4A, 8).

SPI is a composite trait, made up of SD and GCL. When I evaluated correlations based on these two separate components, I gained additional insight into how other traits Were related to SPI (fig. 4B, Appendix 2.3). A_{max} was negatively correlated with SD, but not related to GCL, while g_s was marginally negatively correlated with SD, and weakly positively related to GCL. GCL was negatively correlated with SD and $K_{t\text{-}mr}$, but positively correlated with palisade thickness.

How do $\delta^{13}C$ and Ψ_{min} relate to the structures and functions important for gas exchange *and hydraulic capacity?*

$\delta^{13}C$ and Ψ_{min} were unrelated to each other, but shared a common correlation with GCL (fig. 4C). $\delta^{13}C$ was correlated with all of the traits of the LES, as well as the leaf structural traits SD and $K_{t\text{-}mr}$ (figs. 4B and 9). In contrast, Ψ_{min} was correlated with palisade thickness, K_{leaf} and GCL (figs. 4B and 10).

How do leaf traits change with leaf size across Dioscorea *species?*

Lamina area was significantly positively correlated with SD and negatively correlated with GCL, even while being independent of SPI (figs. 4D and 11, Appendix 2.3). Lamina area was also negatively correlated with g_s, and positively correlated with $\delta^{13}C$ and $K_{t\text{-}mr}$. Regression analysis showed that investment in support did not increase, and may have decreased, with leaf size (Table 2). LMA had a non-significant regression coefficient with lamina area, and lamina mass increased isometrically (with a slope of 1) with lamina area. Lamina mass increased isometrically with petiole cross-sectional area, but increased with petiole length with a slope of approximately 2, indicating that investment in petiole mass decreased with leaf size. The slope of the regression of PVD on lamina area was negative, suggesting that investment in the largest veins also decreased with increasing leaf size.

All measures of xylem cross-sectional areas scaled positively with leaf size (Table 2). Pet VB area and MR VB area increased isometrically with lamina area. MR TE diameter increased with lamina area with a slope less than one, but significantly greater than zero, indicating that larger leaves should have proportionally greater water delivery capacity in their petioles and mid-ribs. Consistent with this, $K_{t\text{-}mr}$ increased with lamina area with a slope of 1.42. The confidence intervals of this slope included 1 (Table 2), so I cannot rule out an isometric increase in mid-rib conductive capacity with leaf size.

Discussion

My goal was to determine if trait correlations within one genus of monocot vines were consistent with the expectations from studies at larger phylogenetic scales. Many of the results matched my predictions, such as the relationships among leaf economic traits, but I also found some surprises. Contrary to my expectations, fluxes (A_{max} and g_s) were negatively related to most of the structural traits that determine the flow of water through at leaf (SPI, $K_{t\text{-}mr}$ and MVD). K_{leaf} was independent of A_{max}, g_s, and most leaf structures. Among *Dioscorea* species, I found two suites of leaf traits. One included the traits that were correlated with $\delta^{13}C$, and appears to be based on the relationships between SD and g_s. The other included the traits that were correlated Ψ_{min}, and appears to be based on cell size. The significant relationships among lamina area, SD, and GCL connected leaf size to of both suites of traits. While support investment in leaves remained constant or decreased with size, conductive capacity of the petiole and midrib increased. The larger xylem elements in large-leaved *Dioscorea* species may select for heightened stomatal sensitivity and lead to selection for correlations among leaf size, SD, and GCL. *Dioscorea* species appear to have evolved idiosyncratic mechanisms for coping with the conflicting demands of CO_2 gain and H_2O loss that are appropriate for herbaceous, monocot vines.

Variation in leaf traits correlations across phylogenetic scales

Correlations among leaf traits observed at large phylogenetic scales are thought to represent adaptive solutions to the physical, physiological, and environmental demands placed on leaves (Wright et al. 2004; Istoby and Wright 2006). At finer phylogenetic scales, various constraints may prevent the evolution of expected trait correlations. Some constraints appear to be present in *Dioscorea*, since several of the correlations I predicted were absent or in the opposite direction. Using phylogenetically controlled analyses, I found negative correlations between leaf structures and fluxes, weak relationships between MVD and fluxes, and no correlations between K_{leaf} and leaf structures (SPI, K_{t-mr}, and MVD) or other fluxes. Although *Dioscorea* species with the highest SPI, MVD and K_{t-mr} should theoretically have the highest potential for gas exchange and conductance, they in fact have the lowest. These results contradict earlier studies from larger phylogenetic scales (Galmes et al. 2007, Brodribb et a. 2005; Brodribb et al. 2007; Sack et al. 2003; Sack and Tyree 2005) and provide little evidence for the evolution of coordinated hydraulic supply and demand in *Dioscorea* . Within angiosperms, the relationship between K_{leaf} and A_{max} appears weaker than across all land plants (Fig. 1A, Brodribb et al. 2007), which I interpret as a possible constraint within that group. Earlier studies have reported coordination of hydraulic supply and demand within genera, but their conclusions are based on measurements of the structures responsible for water delivery and photosynthetic capacity, not actual measurements of fluxes (Edwards 2006; Dunbar-Co et al. 2009). Since A_{max} varied positively with g_s, both across and within species (personal observation, R. Walls), I know that photosynthesis in *Dioscorea* responds normally to CO_2 limitation (Farquhar and Sharkey 1982; Sharkey 1985), and my results are not anomalous. Instead, my results indicate that variation in fluxes across *Dioscorea* species is controlled by regulation of the stomata rather than their total area, and that species with the highest stomatal area regulate their stomata in the most conservative manor.

Instantaneous measures of g_s are often positively correlated with SD (Ter Steege 1994; Hovenden and Brodribb 2000; Woodward et al. 2002; Pearce et al. 2006; Lake and Woodward 2008; Xu and Zhou 2008), but across many studies, the relationship between SD and g_s is inconsistent. Many show positive relationships (see above), some show no relationship (Bettarini et al. 1998; Liao et al. 2005), and this study shows a negative relationship. Galmes et al. (2007), observed that some species use high SD to allow high transpiration and luxury water consumption [see also (Nicotra et al. 2008)] while in other species, high SD is associated with low transpiration and high WUE. Thus, it may be difficult to generalize about the effects of stomatal size and density across taxa. Studies that make assumptions about how SD or SPI will affect fluxes within one taxon, based on large-scale correlations or other taxa, therefore may be inaccurate.

Across *Dioscorea* species, high SD was also associated with low lifetime conductance, based on the positive correlation between $\delta^{13}C$ and SD. A positive relationship between SD and $\delta^{13}C$ or other measures of WUE is not novel. Plants with smaller, denser stomata are able open and close the stomata more easily (Aasamaa et al. 2001; Hetherington and Woodward 2003) or experience stomatal oscillations that allow them to respond to water stress more quickly (Yang et al. 2005). However, these mechanisms are based on guard cell size, rather than higher SD per se. Although $\delta^{13}C$ was associated with both SD and GCL in *Dioscorea*, g_s and WUE_{inst} were significantly correlated only with SD (Appendix 2.3). This suggests that stomatal behavior is linked directly to SD, rather than stomatal size, but I are unaware of any mechanisms that specifically link SD to g_s or stomatal behavior. In *Dioscorea*, WUE may be linked SD through its correlations with leaf size, which I discuss more below (see *Relationships between leaf size and other leaf traits*).

There are several reasons why I may not have found many significant relationships between K_{leaf} and other traits. Earlier studies that report correlations between K_{leaf} and other traits were at broader taxonomic scales, and had a larger range of values than I found (Sack et al. 2003; Brodribb et al. 2005; Sack and Holbrook 2006; Brodribb et al. 2007). My values for A_{max} and K_{leaf} were within the range reported for angiosperms (Brodribb et al. 2007), but at the low end, especially for K_{leaf}. The low variation in fluxes within one genus makes correlations more difficult to detect, although I was able to find significant correlations among many other traits that had similar levels of variability. Differences in measurement methods and growth form may also explain why my results differ from previous work. Several previous studies used highly controlled conditions, such as a high-pressure flow meter (Sack et al. 2003; Sack and Frole 2006), or calculated maximum K_{leaf} based on regression analysis (Brodribb et al. 2007). My measurements, which are based on ambient conditions, reflect the values of K_{leaf} that plants are experiencing under conditions that normally lead to maximum gas exchange (late morning under full sun). The lack of correlations with leaf structures under these conditions, compared to significant correlations under more controlled conditions, suggests that leaves may be overbuilt for their hydraulic demands. This is consistent with the fact that *Dioscorea* species with the highest structural capacity to deliver water appear to regulate their stomata in the most conservative manner (see above). Brodribb et al. (2005) found correlations between K_{leaf} and A_{max} using rehydration kinetics, which, like my method, assesses K_{leaf} under conditions close to ambient. However, within angiosperms, they only measured trees. They found little variation among those trees in the drop in water potential from soil to leaves ($\Delta\Psi$). I found variation in Ψ_{min} (Appendix 2.2), which implies variation in $\Delta\Psi$, since all plants were growing at the same soil moisture. Unlike trees, herbaceous vines may operate at a much broader range of $\Delta\Psi$. This would allow for greater variation in g_s for a given stem and leaf conductive capacity, in effect preventing a correlation between K_{leaf} and g_s or A_{max}. Regardless of the reason for the discrepancy, my results demonstrate that using SD or SPI as a surrogate for K_{leaf} may be invalid within some taxa.

I did find one set of relationships that was consistent with large-scale observations: the correlations among leaf economic traits. Similar relationships across phylogenetic scales suggest that there are limited genetic constraints on the evolution of this suite of traits, and lends support to the idea that the correlations represent adaptive combinations of traits (Armbruster et al. 2004). In fact, all correlations among leaf economic trait were significant only when using phylogenetically controlled methods (Appendix 2.3), suggesting that the relationships are driven by divergences among close relatives. Although they had similar r^2 values, the slope of the relationship between A_{max} and LMA was shallower in *Dioscorea* [slope and confidence interval: -0.22 (-0.37, -0.06)] than in the global data set [slope and confidence interval: -0.75 (-0.79, -0.72), Wright et al. 2004]. This indicates less variation in LMA than in A_{max} within *Dioscorea*, and shows that variation in A_{max} is less constrained by variation in LMA within this genus than across all plants. Different constraints on leaf economic traits may be present in other taxa or at even finer scales. Two studies within genera failed to find relationships among leaf economic traits, but they both examined Hawaiian lineages that underwent very rapid diversification (Givnish et al. 2004; Dunbar-Co et al. 2009). Among *Pelargonium* species, A_{max} appears to be positively correlated with LMA, due to the unusual demand of seasonal rainfall patterns and the association between leaf dissection and A_{max} (Nicotra et al. 2008). Thus, the relationships of the LES may also vary across taxa or phylogenetic scales.

Plant water use and suites of correlated leaf traits

I found two main suites of leaf traits in *Dioscorea* (fig. 12): those related to $\delta^{13}C$ and those related to Ψ_{min}. The first suite of traits relates to WUE and includes two modules, the leaf economic traits, and, since the LES includes A_{max} and g_s, the structures that determine CO_2 and water flux capacity. Although leaf traits associated with maximum fluxes appear to be independent of the LES at large phylogenetic scales (Sack et al. 2003; Sack and Holbrook 2006), I found multiple correlations among them (fig. 4B). While I did find independence of K_{leaf} and the LES, I also found that K_{leaf} was independent of the entire suite of traits linked to flux rates. The correlations among SD and leaf economic traits, the leaf structural trait module, and $\delta^{13}C$ suggest that this suite of traits is based on the relationship between SD and g_s. The relationship between SD and g_s appears to be based on the unusual stomatal behavior inferred across *Dioscorea* species (see above), and may be linked to leaf size. SD is also correlated with GCL, forming a bridge between the two suites of traits.

The second suite of traits included all the traits that were correlated with Ψ_{min}. This suite of traits appears to be linked to cell size, since the two structural traits associated with this suite were GCL and palisade thickness, both indicators of cell size (all palisade mesophylls except *D. trifida* were one cell thick). To my knowledge, this is the first study to identify a relationship between cell size and Ψ_{min} across species. While I did not specifically predict this relationship, the correlation is consistent with what is known about cell size and water potential from studies within species. Plants with smaller cells have a higher percentage of total volume in cell walls and can remain turgid under lower water potentials (Cutler et al. 1977). At least within species, this allows plants with smaller cells to better resist or tolerate drought (Martinez et al. 2007). The positive correlation between K_{leaf} and Ψ_{min} indicates that across species, the ability to deliver water is linked to the water status of their leaves. This may arise from the need for fast water delivery within the leaf to maintain high water potential in species with larger cells, since like Ψ_{min}, K_{leaf} was positively correlated with palisade thickness. Although K_{leaf} is only weakly correlated with GCL (r=0.29, table 3), I cannot rule out a direct link between cell size and hydraulic conductance. The correlation between palisade thickness and K_{leaf} (and lack of correlations with any other structures) suggests that mesophyll resistance is the largest component of leaf resistance. The importance of mesophyll resistance, as a component of total leaf resistance, has been observed in other species (Cochard et al. 2004; Sack et al. 2004).

These two suites of traits suggest that, despite the significant correlation between SD and GCL, other traits correlated with them are fairly independent. Ψ_{min}, which was correlated with GCL, was not correlated with A_{max} or other traits linked photosynthesis

(%N, LMA, SD), while A_{max} was correlated with SD but not GCL. $\delta^{13}C$, which was correlated with both SD and GCL, is also correlated with A_{max} and g_s, but not Ψ_{min}. Like most physiological functions,Ψ_{min}, $\delta^{13}C$, and A_{max} are determined by multiple structures and by other physiological functions. Because of this, multiple combinations of traits can lead to similar values of those functions. My results are consistent with the idea of many-to-one mapping, which allows physiological traits to remain coupled to multiple structural traits, but evolve semi-independently from any particular structure (Alfaro et al. 2005; Wainwright et al. 2005). Given the complex network of interactions among leaf structural and functional traits, this flexibility may be crucial for maintaining viability in an organ that needs to meet multiple functional demands.

Relationships between leaf size and other leaf traits

I found a significant positive correlation between lamina area and $\delta^{13}C$, a negative correlation between lamina area and g_s, and a marginally significant correlation between lamina area and WUE_{inst} (Appendix 2.3). It seems counter-intuitive for larger-leaved species to have evolved the highest WUE, since they come from wet tropical areas (Table 1), and their larger K_{t-mr}, higher SD, marginally higher MVD indicate that large-leaved species potentially have the highest capacity for moving water through their leaves. The correlation between lamina area and SD or GCL suggests that larger-leaved *Dioscorea* species have higher $\delta^{13}C$ and WUE_{inst} due to low g_s associated with smaller, more dense stomata. There are several alternative hypotheses to explain the relationships among lamina area, SD, GCL, g_s and $\delta^{13}C$. Selection for consistently high g_s in small-leaved species from arid or temperate areas may allow them to utilize limited water whenever it becomes available (Nicotra et al. 2008). If these species only put on leaves during periods of rainfall, then leaf tissue will form under conditions of high transpiration, leading to low $\delta^{13}C$ in small-leaved species (Mitchell et al. 2008). Rapid growth during periods of high rainfall is a property shared by dry tropical and mesic temperate plants, and could explain convergence of leaf form in *Dioscorea* species from these two habitats. Alternatively, selection for conservative stomatal behavior may not be directly related to WUE, but instead to the evolution of an isohydric strategy that reduces the water potential gradient from root to leaf (Franks et al. 2007). Selection for isohydric behavior is associated with low drought tolerance and less resistant to embolism (Vogt 2001; Schultz 2003; McDoIll et al. 2008), characteristics could to be more adaptive in wet tropical environments. Since larger-leaved *Dioscorea* species have larger xylem elements in both their leaves (fig. 11) and stems (unpublished data, R. Walls), they may have evolved more conservative stomatal behavior to reduce the risk of embolism. More detailed data on the environmental conditions experienced by *Dioscorea* species in their native environments, including measurements of water use in the field, are needed to assess the alternative hypotheses for the relationships between lamina area and $\delta^{13}C$. Future greenhouse studies measuring stomatal responses to water availability and VPD would also be informative. Whatever the explanation, the strong association between SD or GCL and leaf size, coupled with the functional relationship between SD and g_s means that any selection on leaf size is also likely to impact photosynthetic capacity and WUE.

Despite the significant correlation between lamina area and GCL, and the significant correlation between GCL and Ψ_{min}, there was no relationship between lamina area and Ψ_{min}. Selection for smaller leaves in cooler and drier climates (Dudley 1996;

McDonald et al. 2003; Meier and Leuschner 2008) would lead to larger guard cells in those climates, because of the negative correlation between GCL and lamina area. This would give rise to a negative relationship between lamina area and Ψ_{min}, but the combination of small leaves with high Ψ_{min} is unlikely to be adaptive in dry tropical areas. Warm, wet climates may select for both higher Ψ_{min} and larger leaves, leading to a positive correlation between lamina area and Ψ_{min}. In this case, the negative correlation between GCL and leaf size would conflict with the positive correlation between Ψ_{min} and GLC. I found a marginally significant negative correlation between Ψ_{min} and lamina area using standard methods, which became non-significant using PGLS methods, but the negative correlation between lamina area and GCL was significant under PGLS methods (Appendix 2.3). Selection (or constraint) for a negative relationship between GCL and lamina size appears to supersede any possible selection for a positive relationship between Ψ_{min} and lamina area, while the relationship between Ψ_{min} and GCL remains intact because of the functional dependence of Ψ_{min} on cell size.

Changes in support and conductive capacity with leaf size

In contrast to other studies showing that larger leaves invest disproportionately more in support tissue (Givnish 1986a; Niinemets et al. 2007; Niklas et al. 2007), I did not find any evidence of increased support investment with increasing leaf size among *Dioscorea* species. Larger leaves have lower PVD, although this may be countered by the presence of ladder-like secondary veins (fig. 1), or by larger primary veins. However, the lack of a relationship between LMA and leaf size, and the isometric relationship between lamina area and lamina mass (Table 2), indicate that there are similar levels of support investment within the lamina across leaf sizes. Milla and Reich (2007) showed that within most species, specific leaf area (the inverse of LMA) declines with mass, but it increases for some species and remains constant for others. This, combined with my results, suggests that the relationship between LMA and leaf size may be taxon specific, despite the overall trend at large phylogenetic scales (Milla and Reich 2007; Niinemets et al. 2007).

There were increases in petiole dimensions with leaf size. However, investment in petioles does not appear to increase proportionally more than lamina mass in larger-leaved *Dioscorea* species. The log-scale slope of the regression of lamina mass on petiole length was approximately 2, indicating that lamina mass scales to petiole length as square root of length (Table 2). This is consistent with the relationship found across 19 diverse species (Niklas 1994) and suggests that my results are not exceptional. Lamina mass increased isometrically with petiole cross-sectional area. This relationship was weaker than the petiole length-lamina mass relationship, probably because the petioles of different species have different cross-sectional shapes and thus different flexural stiffness (Niklas 1994). Assuming that petiole mass is directly proportional to petiole length and cross-sectional area (that is, there are no changes in density with petiole size), then the investment in petiole tissue decreased with leaf size. Despite lower biomass investment in petioles with increasing leaf size, there is no loss of conductive capacity, since Pet VB area increases isometrically with leaf size (Table 2).

My data suggest that within the genus *Dioscorea*, there are minimal constraints on the evolution of larger leaves due to the cost of constructing support tissue. Instead, the evolution of leaf size may be constrained more by the trade-off between water transport efficiency and protection from embolism. My results suggest an increase in conductivity of the mid-rib with leaf size, since MR TE diameter and $K_{t\text{-}mr}$ increased with lamina area (Table 2). This makes sense, since larger leaves have lower PVD, meaning each vein has to supply a larger area. The larger size of xylem elements in the primary veins of larger leaves is likely to come with an increased risk of embolism (Jarbeau et al. 1995; Hacke and Sperry 2001), which is consistent with the apparently increased stomatal sensitivity of larger-leaved species (higher $\delta^{13}C$, see discussion above). A trade-off between increased conductivity of large veins and sensitivity to drought-induced embolism may be one factor preventing runaway evolution of leaf size in *Dioscorea* or other taxa.

Conclusions

By examining relationships among a large set of interdependent leaf traits, I was able to develop an integrated view of how evolutionary changes in leaf structures affect their functions. The unexpected form-function relationships and the deviations from large-scale patterns that I found may have arisen because of the unique ecological challenges and combinations of traits found in herbaceous vines, such as the need for rapid seasonal growth and high stem conductivity (Ewers and Fisher 1991; Putz and Mooney 1991). *Dioscorea* may face additional challenges because it is a monocot, and therefore has no secondary growth in its stems. A constraint on the ability to increase stem conductivity relative to leaf area could contribute the heightened stomatal sensitivity of large-leaved *Dioscorea* species and select for the negative correlation between SD and lamina area. This same stomatal sensitivity could be driving the negative relationship between SD and g_s or A_{max}. My results highlight the need to use caution when extrapolating structure-function relationships from one functional or taxonomic group to another. They also demonstrate the importance of considering the effects of multiple traits when interpreting structure-function relationships. On its own, the relationship between SD and g_s seems anomalous, but it is consistent with all of the other leaf traits relationships.

Similarities and difference in trait correlations across phylogenetic scales provide insight into the evolutionary processes that lead to large-scale correlations. As more studies within genera are published, a general pattern is emerging of variable leaf trait relationships among taxa. Leaf-level gas exchange or water use strategies appear to be taxon specific, depending on the growth form and ecology of the plant (Edwards 2006), and combinations of traits that make sense at larger phylogenetic scales are not necessarily adaptive among close relatives. Even though coordinated hydraulic supply and demand, or a negative relationship between LMA and A_{max} may work as general optimization principles, the functional demands of a particular growth form may override those principles. Constraints among close relatives, such as the inability to change overall leaf or growth form, may lead to the evolution of alternative strategies and unusual combinations of leaf traits. The scatter in large-scale relationships like the LES (Wright et al. 2004) provides plenty of room for alternative relationships within its range of values. More systematic investigations of leaf trait variation across phylogenetic scales could help to clarify which factors promote or prevent changes in trait correlations across scales.

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Tables

Table 1. Study species and their native ranges (Burkill 1960; Ayensu 1972; Burkill 1985).

Species and authority	Section	Native Range
Dioscorea alata L.	Enantiophyllum	East Asia, Pan-Tropic in cultivation
Dioscorea batatas Dcne. (syn.: *D. oppositifolia* L.)	Enantiophyllum	Subtropical eastern Asia, sometimes cultivated
Dioscorea bulbifera L.	Opsophyton	Old World tropics, introduced, sometimes cultivated
Dioscorea cotinifolia Knuth	Opsophyton	Sub-tropical southern Africa
Dioscorea dodecaneura Vell.	Lasiogyne	Tropical South America
Dioscorea dumetorum Knuth	Lasiophyton	Tropical Africa, sometimes cultivated
Dioscorea macrmya Harms	Macrmya	Tropical Africa
Dioscorea nipponica Makino	Stenophora	Japan and nearby mainland Asia
Dioscorea pentaphylla L.	Lasiophyton	Tropical Asia and Pacific Islands, cultivated
Dioscorea sylvatica Ecklon	Testudinaria	Sub-tropical southern Africa
Dioscorea trifida L.	Macrogynodium	West Indies and northern South America, cultivated
Dioscorea villosa L.	Stenophora	Eastern United States

Table 2. Regression coefficients with confidence intervals (CI) of lamina area and mass against selected leaf traits. Slopes in bold are significantly different from one, or, in the case of Lamina Area versus Petiole VB area, Midrib VB are MR TE area, significantly different from zero. Intercepts in bold are significantly different from zero.

Trait 1 (X)	Trait 2 (Y)	r^2	Intercept	CI Intercept	Slope	CI Slope
Log Lamina Area	Log Lamina Mass	98.96	**-2.54**	-2.81 – -2.26	1.02	0.95 – 1.10
Log Lamina Area	Log LMA	8.47	**1.46**	1.21 - 1.71	0.03	-0.04 – 0.09
Log Lamina Area	Log Primary Vein Density	75.53	**0.71**	0.09 – 1.33	**-0.42**	-0.58 – -0.26
Log Petiole Length	Log Lamina Mass	93.66	**-2.00**	-2.64 – -1.37	**1.90**	1.57 – 2.24
Log Petiole Area	Log Lamina Mass	85.65	**-1.57**	-2.50 – -0.64	1.15	0.81 – 1.48
Log Lamina Area	Log Petiole VB Area	47.53	0.68	-1.07– 2.43	**0.71**	0.25 – 1.17
Log Lamina Area	Log Midrib VB Area	53.85	-0.08	-1.59 – 1.43	**0.76**	0.36 – 1.16
Log Lamina Area	Log Midrib TE Diameter	81.12	**-2.28**	-2.70 – -1.86	**0.33**	0.22 – 0.44
Log Lamina Area	Log $K_{t\text{-}mr}$	81.29	**-2.30**	-4.09 – -0.52	**1.42**	0.95 – 1.89

Figure Legends

Fig. 1. Predicted suites of traits based on studies at medium to large phylogenetic scales (see text for references). **A.** Global scale relationships among the traits of the leaf economic spectrum. A_{max}, g_s, and %N are all positively correlated with each other, and negatively correlated with LMA, as a result of a trade-off between high physiological activity and long leaf life span. **B.** Traits that describe the maximum fluxes of water or CO_2 through a leaf have been shown to correlate with each other across taxa. This includes A_{max}, g_s or transpiration, and K_{leaf}. Correlations among fluxes are thought to arise from selection for coordinated leaf hydraulic supply and demand. This selective pressure leads to **C.**, positive correlations between fluxes and the structural traits that physically control the flow of water or CO_2 through the leaf (arrow with + sign) and among those structures. These include SPI, MVD, palisade thickness, and measures of the mid-rib conduit diameters, such as theoretical mid-rib conductivity ($K_{t\text{-}mr}$).

Fig. 2. *Dioscorea bulbifera* leaf showing primary veins that originate from the lamina/petiole junction. Note the prominent, ladder-like secondary veins between the primaries. Arrow points to large secondary vein that branches from the first primary vein near the base of the leaf, that was included in calculations of primary vein density.

Fig. 3. Bootstrap consensus tree from maximum parsimony (MP) analyses of data from matK, ndhF, rbcL and atpB- rbcL spacer (4533 sites), with branch lengths generated by maximum likelihood analysis. Numbers on branches are MP/ML bootstrap numbers.

Fig. 4. Compare to fig. 1 for predictions. Solid lines represent positive correlations, dashed lines negative correlations, thick lines $P<0.05$, thin lines $0.05<P<0.08$. **A.** Only the suite of traits associated with the leaf economic spectrum was well supported among *Dioscorea* species (gray oval on left; mutual correlations among A_{max}, g_s, %N, and LMA). There was no evidence for the suite of traits representing the coordination of hydraulic supply and demand. Although A_{max} and g_s were positively correlated, neither was correlated with K_{leaf}. There was limited evidence for the suite of structural traits that control flux rates (gray oval on the right). Only SPI and MVD were significantly correlated with each other, but there were trends for positive correlations with $K_{t\text{-}mr}$ (Appendix 2.3). Palisade thickness was independent of the other structures. A_{max} and g_s were negatively correlated with structures, the opposite of the prediction, but K_{leaf} was positively correlated with palisade thickness, as predicted. **B.** Separating SPI into its components, SD and GCL, revealed additional correlations among structures (gray oval on the right), as well as a correlation between g_s and SD. **C.** $\delta^{13}C$ was correlated with all of the traits from the LES, plus GCL, SD, and $K_{t\text{-}mr}$. Ψ_{min} was correlated with palisade thickness, GCL and K_{leaf}. **D.** Lamina area was correlated with $\delta^{13}C$, g_s, SD, GCL, and $K_{t\text{-}mr}$.

Fig. 5. Scatter plots of all pair-wise combinations of species' means and standard errors for A_{max}, g_s, %N, and LMA. Correlation coefficients are PGLS-r (bold,* $P<0.05$; underlined, + $0.05<P<0.08$).

Fig. 6. Scatter plots of species' means and standard errors for K_{leaf} versus A_{max}, and g_s. Correlation coefficients are PGLS-r (bold,* $P<0.05$; underlined, + $0.05<P<0.08$).

Fig. 7. Scatter plots of all pair-wise combinations of species' means and standard errors for SPI, MVD, K_{t-mr} and palisade thickness. Correlation coefficients are PGLS-r (bold,* $P<0.05$; underlined, + $0.05<P<0.08$).

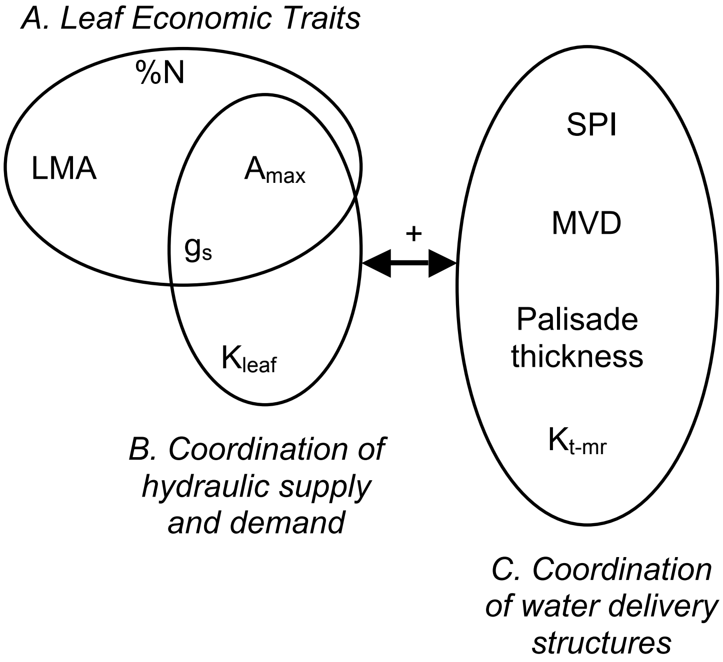
Fig. 8. Scatter plots of species' means and standard errors for fluxes (A_{max}, g_s , and K_{leaf}) versus leaf structures (SPI, MVD, palisade thickness, K_{t-mr}). Correlation coefficients are PGLS-r (bold,* $P<0.05$; underlined, + $0.05<P<0.08$).

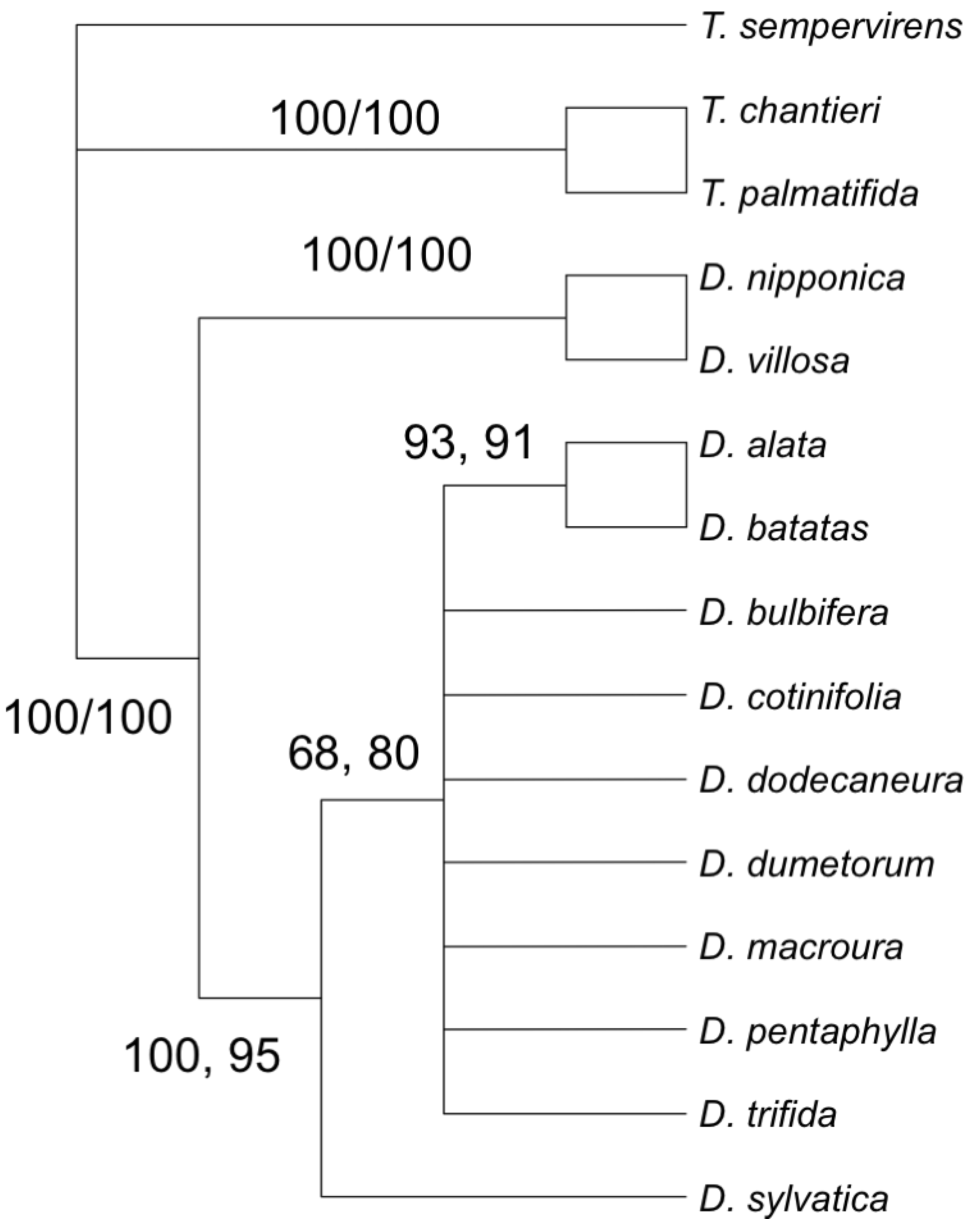
Fig. 9. Scatter plots of species' means and standard errors for $\delta^{13}C$ versus leaf structure (SD, GCL, MVD, and K_{t-mr}) and leaf economic traits (A_{max}, g_s, %N, and LMA) . $\delta^{13}C$ was not significantly correlated with SPI or Ψ_{min}, so plots are not shown. Correlation coefficients are PGLS-r (bold,* $P<0.05$; underlined, + $0.05<P<0.08$).

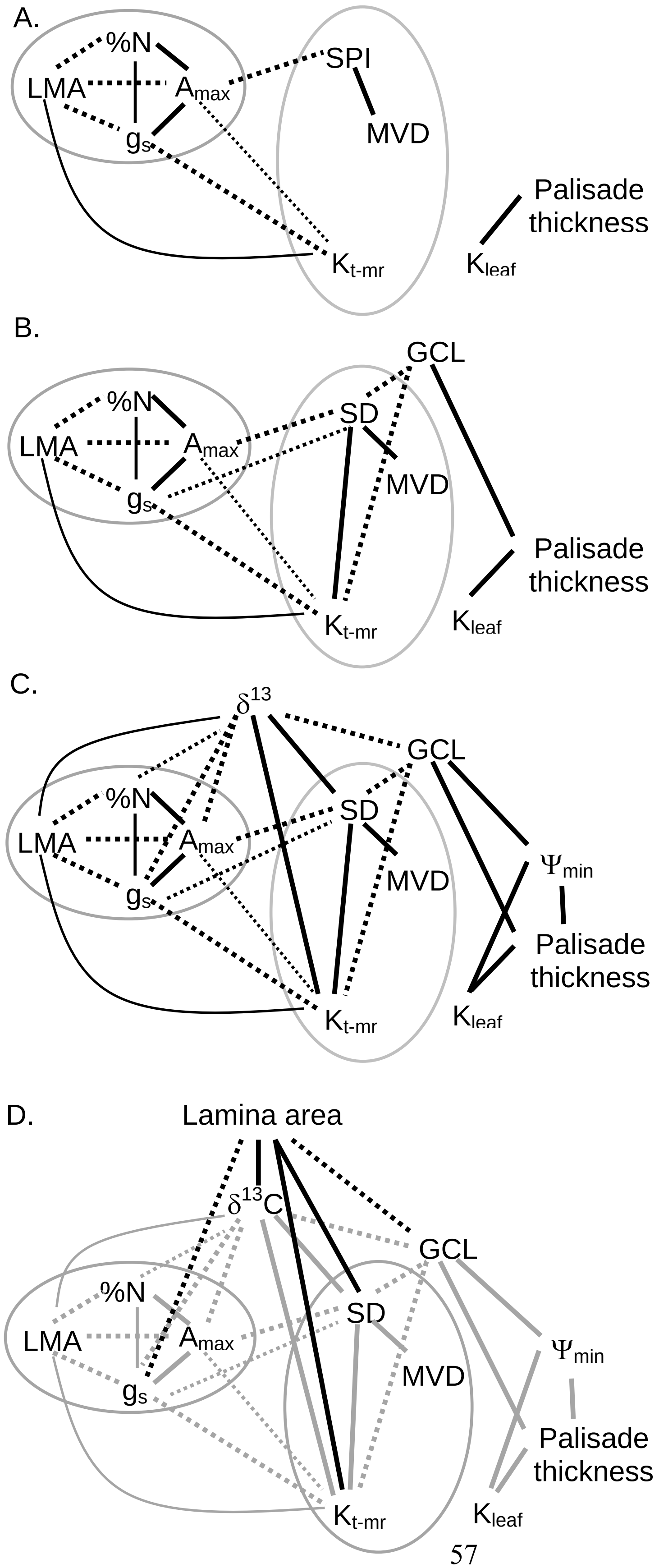
Fig. 10. Scatter plots of species' means and standard errors for Ψ_{min} versus GCL, SD, palisade thickness, and Kleaf. Ψ_{min} was not significantly correlated with other traits, so plots are not shown. Correlation coefficients are PGLS-r (bold,* $P<0.05$).

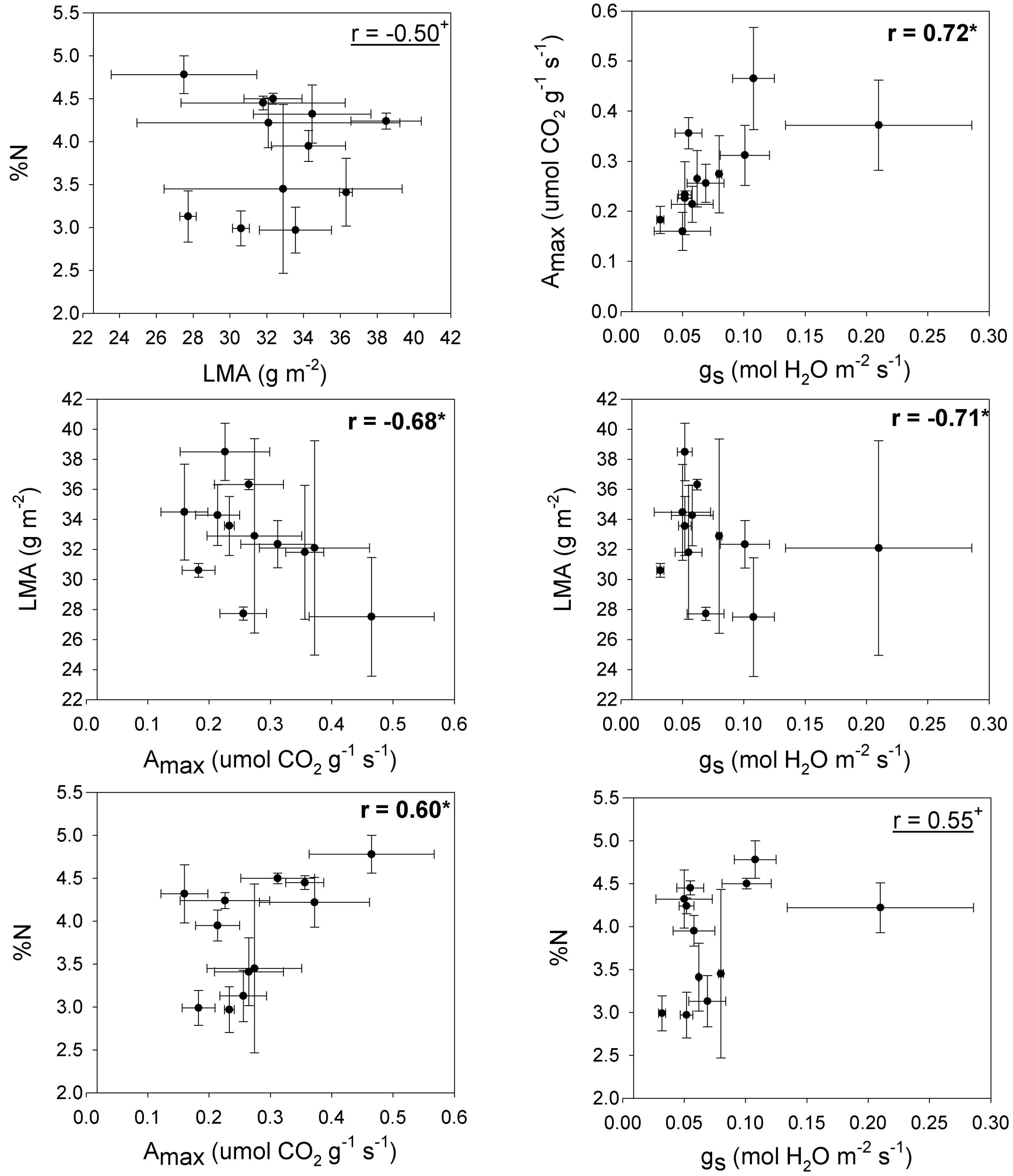
Fig. 11. Scatter plots of species' means and standard errors for lamina area versus leaf structures (GCL, SD, SPI, MiVD, and K_{t-mr}), leaf functions (A_{max}, g_s, Ψ_{min}, $\delta^{13}C$, and K_{leaf}). Correlation coefficients are PGLS-r, except where marked as r_s, which are standard correlations coefficients (bold,* $P<0.05$; underlined, + $0.05<P<0.08$).

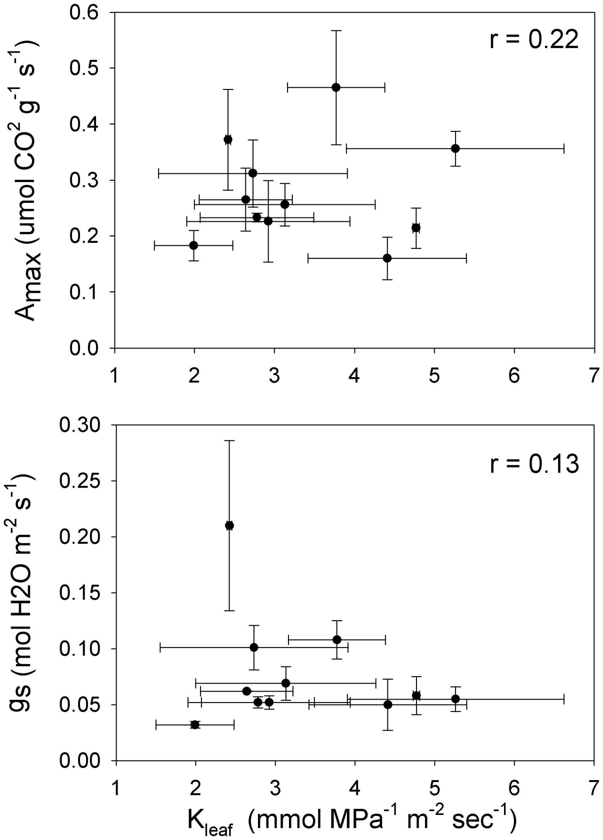
Fig. 12. *Dioscorea* leaf traits formed two relatively independent suites of traits. The first was comprised all of the traits that were correlated with $\delta^{13}C$ (gray oval on left). This included the leaf economic traits and most of the leaf structures. MVD is associated with this suite of traits because of its significant correlation with SD, but its relationships to other traits were weak. The link between $\delta^{13}C$ and leaf economic traits, and the negative relationship between fluxes and structures are contained within this suite. I hypothesize that all of the traits in this suite share a dependence on SD, through its effect on g_s. I hypothesize that the second suite of traits (gray oval on right) is comprised of the traits that depend on cell size. This includes direct measures of cell size (GCL and palisade thickness), as well as the functions that may have a mechanistic link to cell size (Ψ_{min} and K_{leaf}). Stomatal characteristics form a bridge between the two suites of traits, because of the significant negative correlation between SD and GCL.

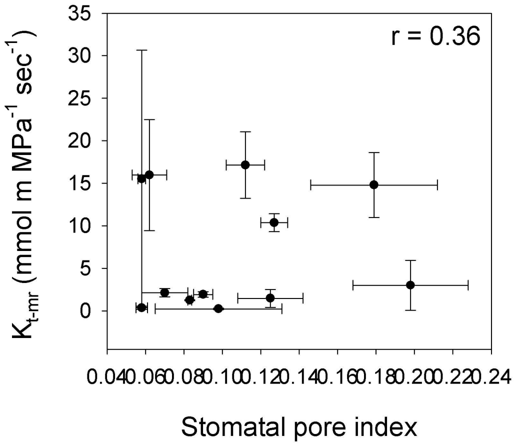

A. Leaf Economic Traits
%N
LMA
A_{max}
g_s
K_{leaf}
B. Coordination of hydraulic supply and demand
+
SPI
MVD
Palisade thickness
$K_{t\text{-}mr}$
C. Coordination of water delivery structures

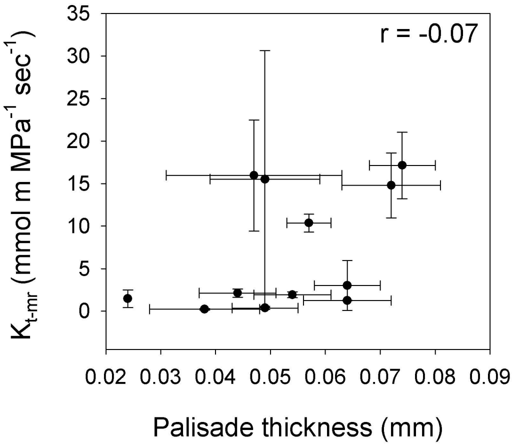

T. sempervirens
100/100
T. chantieri
T. palmatifida
100/100
D. nipponica
D. villosa
93, 91
D. alata
D. batatas
D. bulbifera
D. cotinifolia
100/100
68, 80
D. dodecaneura
D. dumetorum
D. macroura
D. pentaphylla
100, 95
D. trifida
D. sylvatica

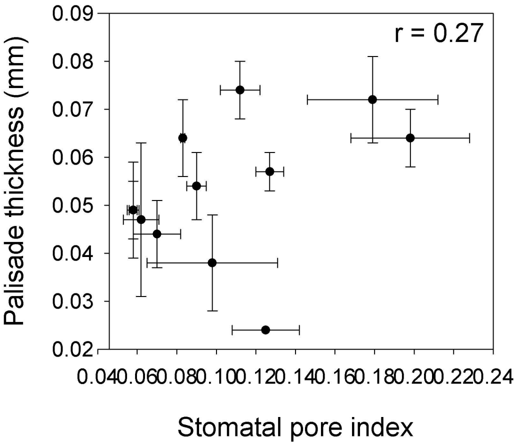

A.
%N
LMA
Amax
gs
SPI
MVD
Palisade thickness
Kt-mr
Kleaf
B.
GCL
%N
SD
LMA
Amax
MVD
gs
Palisade thickness
Kt-mr
Kleaf
C.
δ13
GCL
%N
SD
LMA
Amax
Ψmin
gs
MVD
Palisade thickness
Kt-mr
Kleaf
D.
Lamina area
δ13C
GCL
%N
SD
LMA
Amax
Ψmin
MVD
gs
Palisade thickness
Kt-mr
Kleaf

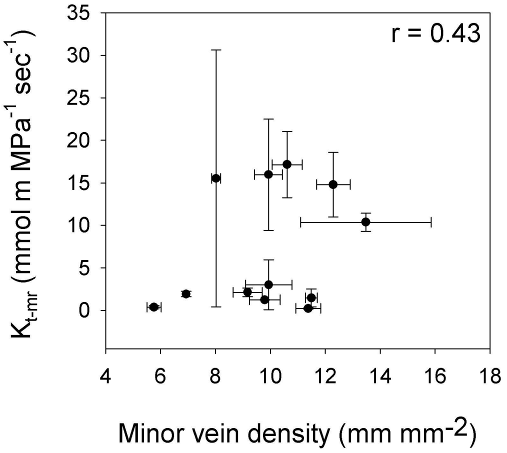

r = -0.50+
%N
LMA (g m-2)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
22
24
26
28
30
32
34
36
38
40
42
r = 0.72*
Amax (umol CO2 g-1 s-1)
gs (mol H2O m-2 s-1)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
r = -0.68*
LMA (g m-2)
Amax (umol CO2 g-1 s-1)
r = -0.71*
LMA (g m-2)
gs (mol H2O m-2 s-1)
r = 0.60*
%N
Amax (umol CO2 g-1 s-1)
r = 0.55+
%N
gs (mol H2O m-2 s-1)

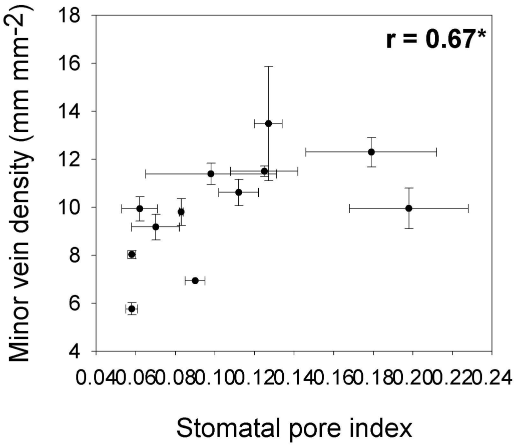

r = 0.22
Amax (umol CO2 g-1 s-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
1
2
3
4
5
6
7
r = 0.13
gs (mol H2O m-2 s-1)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
1
2
3
4
5
6
7
Kleaf (mmol MPa-1 m-2 sec-1)

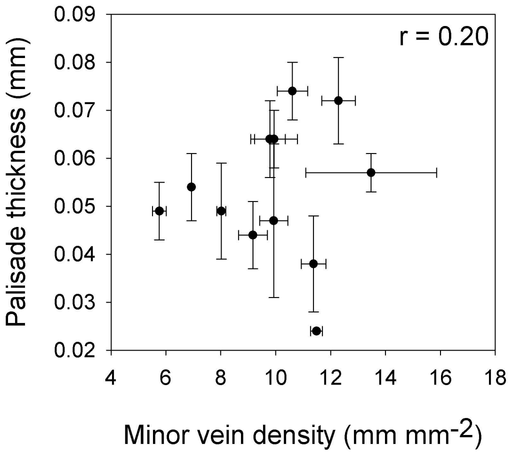

r = 0.36
$K_{t\text{-}mr}$ (mmol m MPa^{-1} sec^{-1})
35
30
25
20
15
10
5
0
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
0.20
0.22
0.24
Stomatal pore index

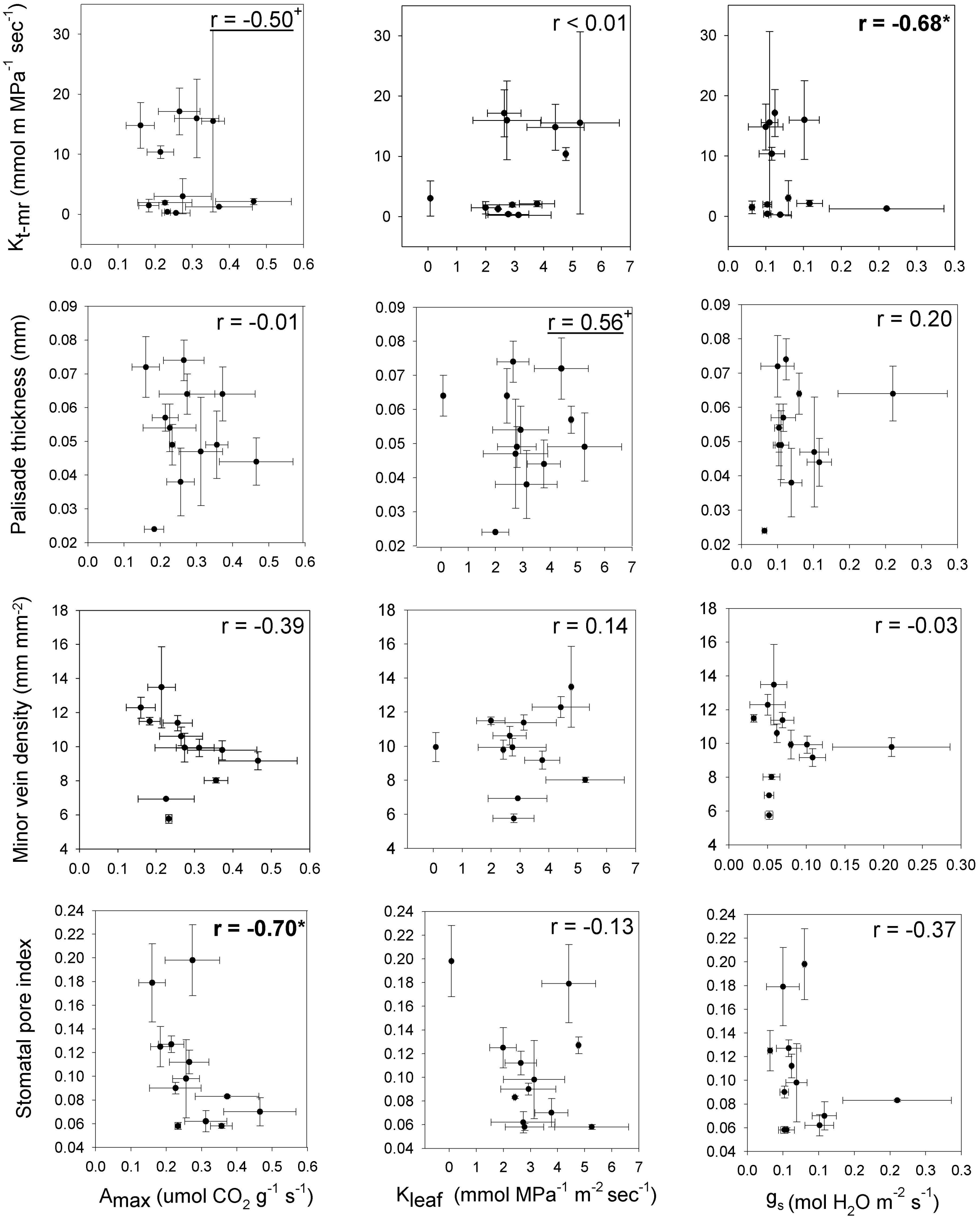

r = -0.07
$K_{t\text{-}mr}$ (mmol m MPa^{-1} sec^{-1})
35
30
25
20
15
10
5
0
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
Palisade thickness (mm)

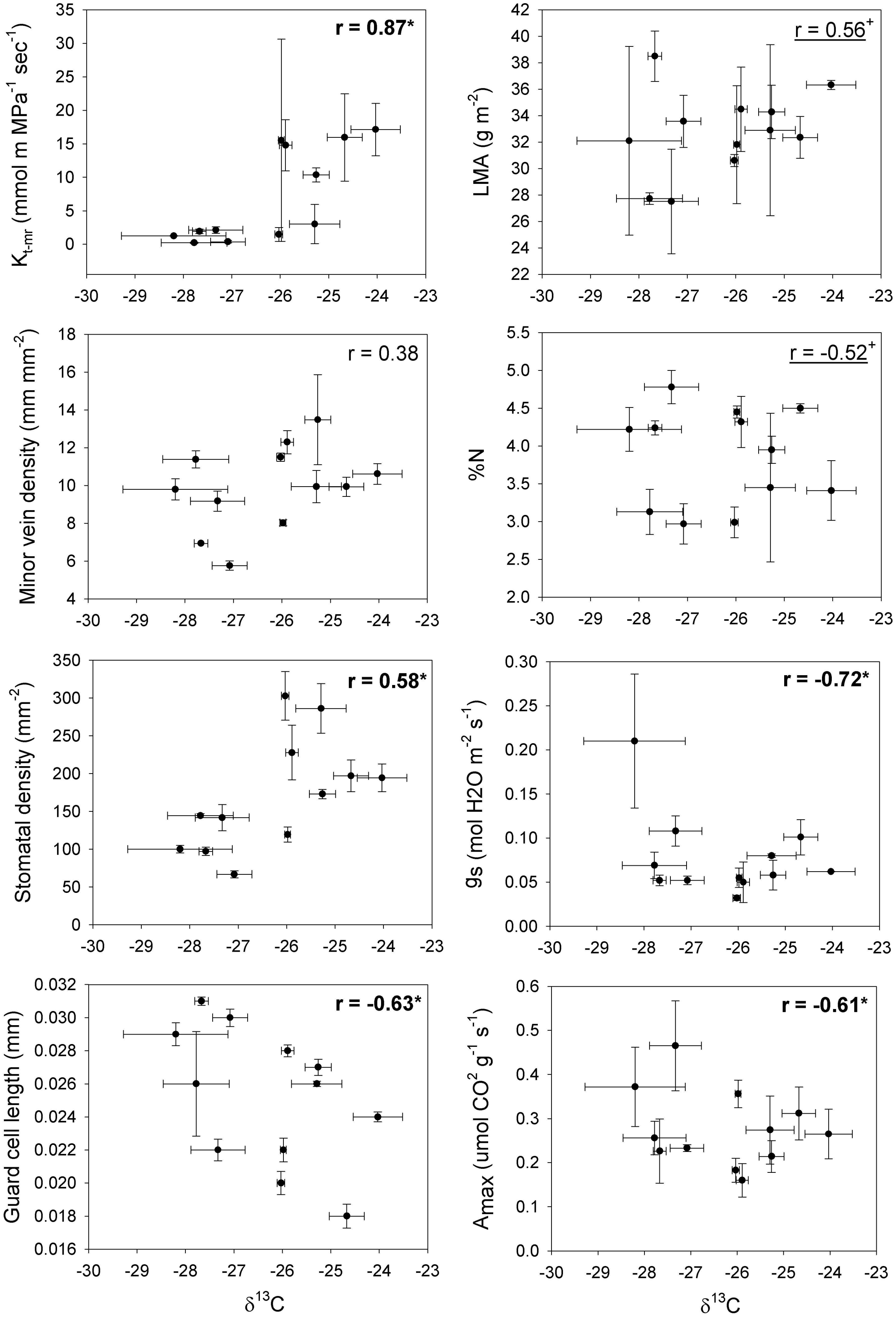

r = 0.27
Palisade thickness (mm)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
0.20
0.22
0.24
Stomatal pore index


r = 0.43
$K_{t\text{-}mr}$ (mmol m MPa^{-1} sec^{-1})
35
30
25
20
15
10
5
0
4
6
8
10
12
14
16
18
Minor vein density (mm mm^{-2})

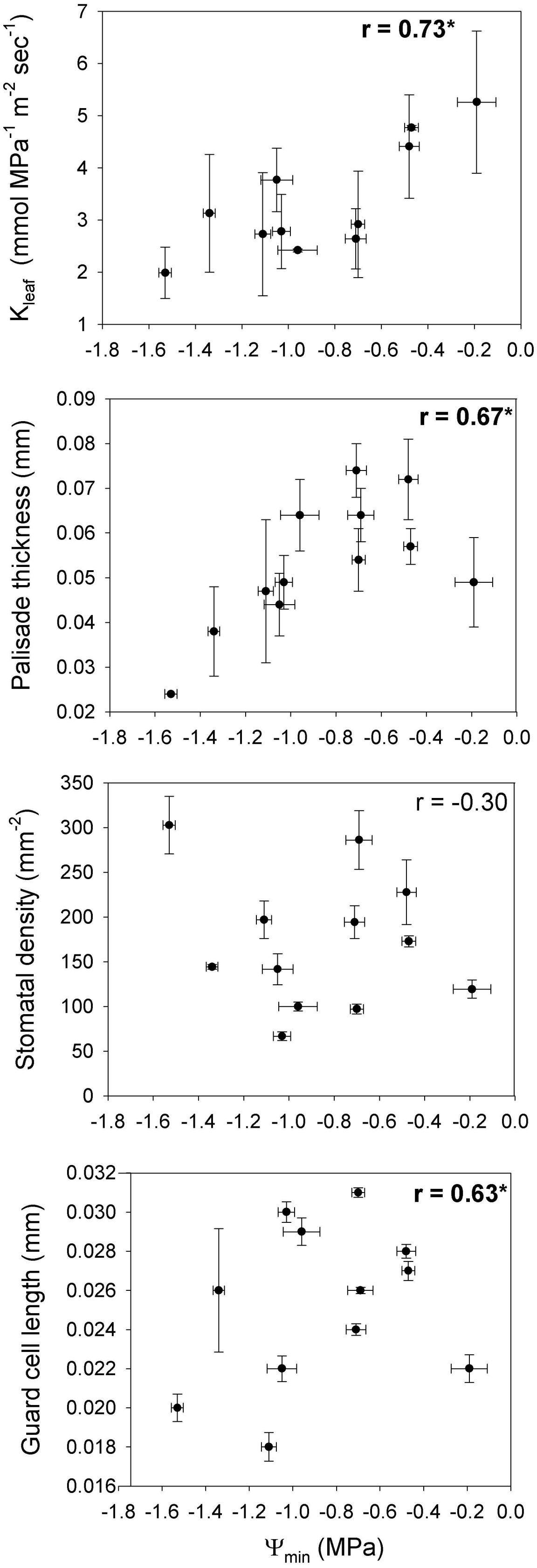

r = 0.67*
Minor vein density (mm mm^{-2})
18
16
14
12
10
8
6
4
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
0.20
0.22
0.24
Stomatal pore index

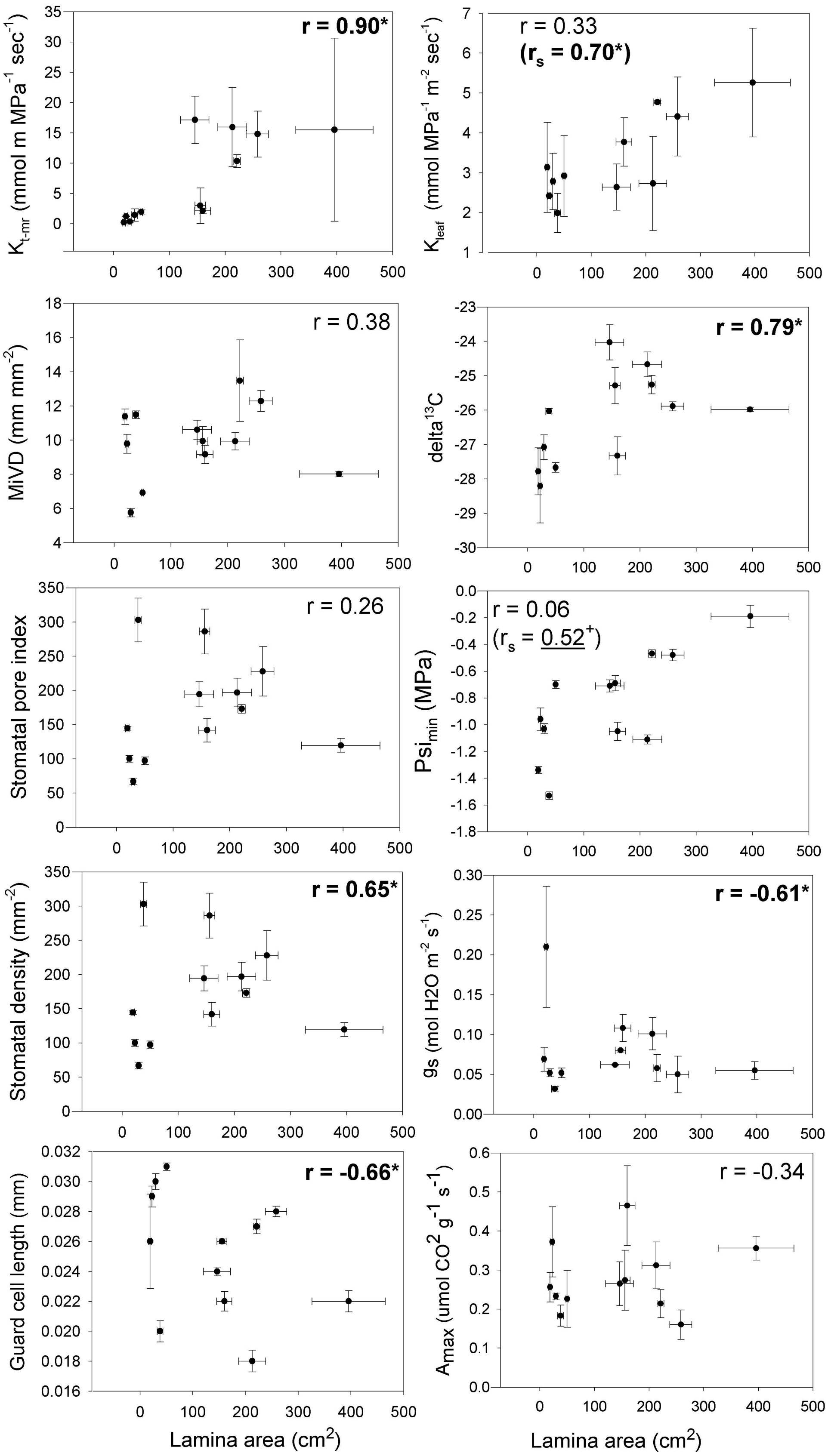

r = 0.20
Palisade thickness (mm)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
4
6
8
10
12
14
16
18
Minor vein density (mm mm^{-2})


Kt-mr (mmol m MPa-1 sec-1)
Palisade thickness (mm)
Minor vein density (mm mm-2)
Stomatal pore index
Amax (umol CO2 g-1 s-1)
Kleaf (mmol MPa-1 m-2 sec-1)
gs (mol H2O m-2 s-1)
r = -0.50+
r < 0.01
r = -0.68*
r = -0.01
r = 0.56+
r = 0.20
r = -0.39
r = 0.14
r = -0.03
r = -0.70*
r = -0.13
r = -0.37

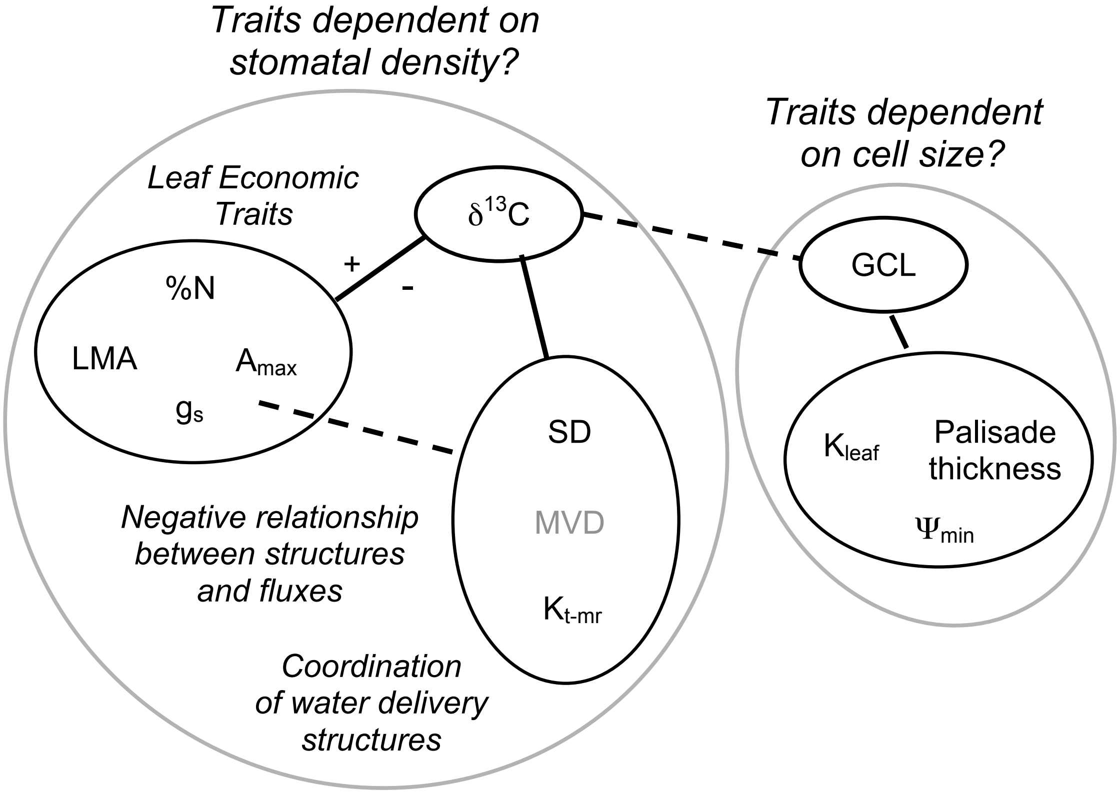


K_{t-mr} (mmol m MPa^{-1} sec^{-1})
r = 0.87*
LMA (g m^{-2})
r = 0.56$^+$
Minor vein density (mm mm^{-2})
r = 0.38
%N
r = -0.52$^+$
Stomatal density (mm^{-2})
r = 0.58*
gs (mol H2O m^{-2} s^{-1})
r = -0.72*
Guard cell length (mm)
r = -0.63*
Amax (umol CO^2 g^{-1} s^{-1})
r = -0.61*
-30
-29
-28
-27
-26
-25
-24
-23
δ^{13}C
δ^{13}C


r = 0.73*
K_leaf (mmol MPa^-1 m^-2 sec^-1)
1
2
3
4
5
6
7
r = 0.67*
Palisade thickness (mm)
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
r = -0.30
Stomatal density (mm^-2)
0
50
100
150
200
250
300
350
r = 0.63*
Guard cell length (mm)
0.016
0.018
0.020
0.022
0.024
0.026
0.028
0.030
0.032
-1.8 -1.6 -1.4 -1.2 -1.0 -0.8 -0.6 -0.4 -0.2 0.0
Ψmin (MPa)



$K_{t\text{-}mr}$ (mmol m MPa^{-1} sec^{-1})
r = 0.90*
K_{leaf} (mmol MPa^{-1} m^{-2} sec^{-1})
r = 0.33
(r_s = 0.70*)
MiVD (mm mm^{-2})
r = 0.38
delta^{13}C
r = 0.79*
Stomatal pore index
r = 0.26
Psi$_{min}$ (MPa)
r = 0.06
(r_s = 0.52$^+$)
Stomatal density (mm^{-2})
r = 0.65*
gs (mol H2O m^{-2} s^{-1})
r = -0.61*
Guard cell length (mm)
r = -0.66*
Amax (umol CO^2 g^{-1} s^{-1})
r = -0.34
0
100
200
300
400
500
Lamina area (cm^2)
Lamina area (cm^2)


Traits dependent on stomatal density?
Traits dependent on cell size?
Leaf Economic Traits
$\delta^{13}C$
+
-
%N
LMA
A_{max}
g_s
GCL
SD
MVD
$K_{t\text{-}mr}$
K_{leaf}
Palisade thickness
Ψ_{min}
Negative relationship between structures and fluxes
Coordination of water delivery structures

CHAPTER 3

LEAF FUNCTIONAL TRAITS, CLIMATE NICHES, AND PHYLOGENETIC CONSERVATISM IN MEXICAN *DIOSCOREA* SPECIES

Abstract

I assessed the role of natural selection by climate in the diversification of Mexican *Dioscorea* species and their leaf traits. I used both standard and phylogenetic generalized least squares (PGLS) methods to analyze correlations between leaf functional traits and regional-scale variation in temperature and precipitation, or climate niches, and examined the phylogenetic patterns of leaf traits and climate niches. Lamina area, leaf mass per area, length: width ratio, water content, and minimum conductance were significantly correlated with climate niches using standard methods, but not using PGLS. All of these traits were correlated with each other, and all except length:width were phylogenetically conserved. Climate niches (measured as mean annual temperature, annual precipitation, and altitude) were also phylogenetically conserved. These results suggest that while these leaf traits are important for adaptation to climate, their current association with climate is a result of earlier adaptation followed by niche conservatism, rather than repeated adaptive evolution. The traits that I expected to be under selection by micro-environmental factors – maximum photosynthetic rate, stomatal density, guard cell length, and stomatal conductance – were not significantly correlated with climate parameters using either method. None of these traits was phylogenetically conserved. Minimum water potential was an exception, since it was phylogenetically conserved, but not correlated with climate parameters. The striking relationship between whether or not traits were correlated with climate and whether or not they were phylogenetically conserved supports the notion that niche conservatism is tightly linked to functional trait conservatism. The combination of conserved traits and niches at one scale with labile traits and niches at another could provide a general mechanism for high species diversity in biodiversity hotspots like Mexico.

Introduction

Mexico is a biodiversity hotspot, with exceptionally high diversity and endemism of many taxa (Myers et al. 2000), including plants of the monocot vine genus *Dioscorea*. Of the approximately 500 species in the genus worldwide (World Checklist of Selected Plant Families 2009), approximately 80 are found in Mexico, of which 48 are endemic or nearly endemic (personal communication, O. Telléz-Valdés). Mexico's high spatial variation in temperature and precipitation within a relatively small area suggests that natural selection by climate factors may have contributed to its biological diversity. Correlations between species' traits and climate (Bailey and Sinnott 1916; Wolfe 1995; Wright et al. 2005), coupled with associations between species distributions and climate (the basis for species distribution modeling; Graham et al. 2004; Heikkinen et al. 2006), support the hypothesis that morphological and physiological variation among species is associated with adaptation to climate. In this study, I assess the role of natural selection by regional climate factors in the diversification of Mexican *Dioscorea* species and their leaf traits. I use the term climate niche to describe regional-scale variation in temperature and precipitation. This is similar to the beta niche (Ackerly et al. 2006; Silvertown et al. 2006). I also examine several functional traits that I expect to be independent of climate parameters, but important for adaptation to local environmental conditions, or the alpha niche. The different spatial scales of these two types of niches may result in different evolutionary patterns for the traits that are important for each type of niche.

Leaves are a major interface between plants and the environment, so species' values of leaf traits should be important for determining their climate tolerances. All *Dioscorea* species in Mexico are drought deciduous, use tubers to survive the dry season, and have a herbaceous, vining growth form. Because of these similarities in form, leaf traits provide a major axis of variation in Mexican *Dioscorea*. Another major axis of variation is plant size, with species ranging from dwarf, prostrate plants to large twiners that may climb up to 20 meters into the canopy. Since leaf size is correlated with plant size (personal observation, R. Walls), leaf form also captures some of this second axis of variation. For this study, I measured eleven leaf functional traits that are related to water use and gas exchange. Leaf functional traits include physiological, morphological or anatomical traits that are important for plant survival, growth, and ultimately fitness (Geber and Griffen 2003). Cross-species correlations between functional traits and climate are consistent with adaptation to climate conditions, and provide a mechanistic basis for analyses of species distributions (Graham et al. 2004; Guisan and Thuiller 2005; Kearney 2006).

I used both standard and phylogenetic methods to test for correlations between leaf functional traits and climate. The inclusion of phylogenetic information allowed me to make additional inferences about which evolutionary processes are likely to have resulted in current relationships between leaf traits and climate (Harvey and Pagel 1991; Westoby et al. 1995). The different possible outcomes are described in Table 1. Phylogenetically-based correlations between functional traits and climate parameters would suggest that evolutionary change in leaf functions co-occurred repeatedly with changes in species' climate niches (Table 1, upper row). This relationship should be present when traits and niches are evolutionarily labile (Table 1, upper row, right column), but difficult to detect when there is conservatism of traits or niches (Table 1, upper row, left column). Significant PGLS correlations would support the hypotheses that the functions I measured are important for adaptation to climate, and that repeated evolution into new climate niches contributed to leaf trait and species diversification in Mexican *Dioscorea*. Correlations between traits and climate variables that are significant using standard methods, but not using phylogenetic methods, would suggest that the relationship between traits and climate did not arise through correlated evolutionary change (Table 1, lower row, left column). This pattern would be consistent with adaptation to past climate conditions, or exaptation (Gould 1997), followed by phylogenetic conservatism of climate niches and/or traits. This would suggest that there are limits to the climate niches that particular clades can occupy, associated with their evolutionary history. If traits are not correlated with climate niches using phylogenetic or standard methods, this would suggest that the traits I measured are not important for species adaptation to climate variation and/or that climate is not the dominant selective force acting on species and leaf trait variation (Table 1, lower row, right column). Instead, these traits may be under selection by factors that are relatively independent of regional-scale climate variation, such as light level or competition from co-occurring species.

In order to distinguish among the scenarios described above, I tested for phylogenetic conservatism of the climate niches and leaf functional traits of Mexican *Dioscorea* species. The role of phylogenetic conservatism versus rapid evolution of in plant species diversification has not been widely studied, and this is one of a handful of studies that examine the evolutionary patterns of physiological traits among a group of close relatives (Cavender-Bares et al. 2004b; Edwards and Donoghue 2006; McKown and Dengler 2007). Studies within species have demonstrated rapid evolutionary change in physiological traits related to photosynthesis (Arntz and Delph 2001; Geber and Griffen 2003), while differences in leaf functional traits among large clades such as angiosperms, gymnosperms, and ferns suggest some degree of phylogenetic conservatism (Ackerly and Reich 1999; Boyce 2005; Boyce et al. 2009). Between these two phylogenetic scales, little is known about the evolutionary lability of most plant traits.

Methods

Study system

This study examined 20 Mexican species of the monocot genus *Dioscorea*. All are drought deciduous and use tubers to survive the dry period, although there is considerable variation in their tuber size and shape (personal observation, R. Walls). Most *Dioscorea* species, including Mexican species, grow at forest edges in a range of well-drained soils and rainfall conditions, and total annual rainfall may be a major factor controlling the distribution of the genus (Burkill 1960). There are approximately 80 species of *Dioscorea* distributed throughout Mexico, in habitats ranging from lowland tropical rain forest to temperate pine-oak forest, but they do not occur in the driest, desert locations (unpublished data, R. Walls and O. Telléz-Valdés).

Phylogeny

I collected sequence data for 2 chloroplast gene regions [ndhF (nicotinamide dehydrogenase F subunit) and the spacer between atpB (the beta subunit of chloroplast ATP synthase) and rbcL (the large subunit of ribulose 1,5-bisphosphate carboxylase)] for 24 new species and added them to an existing database of *Dioscorea* sequences (unpublished, R. Geeta). I analyzed a total of 103 species, of which 54 were native to Mexico and 11 were outgroup species. Sequencing methods, primer sequences, and alignment are described in Walls (Chapter 2). I selected the GTR+I model for ndhF and the GTR+G model for atpB-rbcL, based on Mr. ModelTest (Nylander 2004). To generate phylogenies, I ran Mr. Bayes (Ronquist and Huelsenbeck 2003) for 10 million generations, once with two partitions (one for atpB-rbcL and one for ndhF), and once with four partitions (one for atpB-rbcL and one for each codon positions in ndhF), storing every 1000th tree. I chose the four partition model based on the higher Bayes factor (Nylander et al. 2004). I used all 8000 trees generated after the analysis reached stationary (after 2 million generations, standard deviation of splits frequency <0.05) to make a majority rule consensus tree in Mr. Bayes. For comparative analyses, I used the "sample trees from a separate file" function in Mesquite (Maddison and Maddison 2009) to sample 100 trees of roughly equal probability from those used to construct the consensus tree. I used Mesquite to prune all trees to include only the species used in each analysis.

Plant material and leaf functional traits

I collected tubers from 44 plants of 20 species from throughout Mexico (Table 2, Appendix 3.1). Tubers were grown in a greenhouse on the campus of Stony Brook University, in Stony Brook, New York. The use of a common garden, rather than field measurements, allowed me to measure many physiological traits that cannot practically be measured on a large number of species from throughout a large region. The common garden design also provided an indication of genetic differences among species, separate from plasticity that would be present in the field. Growth conditions are described in Walls (Chapter 2). All measurements took place between July 15 and November 15, 2008. All leaf traits were measured on two to four leaves per plant (three for most traits), using one to six individual plants per species (two for most).

I measured eleven structural and physiological traits that are known to be important for leaf function. As descriptors of leaf structure, I measured leaf size (lamina area), leaf shape (length to width ratio or L:W), stomatal density (SD), guard cell length (GCL), and leaf mass per area (LMA). Leaf area is related to a number of leaf functional traits in *Dioscorea*, including heat tolerance (unpublished data, R. Walls and R. Pearlman) and water use efficiency (WUE) as measured by carbon isotope discrimination (Walls Chapter 2). My field observations suggest that different leaf shapes are associated with different climates and clades (e.g., drip tips in wetter climates, smaller leaves in species of the former genus *Nanarapenta*). SD is correlated with stomatal conductance (g_s), WUE, and photosynthetic capacity, while GCL is correlated with minimum water potential and g_s (Walls Chapter 2). LMA is important for physiological activity and for resistance to physical damage or herbivory (Coley et al. 1985; Wright et al. 2004).

To assess leaf physiological function, I measured minimum or cuticular conductance (g_{min}), minimum water potential (Ψ_{min}), capacitance, leaf water content (WC), maximum photosynthetic rate (A_{max}), and g_s. g_{min} describes the minimum rate of water loss from intact leaves, when their stomata are maximally closed, and may be related to water availability (Kerstiens 1996). Ψ_{min} describes the lowest water potential that leaves experience during the period of highest evaporative demand (generally during mid-day, under full sun). Ψ_{min} measured on a diurnal scale indicates the level of short-term water stress that plants can withstand, and for many species is just above the permanent wilting point. Capacitance is the change in leaf water potential with the change in relative water content (RWC; the mass of water in a leaf at any particular water potential, divided by the mass of water in a fully hydrated leaf), and may be important for tolerating low water stress (Robichaux and Morse 1990; Andrade and Nobel 1997). Leaf water content (WC, distinct from RWC) is the mass of water in a well-hydrated leaf, divided by its total mass. It is important for leaf physiological activity (Lawlor and Cornic 2002). Maximum photosynthetic rate (A_{max}) describes species' photosynthetic capacity, and is positively correlated with g_s and N content in *Dioscorea* and other species (Wright et al. 2004; Walls Chapter 2).

I expect many of these functional traits to be correlated with climate parameters, because of their importance for leaf water use. To the extent that rainfall and temperature determine soil nutrient availability, A_{max} (and the traits correlated with it) may be linked to climate or beta niche. However, I expect them to be more important for microclimatic or alpha niche differentiation, such as differences in light level or fine-scale nutrient availability. LMA may be important for alpha niche differentiation, because of its relationship to photosynthetic capacity (Wright et al. 2004), but its link to water content and regional-scale environmental parameters may also make it important for beta niche differentiation (Niinemets 2001; Wright et al. 2002). I use JMP 5.1 (SAS Institute, Cary, NC) to log transform all trait variables except Ψ_{min} and capacitance, to improve normality.

Lamina area, SD, GCL, LMA, and Ψ_{min} were measured as described in Walls (Chapter 2). L:W was measured from the same images used for lamina area, with length measured from the lamina/petiole junction to the leaf apex and width measured at the widest point of the lamina, perpendicular to the length. I used a Licor LI6400 infrared gas analyzer to measure A_{max}. Conditions were similar to Walls (Chapter 2), except that I maintained the CO_2 input at 400ppm, rather than adjusting input for a constant internal CO_2 concentration. Based on sample light-response curves for all species, I began measurements at 1500 μmol m^{-2} sec^{-1}, waited for conditions to stabilize, increased light to 1750 μmol m^{-2} sec^{-1}, and then measured A_{max} after conditions had stabilized again. I recorded g_s simultaneously with A_{max}.

I measured g_{min} on three leaves per plant, using the bench drying method (Kerstiens 1996; Cornwell et al. 2007). I harvested leaves from well-watered plants, early in the morning, and immediately placed the cut petioles in a beaker of distilled water, covering the laminas with plastic bags. Leaves were held in water in the dark for approximately one hour, to insure that they were in a well-hydrated state, with closed stomata. I then blotted any excess water from the petioles and dipped them into melted paraffin to prevent water loss through the cut end of the petiole. Leaves were placed on a lab bench underneath a cardboard box, to exclude light, and weighed every 10 minutes to the nearest milligram. Temperature and humidity inside the box were monitored throughout the procedure. Temperature and humidity varied from day to day, but generally remained consistent throughout each trial. Raw conductance was calculated as the slope of the rate of water loss over time (change in fresh mass, converted to mmols), divided by the lamina area. To normalize for differences in temperature and humidity on different days (and thus differences in the driving force for evaporation), I divided the raw conductance by the mole fraction water vapor gradient from leaf to air (Pearcy et al. 1991). This was measured as the difference between the vapor pressure in the air inside the box and vapor pressure inside the leaf, assuming that the air inside the leaf was at saturation vapor pressure. Many leaves followed the “normal” pattern of a linear loss of water through time, sometimes showing an initially rapid slope that became shallower and linear after about 30 minutes. Some leaves of some species showed an unusual pattern of an initially shallow, linear slope, which increased to a more rapid linear slope, then later decreased again to the shallower slope. For these species, I used the shallower slope, as it represents the minimum conductance, even though my data suggest that these species may experience higher conductance during the drying phase.

I measured capacitance following (Koide et al. 1991). The night before measurements, plants were watered and covered in black plastic bags, to bring leaves to full hydration. The morning of measurements, I cut leaves from the plants, then immediately placed the petioles in a beaker of distilled water and covered the leaves with plastic bags. I brought the leaves into the laboratory, where I re-cut the petioles under water and put the leaves back under plastic bags in the dark, for up to one hour. At the start of measurements, I cut 1-2 cm of petiole from the leaf, to remove saturated material, weighed the leaf to the nearest mg, then immediately measured the water potential of the leaf using a pressure chamber (PMS Instruments, Corvallis, OR). I repeated the measurements every one to five minutes, depending on the species, holding the leaves on the lab bench between measurements. Calculation of RWC and capacitance requires knowing the mass of the water in the leaf at zero water potential. For some species, hydrated leaves had a water potential close to zero (> -0.5 MPa), but for many species, leaves never reached a water potential above -2 MPa. For *D. subtomentosa*, the water potential would never go above -5 MPa, no matter which techniques I used to hydrate the leaf, so this species was excluded from the analysis. I also excluded three species (*D. matagalpensis*, *D. multinervis*, and *D. urceolata*), because their petioles were too short and weak to withstand repeated measurement in the pressure chamber. For the remaining species, I used the initially linear relationship between fresh mass and water potential to estimate the fresh mass at zero water potential, and used this estimate to calculate RWC. I calculated capacitance as the initial (linear) slope of the relationship between leaf water potential and RWC, pooling measurements from 2-4 leaves per plant. This generally included measurements of RWC >97%, although it included lower RWC for a few species. I calculated WC at full turgor by dividing the actual or estimated mass of water at zero water potential by the leaf fresh weight at the same point.

Climate niches

I collected locality and climate data for 48 species of Mexican *Dioscorea*. This included localities taken from specimens at the Mexico National Herbarium, compiled by my collaborator Oswaldo Telléz-Valdés (~3000 specimens), localities from my own (~200 specimens) and Telléz-Valdés's (~75 specimens) leaf collections, plus localities from the Tropicos database at the Missouri Botanical Garden (~400 specimens; http://www.tropicos.org). For those species whose ranges extend into Central America, I included locations outside Mexico. I had between one and 581 localities per species, 15-150 for most. There were six species (three unidentified) for which I had only one locality.

I used Worldclim data layers to generate climate data for each locality for 19 bioclimatic variables, plus altitude (Hijmans et al. 2005). These variables include annual measures such as mean annual temperature (MAT) and annual precipitation (AP), as well as quarterly or monthly variables, such as temperature of the warmest month or driest quarter, and measures of variability, such as temperature seasonality. Using all Mexican *Dioscorea* localities, I examined pair-wise correlations among all climate variables. All of the temperature variables were correlated with MAT, and all of the precipitation variables were correlated with AP. I conducted preliminary analyses on three variables that were relatively independent of the remaining variables, but still correlated with MAT or AP: maximum temperature of the warmest months, precipitation of the wettest quarter, and precipitation of the warmest quarter. These variables should be more important for *Dioscorea* distributions than those that describe temperature or precipitation during dry or cool periods, because *Dioscorea* is dormant during dry periods and never experiences freezing in Mexico (the lowest minimum temperature of any locality was 4.6° C).

However, correlations with functional traits were always as strong or stronger for the annual variables than the monthly/quarterly variables. Therefore, I chose to use MAT and AP, because they summarize variation in temperature and rainfall. I also included altitude in my analysis. Although altitude is not a climate variable, I included it in my analysis because it is a good proxy for atmospheric CO_2 concentration. CO_2 concentration is important for plant gas exchange, and therefore likely to affect some of the functional traits I measured. Furthermore, both MAT and AP were negatively correlated with altitude for Mexican *Dioscorea* localities, which makes altitude a reasonable summary of annual climatic variation. The negative relationship between altitude and precipitation is due to the topography of Mexico, with wet areas near the coasts and dry areas formed by rain shadows from the mountain ranges and the dry central plateau. However, the relationship between precipitation and altitude is weaker than for temperature, because there are high elevation localities for *Dioscorea* that receive high rainfall, such as cloud forests. I also examined correlations between leaf traits and isothermality (daily temperature range divided by annual temperature range). This variable was independent of most other variables, but correlated with temperature seasonality, maximum temperature of the warmest month, and minimum temperature of the coldest month, so it acts as a summary of temperature variability. I $\log_{10}$ transformed altitude and AP to improve normality.

Correlations between leaf traits and climate

I examined correlations between leaf functional traits and climate parameters (log altitude, MAT, log AP, isothermality) for the 20 species for which I had leaf data (16 species for capacitance and WC), using the same methods described in Walls (Chapter 2). Briefly, I used JMP 5.1 (SAS Institute, Cary, NC) to determine product-moment correlations (standard *r*) among all traits and climate variables. I used the phylogenetic generalized least squares (PGLS) method (Martins and Hansen 1997) as implemented in Compare v.4.6b (Martins 2004), to determine phylogenetic correlations (PGLS *r*). I report standard *r* and the average PGLS *r* for 100 equally probable trees from the Bayesian analysis.

Phylogenetic conservatism of niches and traits

I examined relationships between phylogeny and each of the climate and leaf trait variables using the Geiger package in R v. 2.8.1 GUI v. 1.27 Tiger for Macintosh (Butler and King 2004; Harmon et al. 2008; Urbanek and Iacus 2008; Kozak and Wiens unpublished). This test for phylogenetic conservatism, which was recently described by Kozak and Wiens (unpublished), uses maximum likelihood to determine if the distribution of each variable fits best to a model of white noise (all trait values drawn from the same normal distribution), a Brownian motion model (variables evolve along the tree following a random walk model), or a model of the Ornstein Uhlenbeck process (OU; variables evolve toward an optimum following a random walk; models directional or stabilizing selection). A better fit to the Brownian motion model than the white noise or OU models indicates phylogenetic signal in the data, while a better fit to the OU model than the Brownian motion or white noise models indicates that the trait is less variable than if it were evolving under Brownian motion. By including a fit to the OU model in the definition of phylogenetic conservatism, this test can identify conservatisms in cases where evolutionary signal is obscured because of limited trait variation due to stabilizing selection or some other constraint on evolutionary change (Martins and Hansen 1997; Revell et al. 2008; Kozak and Wiens unpublished). A fit to either the Brownian motion model or the OU model is consistent with phylogenetic conservatism, in the sense that species' trait values resemble those of their close relatives more closely than expected by chance. For this analysis, I used a set of 50 trees (every other tree from the set of 100 equally probable trees described above) and chose a model based on the average Aikake information criterion (AIC). I analyzed all climate and leaf variables using the 20 species raised in the greenhouse (16 for capacitance and WC). Since I had climate data for a total of 48 species, I also tested for niche conservatism using the larger group of species.

Correlations among leaf traits

Walls (Chapter 2) described correlations among a number of leaf functional traits within the genus *Dioscorea*. Since the present study is at a smaller spatial and phylogenetic scale and includes several new traits (capacitance, WC, and g_{min}), I examined pair-wise correlation among all leaf traits, to determine relationships at this finer scale. I calculated correlations using standard methods and the PGLS method (Martins 2004), as described above.

I used linear regression to determine if values of traits measured in the greenhouse were good predictors of traits measured on field-collected leaves. I calculated regressions of field measurements on greenhouse measurements for lamina area, L:W, LMA, SD, and GCL using JMP 5.1 (SAS Institute, Cary, NC).

Results

Phylogeny

Fig. 1 shows the majority rule consensus tree from the Bayesian analysis. The results are broadly consistent with an earlier phylogenetic analysis of *Dioscorea* that included only four Mexican species (Wilkin et al. 2005). There was good support for one large clade composed only of Mexican species and several clades composed of predominantly old-world species but containing a few Mexican species. *D. subtomentosa*, *D. bartlettii*, *D. cyphocarpa* and *D. matudae* are contained in predominantly old world and South American clades. There was good support for multiple smaller clades within the larger Mexican clade. One of these matches the section Trigonobasis (*D. convolvulacea* through *D. pilosioscula*), and one matches the section Apodostemon (*D. composita* through *D. sp1*), but the species of the former genus *Nanarapenta* were not monophyletic.

Some leaf functional traits were correlated with climate

Lamina area, LMA, and L:W were significantly negatively correlated with altitude, and positively correlated with MAT and AP, while WC and g_{min} were significantly positively correlated with altitude and negatively correlated MAT and AP, using standard methods (Table 3). Only WC was significantly positively correlated with isothermality. There were no significant correlations between leaf traits and climate using PGLS methods. A_{max}, g_s, SD, and GCL were unrelated to climate parameters, using either standard or PGLS methods. Capacitance and Ψ_{min} also had no significant correlations with climate parameters using either method, although there were trends for a marginally negative correlation between capacitance and MAT and a positive correlation between Ψ_{min} and MAT using PGLS (Table 3).

Climate niches were conserved

Using only the 20 species from the greenhouse, MAT fit best to the OU model, indicating that it is phylogenetically conserved (Table 4). For altitude, AP, and isothermality, the white noise model had the best fit to the data, although AIC values were very similar for both the white noise and OU models. The Brownian motion model had the worst fit for all parameters. Using all 48 species for which climate data were available, altitude, MAT, and AP all had the best fit to the OU model, suggesting that all three parameters are phylogenetically conserved, but that the test with 20 species had insufficient power to detect the relationships for altitude and AP. Isothermality still fit best to the white noise model, even with 48 species.

Appendix 3.2 lists mean values of climate parameters for the species used in this study. Among *Dioscorea* species locations, mean altitude is highly significantly negatively correlated with MAT ($r=-0.94$, $P<0.001$) and AP ($r=-0.58$, $P<0.001$), but independent of isothermality. Isothermality varied less than the other climate parameters (C.V. is an order of magnitude lower than the other climate parameters), as might be expected in a tropical country where daily and seasonal temperature variation is limited.

Some leaf functional traits were phylogenetically conserved

Lamina area and water content fit best to the Brownian motion model of evolution, while LMA, Ψ_{min}, and g_{min} fit best to the OU model, providing evidence for phylogenetic conservatism of these traits (Table 5). L:W, SD, GCL, A_{max}, g_s, and capacitance fit best to the white noise model, suggesting that these traits are not phylogenetically conserved in Mexican *Dioscorea* species.

Patterns of leaf trait co-variation

Appendix 3.3 lists mean values for all functional traits for all species, and Table 6 lists all pair-wise correlations among leaf functional traits. There were more significant correlations using standard methods than PGLS. Using standard methods, lamina area, g_{min}, and WC were significantly correlated with multiple traits. Using PGLS, lamina area and g_{min} were not significantly correlated with any other traits, but WC remained significantly correlated with LMA and Ψ_{min}. There were several correlations among traits that were significant using either method: the expected correlations between SD and GCL and between A_{max} and g_s (Wong et al. 1979; Hetherington and Woodward 2003; Wright et al. 2004; Walls Chapter 2), and the correlations between LMA and capacitance or WC.

Trait values measured in the greenhouse were good predictors of those measured in the field, especially for leaf size, leaf shape, and GCL (fig. 2). The relationship between LMA in the greenhouse and LMA in the field was significant, but weaker, while the relationship between SD in the greenhouse and in the field was not significant (fig. 2).

Discussion

A relationship between evolutionary stasis in functional characters and stasis in species' niches is often assumed in studies of phylogenetic niche conservatism, but rarely tested. This is due in part to the lack of data on physiological traits for many species, but also to the need for phylogenetic information for the same set of species. I found evidence that multiple leaf functional traits correlated with climate parameters in Mexican *Dioscorea*, and that most of those traits were phylogenetically conserved, matching the lower left corner of Table 1. This included strictly physiological traits such as g_{min} and Ψ_{min}, chemical/structural traits such as LMA and WC, and one traditional aspect of plant form, leaf size. These results suggest that while leaf traits are important for adaptation to climate, their current association with climate is a result of earlier adaptation followed by niche conservatism, rather than repeated evolutionary change. The remaining traits – SD, GCL, A_{max}, g_s, capacitance, and Ψ_{min} – were not significantly correlated with climate parameters using either method, and none of them except Ψ_{min} was phylogenetically conserved. These relationships match the lower right corner of Table 1, suggesting that this set of traits is not important for adaptation to climate niches.

Nearly every trait I measured in this study fell into one of two categories: those that were correlated with climate and were phylogenetically conserved, or those that were not correlated with climate and were not phylogenetically conserved. The relationship between whether or not traits were correlated with climate and whether or not they were phylogenetically conserved supports the notion that phylogenetic niche conservatism (when the niche is defined in terms of climate) is tightly linked to functional trait conservatism. This reduces the likelihood that leaf traits will repeatedly evolve to match climate conditions, and makes it difficult to detect correlations between climate and traits using phylogenetic methods (fig. 3).

The role of trait and niche conservatism in the diversity of Mexican Dioscorea

The fact that many correlations were significant using standard methods but not using PGLS methods, coupled with the fact that leaf traits and climate had significant phylogenetic signal, suggests a limit on species' ability to evolve into new niches. Knowledge of the phylogenetic patterns of niches and traits makes this interpretation possible, but does not allow me to determine whether niche conservatism is leading to trait conservatism through stabilizing selection, or whether trait conservatism, due to some intrinsic inability to evolve new trait values, is leading to climate niche conservatism. Nonetheless, it does provide insight into the likely pattern of diversification of Mexican *Dioscorea*. My results suggest that diversification first occurred along regional-scale axes of temperature and precipitation (as well as other possible factors represent by altitude), followed later by diversification along microclimate environmental axes. This is consistent with Silvertown et al.'s (2006) findings in British meadow communities, at a larger phylogenetic scale, but the differs from the pattern found in California *Ceanothus* (Ackerly et al. 2006). My results are consistent with the patterns found in Floridian *Quercus* by Cavender-Bares et al. (2004a), in the sense that I found different patterns of conservatism at different phylogenetic scales, but not directly comparable, since they were working at a much smaller scale, and did not examine regional-scale climate factors.

Niche and trait conservatism implies that species' evolutionary history is likely to be important for where they can occur. For example, there is a well-supported clade of small-leaved species (*D. berenaica* through *D. tamoides*, fig. 1, *D. matagalpensis* through *D. sumiderensis*, fig. 3), which includes the dwarf species that were formerly classified as the genus *Nanarapenta*. Since lamina area is correlated with altitude and temperature, species in this clade should occur at higher, cooler sites, as most of the species do. However, a few species occur at lower, warmer sites and have somewhat larger leaves (*D. matagalpensis* and *D. igualamontana*, fig. 3), although they still have smaller leaf area compared to low elevation species from other clades (such as *D. composita* or *D. gomez-pompei*). This adds further credence to the adaptive value of leaf size in different climates, and shows that constraints on leaf size evolution are present, but not absolute. Species may be able to overcome constraints on changing leaf size by varying other traits, such as shape, here measured as L:W. L:W is the only trait I measured that is significantly correlated with climate, but not phylogenetically conserved.

Factors other than climate are probably contributing the high levels of diversity in Mexican *Dioscorea*. These may include soil type and geographic isolation at a regional scale, soil and microclimate variation at a local scale, and biotic interactions at both scales (Rausher 1978; Peeters 2002; Kearney 2006). The absence of correlations between climate parameters and traits that are important for photosynthesis (A_{max}, g_s, and stomatal traits) suggests that factors such as light or nutrient availability are driving interspecific variation in photosynthetic ability. The lack of phylogenetic signal in these traits suggests that species are free to adapt to differences in these microclimate parameters, which is consistent with several earlier studies (Arntz and Delph 2001; Geber and Griffen 2003). This lability would have allowed close relatives to diversify along microclimatic environmental axes, and is consistent with my observation that closely related *Dioscorea* species co-occur in many locations. Diversification within clades occupying the same climate niche could also be driven by geographic isolation. The large-scale geographic distribution of most clades does not appear to be limited by biogeographic barriers, since most large clades occur throughout the country (unpublished data, R. Walls). Yet within any clade, Mexico's mountainous topography could limit the dispersal of species that are unable to cross from one hospitable area to another, due to inhospitable conditions in between. In this case, niche conservatism could have contributed to allopatric speciation and diversification (Kozak and Wiens 2006). A more detailed analysis of the geographic distributions of species and clades will allow me to test hypotheses relating to diversification.

The role of leaf functional traits in adaptation to climate

The relationships between leaf traits and climate in Mexican *Dioscorea* support the hypothesis that leaf trait variation is important for adaptation to climate variation. Associations between leaf traits and climate have been observed at larger phylogenetic scales, such as the relationship between leaf size and temperature (Wolfe 1995; Wilf et al. 1998). However, a positive relationships between leaf size and precipitation has also been observed (Wolfe 1995; Wilf et al. 1998; McDonald et al. 2003), but was absent in Mexican *Dioscorea*. In this study, it is difficult to determine the selective pressure of individual environmental factors on leaf traits, since altitude, temperature, and precipitation were all correlated with each other, and most traits were correlated with all three parameters. The mutual correlations among many of the traits (Table 6) also make it difficult to determine whether selection was acting directly or indirectly on any particular trait.

In some cases, the adaptive significance of leaf traits can be inferred, such as positive correlations between L:W and MAT or AP, which may be driven by the presence of drip tips on species from warm, wet climate. Other relationships are less obvious, such as the relationship between climate and LMA. High LMA is often found in species from dry climates (Wright et al. 2005; Valladares and Sanchez-Gomez 2006), but in Mexican *Dioscorea*, LMA was positively correlated with AP and MAT. The relationship between LMA and other leaf traits was different for this group of species than for a sample of *Dioscorea* taken from throughout the world (Walls Chapter 2). In the broader sample, LMA was negatively correlated with A_{max}, as in other large-scale studies (Wright et al. 2004), while in Mexico, there was no relationship between LMA and A_{max}. This suggests that selection on LMA for climate tolerance at this scale is more important than its association with photosynthetic capacity or microclimate differentiation. There was a negatively correlation between LMA and WC, which was not surprising, since leaves with higher dry matter content per area (LMA) must have lower water content per area, and may therefore have lower water content on a mass basis (Niinemets 2001). This relationship may be driving the unexpected correlation between LMA and AP. High water content (and associated low LMA, Table 3) in low rainfall areas could be an indication of succulence, a trait that can be adaptive under drought stress (Ober et al. 2005; Omae et al. 2007).

I was surprised to find that capacitance had no significant relationships to climate variables, despite its correlation with LMA, since capacitance has been shown to be important for variation in drought tolerance (Andrade and Nobel 1997). There was a trend for a negative correlations between capacitance and MAT (Table 3), so there may be a relationship that I was unable to detect due to insufficient power (I only had 16 data points for capacitance, compared to 20 for most traits). However, I am not aware of any theoretical or empirical work that describes why lower capacitance should be advantageous under higher temperature.

g_{min} is thought to confer drought tolerance (Kerstiens 1996), but in *Dioscorea*, it was negatively correlated with AP and MAT, indicating that species from drier or cooler climates had a higher loss of water through their cuticles and closed stomata. The positive correlation between g_{min} and GCL suggests that species with larger stomata may not be able to close their stomata as completely, or that stomatal size is associated with difference in cuticle properties. The positive correlation between g_{min} and WC suggests a strategy whereby species with high water content maintain higher conductance through their stomata and/or cuticles under drought stress, because they are buffered by the high water content. This putative property could help to explain why leaves from drier climates have a higher g_{min}.

The only trait that was correlated with isothermality was WC (Table 3). Unlike MAT and AP, there was no evidence for phylogenetic conservatism of isothermality, despite the fact that it was much less variable than the other climate traits (C.V. an order of magnitude lower). Since there is little annual variation in temperature in a tropical country like Mexico, what variation does exist should come from daily temperature variation (isothermality is the ratio of daily temperature variation to annual temperature variation). The positive correlation between isothermality and WC suggests that having higher water content is helpful in dealing with larger daily swings in temperature, such as might be experienced at higher elevations. This is consistent with the strong positive correlation between WC and altitude, despite the fact that isothermality and altitude were not correlated.

Since many of the traits I measured are important for water use, I expected more significant correlations with AP than temperature. In part, correlations with temperature may arise because of the co-variation between temperature and precipitation in Mexico. However, this cannot completely explain the importance of temperature, since most traits actually had stronger correlations with MAT than with AP, and phylogenetic conservatism of temperature was stronger than for precipitation, that is, it was easier to distinguish it from the white noise model using only 20 species (Table 4). The distribution of *Dioscorea* throughout Mexico, showing that it doesn't occur in the driest areas, supports Burkill's hypothesis that there is a minimum amount of rainfall below which *Dioscorea* cannot occur (Burkill 1960). It appears that within the appropriate rainfall range, temperature may be more important for species diversification, while conservatism of the precipitation niche may be more important for the overall distribution of the genus.

The set of traits that were not correlated with climate is as noteworthy as those that are. As I predicted, A_{max} was not related to climate. Although g_s is important for water use, it is also tightly linked to A_{max}, especially when measured under conditions of maximum photosynthesis, as I did in this study. Therefore, g_s is likely to be under selection by similar factors as A_{max}. SD and GCL, which were significantly negatively correlated with each other, also bore no relationship to climate. Although there was no relationship between stomatal traits and A_{max} or g_s in this group of species, both SD and GCL have been shown to correlate with g_s across a broader sample of *Dioscorea* species (Walls Chapter 2). My results suggest that traits that are important for gas exchange are unimportant for climate tolerance, and instead may be important for microhabitat differentiation. This is consistent with large-scale studies that have found weak relationships between A_{max}, g_s or N content and MAT or AP, and high variation in these traits within one site (Wright et al. 2004).

Intraspecific variation and plasticity

I focused on species-level differences measured in a common garden, but plasticity and intraspecific variation may also be important for climate tolerances. There was intraspecific variation for all traits measured in the greenhouse. If some of that variation matches the patterns found across species, then the relationships between leaf traits and climate could be even stronger than detected here. My observations suggest that species with broad distributions, such as *D. floribunda*, have high variation in leaf traits such as size or L:W, most of which was maintained in the greenhouse and appears to have a genetic basis (unpublished data, R. Walls).

The importance of phenotypic plasticity in environmental tolerance has been well documented (Schlichting and Pigliucci 2001), and *Dioscorea* is no exception. Two traits that are known to be very plastic, LMA and SD, had weaker correlations between the field and greenhouse than the other leaf traits (fig. 2). Although I used different techniques to measure LMA in the two environments (whole leaves in the greenhouse and leaf disks in the field), I had 21 field-collected populations that I measured using both techniques. The correlation between the two measurement techniques was strong (r^2=83), so measurement method is not the main source of variation. Despite the plastic variation, LMA still had a species level signal, since the correlation between field and greenhouse leaves was significant, and LMA was significantly correlated with climate. SD in the greenhouse, on the other hand, was not significantly correlated with SD in the field, had no phylogenetic signal, and was not related to climate. SD is clearly highly responsive to local environmental conditions. For example, *D. multinervis* occurs at very high elevations, and has the highest stomatal density of any species measured in the field, but one of the lowest densities when measured in the greenhouse (circled dot, fig. 2 D). It is also the only species with stomata on both surfaces, a trait it maintained in the greenhouse. Since the greenhouse is near sea-level, the response in SD may be due to differences in ambient CO_2 concentration between the two locations (Woodward and Bazzaz 1988), while the presence of stomata on the upper or adaxial leaf surface is genetically controlled.

Conclusions

In this study, I not only found that leaf functional traits were correlated with climate parameters in Mexican *Dioscorea* species, and that species climate niches and leaf traits were phylogenetically conserved, I also found a relationship between whether or not traits were phylogenetically conserved, and whether or not they were correlated with regional-scale climate parameters. To my knowledge, this is the first demonstration that the evolutionary lability of species' niches is linked to the evolutionary lability of their traits. Furthermore, there appeared to be a distinction between the types of traits that were conserved (those related to regional-scale variation in temperature and precipitation), and the types that were labile (those related to small-scale variation in light or nutrient availability).

The results of this study have important consequences for understanding the distribution and co-occurrence of closely related species and the mechanisms that generate diversity. The conservatism of climate tolerances and the traits that are important for them means that close relatives need to occur in similar climate zones, which could limit the opportunities for dispersal. This is especially relevant in a mountainous region like Mexico, where climate variation can occur over very small geographical distances. While this trait could contribute to diversification through allopatric speciation (Kozak and Wiens 2006), it may also prevent more distantly related species of the same genus from dispersing the same region, thereby limiting the potential for high diversity. However, the relative lability of traits that are important for microhabitat differentiation may allow close relatives to partition the microclimate space within regional climate niches and co-exist, contributing to sympatric speciation. Although it is too early to determine if this pattern will hold across many taxa or regions, the combination of conserved traits and niches at one scale, combined with labile traits and niches at another scale, could provide a general mechanism for high species diversity in biodiversity hotspots like Mexico.

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Tables

Table 1. Possible outcomes of tests of the relationships between leaf traits and climate and phylogenetic conservatism, plus the implications of those outcomes.

		Are climate niches and/or traits phylogenetically conserved?	
		YES	NO
Are functional traits correlated with climate niche using phylogenetic methods?	YES	Relationships represent repeated adaptive evolution, but there are constraints on the ability to evolve into new climate niches. Phylogenetic conservatism will limit the power to detect this type of relationship.	Relationships represent repeated adaptive evolution, and there are few constraints on the ability to evolve into new climate niches.
	NO	If standard correlations are significant, traits may be important for adaptation to climate niches, but relationships represent past diversification followed by phylogenetic conservatism.	Traits are not important for adaptation to climate niches. Other environmental factors may be more important for selection on these traits.

Table 2. Mexican *Dioscorea* species used in this study. Not all individuals were used for every measurement. Habitat types come from Campbell and Lamar (1989).

Species	# Individuals	Habitat
D. bartlettii	2	Tropical evergreen forest
D. composita	2	Tropical evergreen forest
D. convolvulacea	2	Tropical evergreen forest to tropical deciduous forest
D. floribunda	10	Tropical evergreen forest to tropical deciduous forest
D. gomez-pompei	1	Tropical evergreen forest to tropical deciduous forest
D. guerrerensis	4	Tropical deciduous forest to arid tropical forest
D. igualamontana	2	Arid tropical forest
D. matagalpensis	2	Tropical evergreen forest to tropical deciduous forest
D. mexicana	2	Tropical evergreen forest
D. morelosana	1	Tropical deciduous forest
D. multinervis	1	Pine oak forest
D. pumicicola	1	Tropical deciduous forest to arid tropical forest
D. remotiflora	1	Tropical deciduous forest
D. sp1	2	Tropical deciduous forest
D. sp3	1	Arid tropical forest
D. sp6	2	Arid tropical forest
D. spiculiflora	3	Tropical deciduous forest
D. subtomentosa	3	Tropical deciduous forest
D. sumiderensis	2	Tropical deciduous forest
D. urceolata	1	Pine oak forest

Table 3. PGLS and standard correlations between leaf functional traits and climate parameters for 20 Mexican *Dioscorea* species (16 species for capacitance and water content). Bold, $P<0.05$.

		Log Altitude	Mean annual temperature	Isothermality	Log annual precipitation
log lamina area	PGLS *r*	-0.41	0.52	-0.13	0.36
	standard *r*	**-0.53**	**0.72**	-0.09	0.28
log LMA	PGLS *r*	-0.50	0.35	-0.11	0.46
	standard *r*	**-0.57**	**0.54**	-0.16	**0.45**
log L:W	PGLS *r*	-0.31	0.33	0.14	0.39
	standard *r*	**-0.55**	**0.55**	-0.22	**0.52**
log SD	PGLS *r*	0.04	0.14	0.04	-0.06
	standard *r*	-0.12	0.25	0.09	0.05
log GCL	PGLS *r*	0.00	-0.08	0.01	-0.13
	standard *r*	0.27	-0.21	0.11	-0.34
log Amax	PGLS *r*	0.30	0.10	0.37	-0.14
	standard *r*	0.35	0.07	0.31	-0.33
log g_s	PGLS *r*	0.43	-0.11	0.21	-0.26
	standard *r*	0.45	-0.14	0.17	-0.4
log water content	PGLS *r*	0.26	-0.22	0.42	-0.08
	standard *r*	**0.66**	**-0.55**	**0.48**	**-0.50**
log g_{min}	PGLS *r*	0.28	-0.19	0.11	-0.34
	standard *r*	**0.61**	**-0.51**	0.25	**-0.56**
Capacitance	PGLS *r*	0.2	-0.46	-0.06	-0.09
	standard *r*	0.18	-0.44	-0.1	-0.08
Ψmin	PGLS *r*	-0.40	0.50	0.29	0.38
	standard *r*	-0.04	0.09	0.32	0.18

Table 4. Mean AIC values for relationships between climate parameters at species' locations and phylogeny. Bold indicates the model that had the best support based on AIC values Results were the same using likelihood ratio tests.

	Log Altitude	Mean annual temperature	Isothermality	Log annual precipitation
Mean AIC values for 20 species				
White noise	**9.453**	226.329	**101.0436**	**-18.865**
Brownian motion	18.669	41729.7	2298.66	-14.118
OU	9.786	**193.596**	103.255	-18.198
Mean AIC values for 48 species				
White noise	51.427	699.555	**246.476**	-63.783
Brownian motion	44.715	160559.1	4003.6	-56.039
OU	**41.339**	**506.215**	248.795	**-66.154**

Table 5. Mean AIC values for relationships between leaf functional traits and phylogeny. Bold letters indicate the model that had the best support based on AIC values. Results were the same using likelihood ratio tests.

	White noise	Brownian motion	OU
Log lamina area	11.59	**4.42**	4.97
Log LMA	-33.81	-33.96	**-34.03**
Log L:W	**-34.82**	-22.58	-32.85
Log SD	**-14.43**	-5.82	-13.56
Log GCL	**-65.99**	-52.65	-64.44
Log Amax	**-8.66**	-0.71	-7.32
Log g_s	**0.63**	17.99	2.50
Log water content	-69.13	**-77.27**	-76.42
Log g_{min}	4.05	0.62	**0.25**
Capacitance	**-66.18**	-62.22	-64.70
Ψmin	-0.87	-0.29	**-1.82**

Table 6. Correlations among leaf functional traits. Above diagonal, PGLS *r*, below diagonal, standard *r*. Bold numbers indicate *P*<0.05

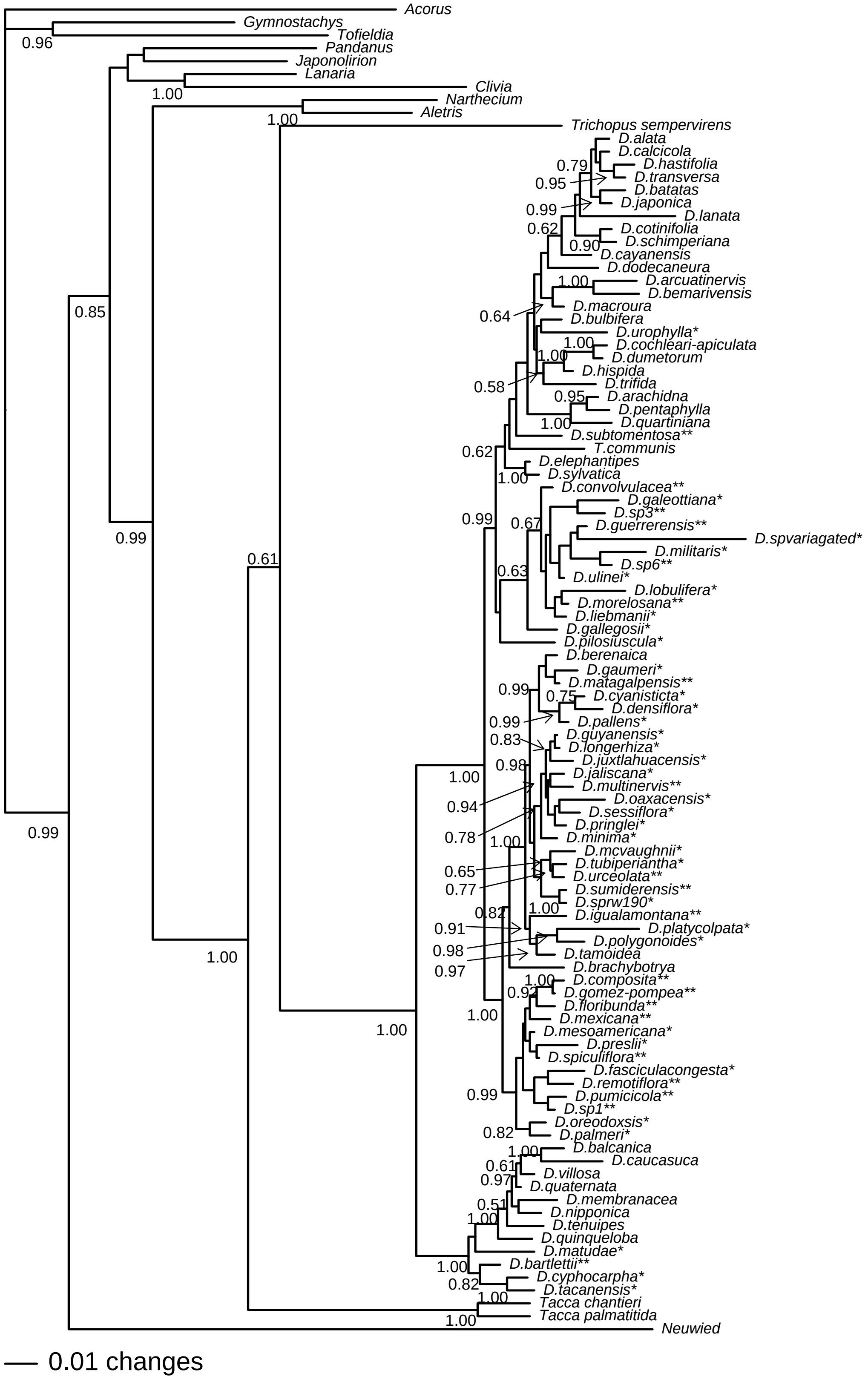
	log area	log LMA	log L:W	log GCL	log SD	log g_{min}	Ψ_{min}	log g_s	log A_{max}	capacitance	log WC
log area	x	0.45	0.23	-0.08	0.01	-0.15	0.03	0.16	0.22	-0.09	-0.41
log LMA	**0.64**	x	0.30	0.16	-0.21	0.02	0.12	-0.35	-0.12	**0.68**	**-0.55**
log L:W	**0.60**	**0.62**	x	-0.08	-0.21	-0.46	0.07	0.09	-0.22	-0.09	-0.38
log GCL	-0.13	0.07	-0.21	x	**-0.75**	0.48	0.20	-0.03	-0.01	-0.07	0.28
log SD	0.13	-0.16	-0.09	**-0.72**	x	-0.35	0.05	0.07	0.08	0.12	-0.16
log g_{min}	**-0.51**	-0.27	**-0.60**	**0.56**	-0.37	x	0.13	0.27	-0.28	0.15	0.39
Ψ_{min}	-0.39	-0.12	-0.25	0.18	0.06	0.38	x	0.05	0.18	-0.44	**0.61**
log g_s	0.12	-0.13	0.08	0.15	0.02	0.39	0.05	x	**0.89**	-0.19	0.44
log A_{max}	0.24	0.09	0.17	0.11	0.06	0.36	0.12	**0.89**	x	-0.05	0.39
capacitance	0.00	**0.70**	0.06	0.22	-0.15	0.20	0.25	-0.04	0.13	x	-0.32
log WC	**-0.70**	**-0.61**	**-0.60**	0.44	-0.25	**0.74**	**0.54**	**0.51**	**0.55**	-0.16	x

Figure Legends

Fig. 1. Majority rule consensus tree from the Bayesian analysis. Branch lengths indicate the expected proportion of changes per site. Numbers on nodes are posterior probabilities.

Fig. 2. Regressions of leaf traits measured in the field on leaf traits measured in the greenhouse. **A.** lamina area, **B.** LMA, **C.** L:W, **D.** stomatal density, **E.** guard cell length.

Fig. 3. Face to face comparison of trees showing mean annual temperature (left, bio 1) and leaf size (right, log lamina area). The significant standard correlation between altitude and lamina area is clear from the matching colors of the branches on the two trees. Similarity in trait values among close relatives (similar colored braches within clades) indicates phylogenetic conservatism, which makes it unlikely that significant correlations will be found among traits using phylogenetic methods. Trees were generated in Mesquite (Maddison and Maddison 2009), using the trace characters function.


Acorus
Gymnostachys
Tofieldia
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Pandanus
Japonolirion
Lanaria
Clivia
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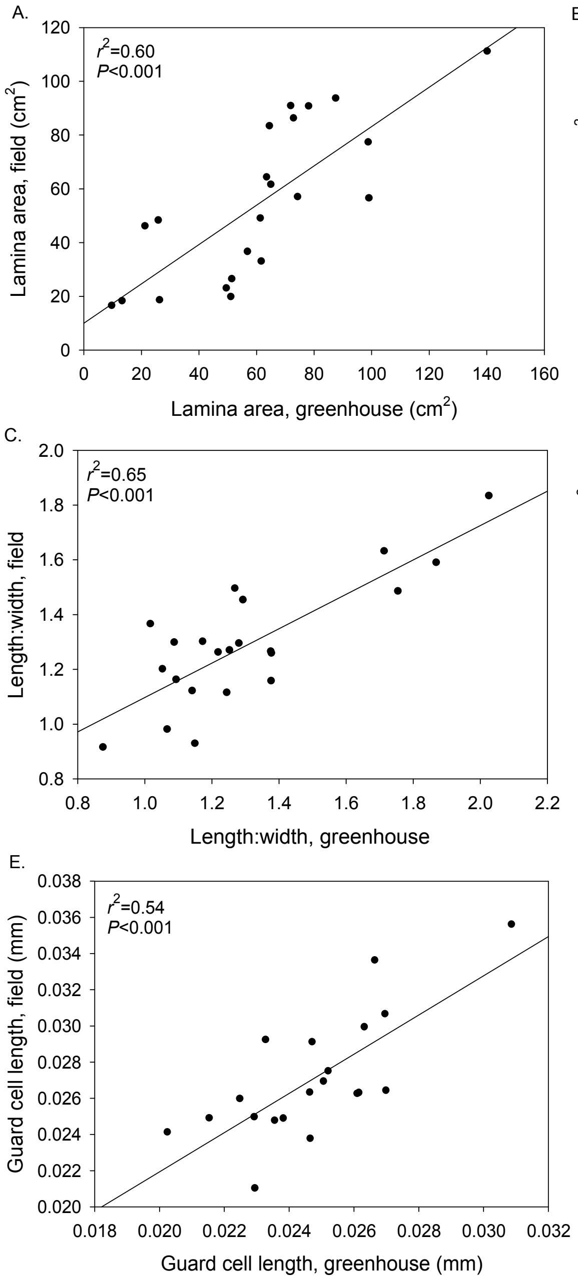
Narthecium
Aletris
1.00
Trichopus sempervirens
D.alata
D.calcicola
0.79
D.hastifolia
0.95
D.transversa
D.batatas
0.99
D.japonica
D.lanata
0.62
D.cotinifolia
0.90
D.schimperiana
D.cayanensis
D.dodecaneura
1.00
D.arcuatinervis
D.bemarivensis
0.85
D.macroura
0.64
D.bulbifera
D.urophylla*
1.00
D.cochleari-apiculata
1.00
D.dumetorum
D.hispida
0.58
D.trifida
0.95
D.arachidna
D.pentaphylla
1.00
D.quartiniana
D.subtomentosa**
0.62
T.communis
D.elephantipes
1.00
D.sylvatica
D.convolvulacea**
D.galeottiana*
0.99
D.sp3**
0.67
D.guerrerensis**
0.99
D.spvariagated*
D.militaris*
0.61
0.63
D.sp6**
D.ulinei*
D.lobulifera*
D.morelosana**
D.liebmanii*
D.gallegosii*
D.pilosiuscula*
D.berenaica
D.gaumeri*
0.99
D.matagalpensis**
0.75
D.cyanisticta*
D.densiflora*
0.99
D.pallens*
0.83
D.guyanensis*
D.longerhiza*
D.juxtlahuacensis*
0.98
1.00
D.jaliscana*
D.multinervis**
0.94
D.oaxacensis*
D.sessiflora*
D.pringlei*
0.99
0.78
D.minima*
1.00
D.mcvaughnii*
0.65
D.tubiperiantha*
D.urceolata**
0.77
D.sumiderensis**
D.sprw190*
0.82
1.00
D.igualamontana**
0.91
D.platycolpata*
D.polygonoides*
0.98
D.tamoidea
0.97
D.brachybotrya
1.00
1.00
D.composita**
0.92
D.gomez-pompea**
D.floribunda**
1.00
D.mexicana**
D.mesoamericana*
1.00
D.preslii*
D.spiculiflora**
D.fasciculacongesta*
D.remotiflora**
0.99
D.pumicicola**
D.sp1**
D.oreodoxsis*
0.82
D.palmeri*
1.00
D.balcanica
D.caucasuca
0.61
D.villosa
0.97
D.quaternata
D.membranacea
0.51
D.nipponica
1.00
D.tenuipes
D.quinqueloba
D.matudae*
1.00
D.bartlettii**
0.82
D.cyphocarpha*
D.tacanensis*
1.00
Tacca chantieri
Tacca palmatitida
1.00
Neuwied
0.01 changes


A.
r^2=0.60
P<0.001
Lamina area, field (cm^2)
Lamina area, greenhouse (cm^2)
0
20
40
60
80
100
120
140
160
B.
r^2=0.24
P=0.03
LMA, field (g m^{-2})
LMA, greenhouse (g m^{-2})
10
20
25
30
35
40
45
50
55
60
C.
r^2=0.65
P<0.001
Length:width, field
Length:width, greenhouse
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
D.
r^2=0.05
P=0.33
Stomatal density, field (mm^{-2})
Stomatal density, greenhouse (mm^{-2})
50
100
150
200
250
300
350
400
E.
r^2=0.54
P<0.001
Guard cell length, field (mm)
Guard cell length, greenhouse (mm)
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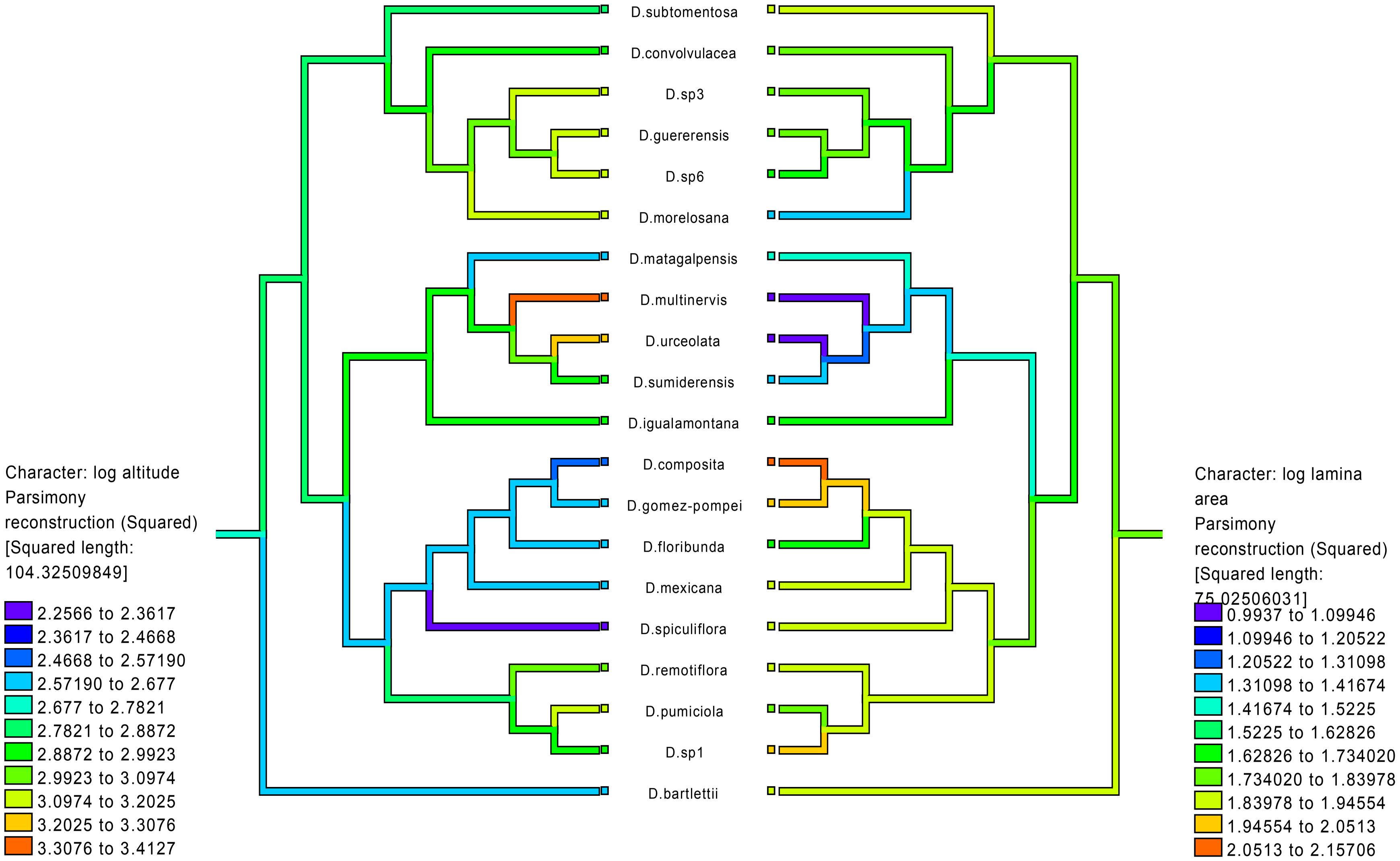
0.020
0.022
0.024
0.026
0.028
0.030
0.032
0.034
0.036
0.038


Character: log altitude
Parsimony
reconstruction (Squared)
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104.32509849]
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2.3617 to 2.4668
2.4668 to 2.57190
2.57190 to 2.677
2.677 to 2.7821
2.7821 to 2.8872
2.8872 to 2.9923
2.9923 to 3.0974
3.0974 to 3.2025
3.2025 to 3.3076
3.3076 to 3.4127
D.subtomentosa
D.convolvulacea
D.sp3
D.guererensis
D.sp6
D.morelosana
D.matagalpensis
D.multinervis
D.urceolata
D.sumiderensis
D.igualamontana
D.composita
D.gomez-pompei
D.floribunda
D.mexicana
D.spiculiflora
D.remotiflora
D.pumiciola
D.sp1
D.bartlettii
Character: log lamina
area
Parsimony
reconstruction (Squared)
[Squared length:
75.02506031]
0.9937 to 1.09946
1.09946 to 1.20522
1.20522 to 1.31098
1.31098 to 1.41674
1.41674 to 1.5225
1.5225 to 1.62826
1.62826 to 1.734020
1.734020 to 1.83978
1.83978 to 1.94554
1.94554 to 2.0513
2.0513 to 2.15706

CONCLUSIONS

The goal of my dissertation research was to bring together information on leaf form and function with information on species' evolutionary history, in order to better understand the evolutionary processes that contributed to angiosperm leaf trait diversity. Each chapter provided some evidence that leaf form variation was the result of adaptive evolution, through natural selection on leaf functions. Each study also showed that historical events, as reflected in phylogenetic patterns, played a part in determining current patterns of leaf form diversity. Looking across all three chapters, my results suggest that trait conservatism (vein patterns in large clades of angiosperms, the monocot, herbaceous, vine form in the clade composed of the genus *Dioscorea*, or traits that are important for climate adaptation in smaller Mexican clades of *Dioscorea*), acted as a constraint on the evolution of form-function relationships. The results suggest that the unique combinations of traits present in different lineages can lead to the evolution of alternative solutions in response to environmental conditions. My research demonstrates how the complementary processes of change (adaptive evolution) and lack of change (phylogenetic conservatism) may act together to generate biodiversity, providing a general mechanism to explain the high diversity of angiosperm leaf forms.

In Chapter 1, I provided the first phylogenetically-based, global-scale demonstration of relationships between vein patterns and leaf functions. I described relationships between major and minor vein patterns and traits from the leaf economic spectrum, that is, traits that represent the evolutionary trade-off between high physiological activity and long leaf life span (Wright et al. 2004). I was able to demonstrate that natural selection on vein patterns, a micro-evolutionary process, may have contributed to global scale patterns of trait-co-variation, a macro-evolutionary pattern. This supports the hypothesis that variation in leaf form arises from natural selection under different environmental conditions. Including data on phylogenetic relationships allowed me to distinguish which relationships represent correlated evolution of structures and functions (secondary vein type and leaf life span, minor vein density and maximum photosynthetic rate) and which appear to be based on particular combinations of traits arising in just a few clades (primary vein type and maximum photosynthetic rate). This demonstrates how historical contingencies (such as the vein pattern of a common ancestor) can contribute to leaf diversity: if species in a particular clade are constrained to have a certain primary vein pattern, then natural selection may act on other traits to compensate for the limitation of that vein pattern, thereby generating multiple combinations of leaf traits that are adaptive to the same set of environmental conditions.

In Chapter 2, I used phylogenetic methods to analyze correlations among multiple leaf traits in species of the monocot vine genus *Dioscorea*. On its own, the pattern of relationships that I found provided unique insight into how species of an individual clade (i.e. *Dioscorea)* respond to the multiple functional demands placed on their leaves. By choosing a set of traits that has been studied extensively at larger phylogenetic scales, I was able to compare my results to many previously-published, large-scale studies. This allowed me to make additional inferences about how the challenges of the monocot, herbaceous vine form can impact leaf structure-function relationships. The implications of the results are that leaf trait correlations from one group of species will not necessarily translate directly into another, and that researchers need to use caution when extrapolating between clades or scales. This study demonstrates what others have suggested: that some of the scatter present in large-scale trait correlations represents alternative strategies present in the individual clades contained within that study (Gutschick 1999; Wright et al. 2004). The unique characteristic of individual clades can act as constraints that impact the evolutionary trajectory of other traits, leading to alternative adaptive solutions and a greater diversity of leaf forms.

In Chapter 3, I demonstrated that leaf functional traits measured in a common garden were correlated with the environmental conditions found in species' native ranges, using Mexican *Dioscorea* species. Nearly every trait I measured in this study fell into one of two categories: those that were correlated with climate and were phylogenetically conserved, or those that were not correlated with climate and were not phylogenetically conserved. The relationship between whether or not traits were correlated with climate and whether or not they were phylogenetically conserved supports the notion that phylogenetic niche conservatism (when the niche is defined in terms of regional-scale climate parameters) is tightly linked to functional trait conservatism. This relationship is often assumed, but rarely tested. This is the first study, to my knowledge, to demonstrate that leaf-level physiological traits (other than C4 photosynthesis) are phylogenetically conserved, a result that has important implications for both plant physiologists and physiological ecologists (Monson 1996). On one hand, the results of this study showed that certain leaf traits were important for adaptation to climate conditions, supporting the hypothesis that natural selection by climate factors played a role in the diversification of Mexican *Dioscorea* leaf traits and species. On the other hand, that same set of traits was phylogenetically conserved, which suggests that evolution into new climate niches was not the sole driver of species diversification in this group. Since the set of traits that were not correlated with climate parameters were not phylogenetically conserved, close relatives may be able to diversify along microclimate axes. This combination of conserved traits at one scale and variable traits and another scale might represent a general way of generating species diversity in areas with high spatial variability of climate conditions.

This dissertation provides a real-world demonstration of earlier theoretic work that work described how the need to simultaneously optimize many functional traits can result in multiple combinations of traits with roughly equal fitness under the same set of environmental conditions (Niklas 1994; Marks and Lechowicz 2006; Marks 2007). In the models, starting combinations of trait values are chosen randomly, but in the systems I described, starting combinations of traits were the result of the independent evolutionary trajectories followed by different lineages. While the models describe species evolving to multiple optima under identical environmental conditions, the patterns I describe (particularly in Chapter 3) certainly allow for small-scale environmental variation within one site, so that a strict model of one optimal phenotype per environment cannot be ruled out. Nonetheless, my research suggests that a debate between convergence on a single optimum due to selection by the environment and divergence to multiple optima due to idiosyncratic historical events is best resolved by allowing for the influence of both factors.

My research, particularly Chapter 2, suggests an additional mechanism for leaf trait variation within one environment: different combination of leaf structural traits can lead to the same value of leaf functions in different organisms. Just as many-to-one mapping of genotypes to phenotypes can allow organisms with different genotypes to express the same phenotype, because most phenotypes are determined by multiple genes, multiple combinations of structural traits can lead to similar values of functions in different species (Alfaro et al. 2005; Wainwright et al. 2005). This is possible in leaves, because most leaf functions are determined by multiple leaf structures. This allows physiological or other functional traits to remain coupled to multiple structural traits but evolve semi-independently from any individual structure. This flexibility may be crucial for maintaining viability in an organ like the leaf that needs to meet multiple functional demands.

The need to meet multiple functional demands may also be responsible for the occasional discrepancy between leaf form and the functions one would predict from that form. This was most obvious in Chapter 2, where I showed that large-leaved *Dioscorea* species appear to be overbuilt for their conductive demand. The conflicting demands of CO_2 uptake and protection from embolism appears to have led to the evolution a negative relationship between flux rates and the structures that deliver water through the leaf in *Dioscorea*. This relationship would not have been predicted from larger-scale studies, and can only be explained when multiple plant traits are taken into consideration. The absence of correlations at small phylogenetic scales, such as the relationship between leaf mass per area (LMA) and maximum photosynthetic rate in Chapter 3, and the correlations between LMA and species' climate parameters, demonstrated that traits that control LMA, such as water content or dry matter content, are important not just for photosynthetic ability, but also for the ability to tolerate variation in temperature and precipitation. Since natural selection acts on all of a leaf's functional demands, it can be difficult to predict the relationship between any one structure and function.

My focus has been on demonstrating how studies of form-function relationships can be used to answer evolutionary questions through the inclusion of phylogenetic information. These studies can also be used to inform ecological or plant physiological questions. Knowing if relationships between leaf traits arise through repeated co-evolution or are the result of a few divergences followed by stasis in trait values is useful for ecologists who study the distribution of traits within a community or in geographic space. It can provide an indication of whether trait values are likely to have arisen *in situ* due to selection by current environmental conditions, or whether they represent exaptations and are more likely to have dispersed into their current location (Ackerly et al. 2006). While phylogenetic conservatism of traits may weaken the ability to predict plant function based on form (as for the relationships described in Chapter 1), it also adds a complementary source of information, by allowing researchers to predict plant function from phylogenetic relationships. This could be useful for researchers who want to predict ecosystem function based on plant traits, paleoecologists who want to study functions of extinct plants, or researchers who want to predict future functional responses of plant communities under climate change. Plant physiologists may be able to use the knowledge that some physiological traits are phylogenetically conserved to identify experimental systems or to aid in the search for shared genetic pathways that control physiological traits (Monson 1996).

By uniting new information on leaf form and function with information on species' evolutionary history, this thesis afforded new insight into the evolutionary processes that contribute to angiosperm leaf trait diversity. I presented several examples of how historical contingencies can affect to current patterns of plant diversity, providing much-needed empirical evidence for the theoretical frame-work of multiple evolutionary optima as a mechanism for biodiversity. The evaluation of different spatial and phylogenetic scales in each chapter showed that phylogenetic conservatism can impact structure function relationships at any scale. I hope that this work will inspire more detailed studies of the evolutionary patterns of leaf functional traits and raise questions about what those evolutionary patterns can tell us about the role of ecology in generating leaf diversity.

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APPENDICES

Appendix 1.1: Coefficients of major axis regression of A_{max} and N content on mass basis on minor vein density, for raw data and independent contrasts (PICs). Bold numbers represent $P<0.05$.

Predictor	**Response**	**r^2 (raw data)**	***P* (raw data)**	**r^2 (PICs)**	***P* (PICs)**
Minor Vein Density	A_{mass}	0.12	**0.001**	0.02	0.235
Minor Vein Density	N_{mass}	0.09	**0.005**	0.01	0.341

Appendix 1.2: Primary vein type, biome, growth form, and C3/C4 status for each of the species used in this study, ordered by family. Biome, growth form and C3/C4 data were compiled from Wright et al. (2004).

Family	**Genus**	**Species**	**Primary vein pattern (for leaflet, if compound)**	**Biome**	**Growth Form**	**Photo-synthetic Pathway**
Aextoxicaceae	*Aextoxicon*	*punctatum*	pinnate	temperate forest	tree	C3
Amaranthaceae	*Amaranthus*	*blitoides*	pinnate	wetland	herb	C4
Amaranthaceae	*Amaranthus*	*retroflexus*	pinnate	wetland	herb	C4
Amaranthaceae	*Atriplex*	*canescens*	pinnate	wetland	herb	C3
Amaranthaceae	*Atriplex*	*halimus*	pinnate	wetland	shrub	C4
Amaranthaceae	*Atriplex*	*stipitata*	palmate	wetland	shrub	C4
Amaranthaceae	*Beta*	*vulgaris*	pinnate	wetland	herb	C3
Amaranthaceae	*Chenopodium*	*album*	pinnate	wetland	herb	C3
Amaranthaceae	*Chenopodium*	*oahuense*	pinnate	temperate rain forest	shrub	C3
Anacardiaceae	*Anacardium*	*excelsum*	pinnate	tropical rain forest	tree	
Anacardiaceae	*Buchanania*	*obovata*	pinnate	tropical forest	tree	C3
Anacardiaceae	*Pistacia*	*lentiscus*	pinnate	wetland	shrub	C3
Anacardiaceae	*Pistacia*	*terebinthus*	pinnate	grassland/meadow	tree	C3
Anacardiaceae	*Rhus*	*sandwicensis*	pinnate	temperate rain forest	tree	C3
Anacardiaceae	*Rhus*	*tomentosa*	pinnate	temperate forest	shrub	C3
Anacardiaceae	*Toxicodendron*	*diversiloba*	pinnate	wetland	shrub	C3
Annonaceae	*Annona*	*coriacea*	pinnate	tropical forest	tree	C3
Annonaceae	*Duguetia*	*furfuracea*	pinnate	tropical forest	tree	C3
Apiaceae	*Bupleurum*	*rigidum*	parallel	grassland/meadow	herb	C3
Apiaceae	*Carum*	*carvi*	pinnate	alpine	herb	C3
Apiaceae	*Daucus*	*carota*	pinnate	grassland/meadow	herb	C3
Apiaceae	*Eryngium*	*campestre*	pinnate	grassland/meadow	herb	C3
Apiaceae	*Ligusticum*	*mutellina*	pinnate	alpine	herb	C3
Apocynaceae	*Aspidosperma*	*album*	pinnate	tropical rain forest	tree	C3
Apocynaceae	*Aspidosperma*	*tomentosum*	pinnate	tropical forest	tree	C3
Apocynaceae	*Wrightia*	*pubescens*	pinnate	tropical forest	tree	C3
Aquifoliaceae	*Ilex*	*verticillata*	pinnate	temperate forest	shrub	C3
Araliaceae	*Astrotricha*	*floccosa*	pinnate	temperate forest	shrub	C3
Araliaceae	*Cheirodendron*	*trigynum*	pinnate	temperate rain forest	tree	C3
Araliaceae	*Didymopanax*	*morototoni*	pinnate	tropical rain forest	tree	
Araliaceae	*Didymopanax*	*vinosum*	pinnate	tropical forest	tree	C3
Asclepiadaceae	*Asclepias*	*syriaca*	pinnate	temperate forest	herb	C3
Asclepiadaceae	*Asclepias*	*tuberosa*	pinnate	temperate forest	herb	C3
Asteraceae	*Achillea*	*erba*	pinnate	alpine	herb	C3
Asteraceae	*Achillea*	*millefolium*	pinnate	alpine	herb	C3
Asteraceae	*Ambrosia*	*artemisiifolia*	pinnate	temperate forest	herb	C3
Asteraceae	*Ambrosia*	*trifida*	palmate	grassland/meadow	herb	
Asteraceae	*Artemisia*	*tridentata*	palmate	wetland	shrub	C3
Asteraceae	*Aster*	*azureus*	pinnate	temperate forest	herb	C3
Asteraceae	*Aster*	*ericoides*	pinnate	temperate forest	herb	C3
Asteraceae	*Aster*	*novae-angliae*	pinnate	temperate forest	herb	C3
Asteraceae	*Baccharis*	*pilularis*	parallel	wetland	shrub	C3
Asteraceae	*Chrysanthemum*	*leucanthemum*	pinnate	alpine	herb	C3
Asteraceae	*Cichorium*	*intybus*	pinnate	wetland	herb	C3
Asteraceae	*Crepis*	*triasii*	pinnate	wetland	herb	C3
Asteraceae	*Dittrichia*	*viscosa*	pinnate	wetland	shrub	C3
Asteraceae	*Echinacea*	*purpurea*	parallel	temperate forest	herb	C3
Asteraceae	*Echinops*	*ritro*	pinnate	grassland/meadow	herb	C3
Asteraceae	*Erigeron*	*uniflorus*	pinnate	alpine	herb	C3
Asteraceae	*Eupatorium*	*rugosum*	palmate	temperate forest	herb	C3
Asteraceae	*Gutierrezia*	*sarothrae*	pinnate	wetland	shrub	C3
Asteraceae	*Helianthus*	*microcephalus*	pinnate	temperate forest	herb	C3
Asteraceae	*Helichrysum*	*apiculatum*	pinnate	wetland	shrub	C3
Asteraceae	*Liatris*	*aspera*	pinnate	temperate forest	herb	C3
Asteraceae	*Petasites*	*frigidus*	palmate	tundra	herb	
Asteraceae	*Piptocarpha*	*rotundifolia*	pinnate	tropical forest	tree	C3
Asteraceae	*Pterocaulon*	*pycnostachyum*	pinnate	temperate forest	herb	C3
Asteraceae	*Silphium*	*integrifolium*	pinnate	temperate forest	herb	C3
Asteraceae	*Silphium*	*terebinthinaceum*	pinnate	temperate forest	herb	C3

Asteraceae	*Solidago*	*nemoralis*	pinnate	temperate forest	herb	C3
Asteraceae	*Solidago*	*rigida*	pinnate	temperate forest	herb	C3
Asteraceae	*Taraxacum*	*alpinum*	pinnate	alpine	herb	C3
Asteraceae	*Taraxacum*	*officinale*	pinnate	alpine	herb	C3
Berberidaceae	*Caulophyllum*	*thalictroides*	palmate	temperate forest	herb	C3
Berberidaceae	*Podophyllum*	*peltatum*	palmate	temperate forest	herb	C3
Betulaceae	*Alnus*	*hirsuta*	pinnate	temperate forest	tree	C3
Betulaceae	*Alnus*	*incana*	pinnate	temperate forest	tree	C3
Betulaceae	*Alnus*	*japonica*	pinnate	temperate forest	tree	C3
Betulaceae	*Betula*	*davurica*	pinnate	temperate forest	tree	C3
Betulaceae	*Betula*	*ermanii*	pinnate	temperate forest	tree	C3
Betulaceae	*Betula*	*glandulosa*	pinnate	tundra	shrub	
Betulaceae	*Betula*	*maximowicziana*	pinnate	temperate forest	tree	C3
Betulaceae	*Betula*	*nana*	pinnate	tundra	shrub	C3
Betulaceae	*Betula*	*nigra*	pinnate	temperate forest	tree	C3
Betulaceae	*Betula*	*papyrifera*	pinnate	temperate forest	tree	C3
Betulaceae	*Betula*	*pendula*	pinnate	temperate forest	tree	C3
Betulaceae	*Betula*	*platyphylla*	pinnate	temperate forest	tree	C3
Betulaceae	*Betula*	*populifolia*	pinnate	temperate forest	tree	C3
Betulaceae	*Betula*	*pubescens*	pinnate	tundra	tree	C3
Betulaceae	*Betula*	*pumila*	pinnate	temperate forest	tree	C3
Betulaceae	*Carpinus*	*betulus*	pinnate	temperate forest	tree	C3
Betulaceae	*Carpinus*	*cordata*	pinnate	temperate forest	tree	C3
Betulaceae	*Corylus*	*americana*	pinnate	temperate forest	shrub	C3
Betulaceae	*Ostrya*	*japonica*	pinnate	temperate forest	tree	C3
Bignoniaceae	*Mansoa*	*verrucifera*	pinnate	tropical rain forest	vine	C3
Boraginaceae	*Eriodictyon*	*californicum*	pinnate	wetland	shrub	C3
Brassicaceae	*Capparis*	*spinosa*	pinnate	wetland	shrub	C3
Brassicaceae	*Capparis*	*verrucosa*	pinnate	tropical rain forest	shrub	C3
Brassicaceae	*Dentaria*	*laciniata*	palmate	temperate forest	herb	C3
Brassicaceae	*Morisonia*	*americana*	pinnate	tropical rain forest	tree	C3
Buxaceae	*Buxus*	*sempervirens*	pinnate	grassland/meadow	shrub	C3
Caprifoliaceae	*Lonicera*	*bella*	pinnate	temperate forest	shrub	C3
Caprifoliaceae	*Sambucus*	*mexicana*	pinnate	wetland	shrub	C3
Caprifoliaceae	*Viburnum*	*cassinoides*	pinnate	temperate forest	shrub	C3
Caprifoliaceae	*Viburnum*	*tinus*	pinnate	grassland/meadow	shrub	C3
Caryocaraceae	*Caryocar*	*brasiliense*	pinnate	tropical forest	tree	C3
Celastraceae	*Maytenus*	*oleoides*	pinnate	temperate forest	shrub	C3
Cercidiphyllaceae	*Cercidiphyllum*	*japonicum*	palmate	temperate forest	tree	C3
Chrysobalanaceae	*Licania*	*heteromorpha*	pinnate	tropical rain forest	tree	C3
Cistaceae	*Cistus*	*albidus*	pinnate	wetland	shrub	C3
Cistaceae	*Cistus*	*monspeliensis*	pinnate	wetland	shrub	C3
Cistaceae	*Cistus*	*salvifolius*	pinnate	wetland	shrub	C3
Clethraceae	*Clethra*	*alnifolia*	pinnate	wetland	shrub	C3
Clusiaceae	*Caraipa*	*heterocarpa*	pinnate	tropical rain forest	tree	C3
Clusiaceae	*Hypericum*	*balearicum*	pinnate	wetland	shrub	C3
Clusiaceae	*Kielmeyera*	*coriacea*	pinnate	tropical forest	tree	C3
Clusiaceae	*Vismia*	*japurensis*	pinnate	tropical rain forest	tree	C3
Clusiaceae	*Vismia*	*lauriformis*	pinnate	tropical rain forest	tree	C3
Combretaceae	*Terminalia*	*ferdinandiana*	pinnate	tropical forest	tree	C3
Connaraceae	*Connarus*	*suberosus*	pinnate	tropical forest	tree	C3
Convolvulaceae	*Convolvulus*	*arvensis*	pinnate	wetland	herb	C3
Cornaceae	*Cornus*	*contraversa*	pinnate	temperate forest	tree	C3
Cornaceae	*Cornus*	*florida*	pinnate	temperate forest	tree	C3
Cornaceae	*Cornus*	*racemosa*	pinnate	temperate forest	shrub	C3
Cunoniaceae	*Eucryphia*	*cordifolia*	pinnate	temperate forest	tree	C3
Cyrillaceae	*Cyrilla*	*racemiflora*	pinnate	wetland	shrub	C3
Diapensiaceae	*Galax*	*aphylla*	palmate	temperate forest	herb	C3
Dilleniaceae	*Dillenia*	*suffruticosa*	pinnate	tropical rain forest	shrub	
Dilleniaceae	*Hibbertia*	*bracteata*	pinnate	wetland	shrub	C3
Dilleniaceae	*Hibbertia*	*huegelii*	pinnate	wetland	shrub	C3
Dilleniaceae	*Hibbertia*	*subvaginata*	pinnate	wetland	shrub	C3
Dipsacaceae	*Cephalaria*	*squamiflora*	pinnate	wetland	shrub	C3
Ebenaceae	*Diospyros*	*cauliflora*	pinnate	tropical rain forest	tree	C3
Elaeagnaceae	*Eleagnus*	*angustifolia*	pinnate	wetland	tree	C3
Ericaceae	*Andromeda*	*glaucophylla*	pinnate	temperate forest	shrub	C3
Ericaceae	*Arbutus*	*menziesii*	pinnate	wetland	shrub	C3
Ericaceae	*Arbutus*	*unedo*	pinnate	wetland	tree	C3
Ericaceae	*Arctostaphylos*	*tomentosa*	pinnate	wetland	shrub	C3

Ericaceae	*Arctostaphylos*	*uva-ursi*	pinnate	temperate forest	shrub	C3
Ericaceae	*Arctous*	*alpina*	pinnate	tundra	shrub	
Ericaceae	*Astroloma*	*xerophyllum*	pinnate	wetland	shrub	C3
Ericaceae	*Conostephium*	*pendulum*	pinnate	wetland	shrub	C3
Ericaceae	*Gaylussacia*	*baccata*	pinnate	temperate forest	shrub	C3
Ericaceae	*Kalmia*	*angustifolia*	pinnate	temperate forest	tree	C3
Ericaceae	*Kalmia*	*latifolia*	pinnate	temperate forest	tree	C3
Ericaceae	*Kalmia*	*polifolia*	pinnate	temperate forest	tree	C3
Ericaceae	*Ledum*	*groenlandicum*	pinnate	temperate forest	shrub	C3
Ericaceae	*Ledum*	*palustre*	pinnate	tundra	shrub	
Ericaceae	*Leucothoe*	*racemosa*	pinnate	wetland	shrub	C3
Ericaceae	*Lyonia*	*lucida*	pinnate	wetland	shrub	C3
Ericaceae	*Rhododendron*	*anthopogon*	pinnate	alpine	shrub	
Ericaceae	*Rhododendron*	*maximum*	pinnate	temperate forest	tree	C3
Ericaceae	*Vaccinium*	*arboreum*	pinnate	temperate forest	shrub	C3
Ericaceae	*Vaccinium*	*corymbosum*	pinnate	temperate forest	shrub	C3
Ericaceae	*Vaccinium*	*myrtilloides*	pinnate	temperate forest	shrub	C3
Ericaceae	*Vaccinium*	*myrtillus*	pinnate	tundra	shrub	C3
Ericaceae	*Vaccinium*	*uliginosum*	pinnate	tundra	shrub	C3
Ericaceae	*Vaccinium*	*vitis-idaea*	pinnate	tundra	shrub	C3
Euphorbiaceae	*Aporosa*	*bracteosa*	pinnate	tropical rain forest	tree	C3
Euphorbiaceae	*Aporosa*	*lunata*	pinnate	tropical rain forest	tree	C3
Euphorbiaceae	*Aporosa*	*microstachya*	pinnate	tropical rain forest	tree	C3
Euphorbiaceae	*Euphorbia*	*margalidiana*	pinnate	wetland	shrub	C3
Euphorbiaceae	*Macaranga*	*heynei*	pinnate	tropical rain forest	tree	
Euphorbiaceae	*Macaranga*	*triloba*	pinnate	tropical rain forest	tree	C3
Euphorbiaceae	*Mallotus*	*paniculatus*	palmate	tropical rain forest	tree	
Euphorbiaceae	*Manihot*	*esculenta*	palmate	tropical rain forest	shrub	C3
Euphorbiaceae	*Micrandra*	*sprucei*	pinnate	tropical rain forest	tree	C3
Fabaceae	*Acacia*	*auriculiformis*	parallel	tropical forest	tree	C3
Fabaceae	*Acacia*	*doratoxylon*	parallel	wetland	shrub	C3
Fabaceae	*Acacia*	*floribunda*	parallel	temperate forest	tree	C3
Fabaceae	*Acacia*	*oswaldii*	parallel	wetland	shrub	C3
Fabaceae	*Acacia*	*suaveolens*	pinnate	wetland	shrub	C3
Fabaceae	*Amorpha*	*canescens*	pinnate	temperate forest	shrub	C3
Fabaceae	*Anagyris*	*foetida*	pinnate	wetland	shrub	C3
Fabaceae	*Astragalus*	*candensis*	pinnate	temperate forest	herb	C3
Fabaceae	*Baptisia*	*leucophaea*	pinnate	temperate forest	herb	C3
Fabaceae	*Bowdichia*	*virgilioides*	pinnate	tropical forest	tree	C3
Fabaceae	*Ceratonia*	*siliqua*	pinnate	wetland	tree	C3
Fabaceae	*Chamaedaphne*	*calyculata*	pinnate	temperate forest	shrub	C3
Fabaceae	*Desmodium*	*canadense*	pinnate	temperate forest	herb	C3
Fabaceae	*Eperua*	*purpurea*	pinnate	tropical rain forest	tree	C3
Fabaceae	*Gompholobium*	*grandiflorum*	pinnate	wetland	shrub	C3
Fabaceae	*Lespedeza*	*capitata*	pinnate	temperate forest	herb	C3
Fabaceae	*Lonchocarpus*	*dipteroneurus*	pinnate	tropical rain forest	tree	C3
Fabaceae	*Lotus*	*scoparius*	pinnate	wetland	shrub	C3
Fabaceae	*Lupinus*	*perennis*	pinnate	temperate forest	herb	C3
Fabaceae	*Maackia*	*amurensis*	pinnate	temperate forest	tree	C3
Fabaceae	*Medicago*	*minima*	pinnate	grassland/meadow	herb	C3
Fabaceae	*Petalostemum*	*purpureum*	pinnate	temperate forest	herb	C3
Fabaceae	*Petalostemum*	*villosum*	pinnate	temperate forest	herb	C3
Fabaceae	*Phyllota*	*phylicoides*	pinnate	wetland	shrub	C3
Fabaceae	*Pithecellobium*	*dulce*	pinnate	tropical rain forest	tree	C3
Fabaceae	*Pultenaea*	*daphnoides*	pinnate	temperate forest	shrub	C3
Fabaceae	*Pultenaea*	*flexilis*	pinnate	temperate forest	shrub	C3
Fabaceae	*Robinia*	*pseudoacacia*	pinnate	temperate forest	tree	C3
Fabaceae	*Senna*	*artemisioides*	pinnate	wetland	shrub	C3
Fabaceae	*Sophora*	*chrysophylla*	pinnate	temperate rain forest	tree	C3
Fabaceae	*Trifolium*	*repens*	pinnate	alpine	herb	C3
Fagaceae	*Castanea*	*dentata*	pinnate	temperate forest	tree	C3
Fagaceae	*Castanopsis*	*sieboldii*	pinnate	temperate forest	tree	C3
Fagaceae	*Fagus*	*grandifolia*	pinnate	temperate forest	tree	C3
Fagaceae	*Fagus*	*sylvatica*	pinnate	temperate forest	tree	C3
Fagaceae	*Nothofagus*	*betuloides*	pinnate	wetland	tree	C3
Fagaceae	*Nothofagus*	*dombeyi*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*acuta*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*alba*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*austrina*	pinnate	temperate forest	tree	C3

Fagaceae	*Quercus*	*chapmanii*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*coccifera*	pinnate	wetland	shrub	C3
Fagaceae	*Quercus*	*coccinea*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*ellipsoidalis*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*faginea*	pinnate	wetland	tree	
Fagaceae	*Quercus*	*falcata*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*geminata*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*hemisphaerica*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*humilis*	pinnate	wetland	tree	C3
Fagaceae	*Quercus*	*ilex*	pinnate	grassland/meadow	tree	C3
Fagaceae	*Quercus*	*incana*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*laevis*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*laurifolia*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*macrocarpa*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*margaretta*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*michauxii*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*minima*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*mongolica*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*myrsinaefolia*	pinnate	temperate forest	tree	
Fagaceae	*Quercus*	*myrtifolia*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*nigra*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*pubescens*	pinnate	grassland/meadow	tree	C3
Fagaceae	*Quercus*	*pumila*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*pyrenaica*	pinnate	wetland	tree	
Fagaceae	*Quercus*	*robur*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*rotundifolia*	pinnate	wetland	tree	
Fagaceae	*Quercus*	*rubra*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*shumardii*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*stellata*	pinnate	temperate forest	tree	C3
Fagaceae	*Quercus*	*suber*	pinnate	wetland	tree	
Fagaceae	*Quercus*	*virginiana*	pinnate	temperate forest	tree	C3
Goupiaceae	*Goupia*	*glabra*	pinnate	tropical rain forest	tree	C3
Grossulariaceae	*Ribes*	*californicum*	palmate	wetland	shrub	C3
Grossulariaceae	*Ribes*	*uva-crispa*	palmate	wetland	shrub	C3
Haemodoraceae	*Anigozanthos*	*humilis*	parallel	wetland	herb	C3
Hydrangeaceae	*Broussaisia*	*arguta*	pinnate	temperate rain forest	shrub	C3
Illiciaceae	*Illicium*	*anisatum*	pinnate	temperate forest	tree	C3
Iridaceae	*Gladiolus*	*caryophyllaceus*	parallel	wetland	herb	C3
Iridaceae	*Patersonia*	*occidentalis*	parallel	wetland	herb	C3
Iteaceae	*Itea*	*virginica*	pinnate	wetland	shrub	C3
Juglandaceae	*Carya*	*ovata*	pinnate	temperate forest	tree	C3
Juglandaceae	*Juglans*	*nigra*	pinnate	temperate forest	tree	C3
Lamiaceae	*Agastache*	*foeniculum*	pinnate	temperate forest	herb	C3
Lamiaceae	*Calamintha*	*nepeta*	pinnate	grassland/meadow	shrub	C3
Lamiaceae	*Lavandula*	*dentata*	pinnate	wetland	shrub	C3
Lamiaceae	*Lavandula*	*latifolia*	pinnate	grassland/meadow	shrub	C3
Lamiaceae	*Lepechinia*	*calycina*	pinnate	wetland	shrub	C3
Lamiaceae	*Phlomis*	*italica*	pinnate	wetland	shrub	C3
Lamiaceae	*Teucrium*	*chamaedrys*	parallel	grassland/meadow	shrub	C3
Lauraceae	*Cinnamomum*	*japonicum*	pinnate	temperate forest	tree	C3
Lauraceae	*Cryptocarya*	*alba*	pinnate	temperate forest	tree	C3
Lauraceae	*Machilus*	*thunbergii*	pinnate	temperate forest	tree	
Lauraceae	*Neolitsea*	*sericea*	pinnate	temperate forest	tree	C3
Lauraceae	*Ocotea*	*costulata*	pinnate	tropical rain forest	tree	C3
Lauraceae	*Persea*	*borbonia*	pinnate	temperate forest	shrub	C3
Lauraceae	*Persea*	*lingue*	pinnate	temperate forest	tree	C3
Lauraceae	*Umbellularia*	*californica*	pinnate	wetland	shrub	
Lecythidaceae	*Planchonia*	*careya*	pinnate	tropical forest	tree	C3
Lentibulariaceae	*Pinguicula*	*vulgaris*	pinnate	tundra	herb	C3
Liliaceae	*Erythronium*	*americanum*	parallel	temperate forest	herb	C3
Liliaceae	*Urginea*	*maritima*	parallel	wetland	herb	C3
Liliaceae	*Veratrum*	*parviflorum*	parallel	temperate forest	herb	C3
Magnoliaceae	*Liriodendron*	*tulipifera*	pinnate	temperate forest	tree	C3
Magnoliaceae	*Magnolia*	*obovata*	pinnate	temperate forest	tree	C3
Malvaceae	*Abutilon*	*theophrasti*	palmate	grassland/meadow	herb	
Malvaceae	*Brachychiton*	*populneus*	pinnate	wetland	tree	C3
Malvaceae	*Lasiopetalum*	*ferrugineum*	pinnate	temperate forest	shrub	C3
Malvaceae	*Lavatera*	*cretica*	palmate	wetland	herb	C3
Malvaceae	*Luehea*	*seemannii*	pinnate	tropical rain forest	tree	

Malvaceae	*Malvastrum*	*rotundifolium*	palmate	desert	herb	
Malvaceae	*Pseudobombax*	*septenatum*	pinnate	tropical rain forest	tree	
Malvaceae	*Tilia*	*cordata*	pinnate	temperate forest	tree	C3
Malvaceae	*Tilia*	*japonica*	pinnate	temperate forest	tree	C3
Marantaceae	*Calathea*	*inocephala*	pinnate	tropical rain forest	herb	
Marantaceae	*Pleiostachya*	*pruinosa*	pinnate	tropical rain forest	herb	
Melanthiaceae	*Trillium*	*grandiflora*	parallel	temperate forest	herb	C3
Melastomataceae	*Clidemia*	*sericea*	parallel	tropical rain forest	tree	C3
Melastomataceae	*Melastoma*	*malabathricum*	parallel	tropical rain forest	shrub	
Melastomataceae	*Miconia*	*albicans*	parallel	tropical forest	tree	C3
Melastomataceae	*Miconia*	*dispar*	parallel	tropical rain forest	tree	C3
Melastomataceae	*Miconia*	*ligustroides*	parallel	tropical forest	tree	C3
Meliaceae	*Synoum*	*glandulosum*	pinnate	temperate forest	tree	C3
Monimiaceae	*Laurelia*	*philippiana*	pinnate	temperate forest	tree	C3
Moraceae	*Castilla*	*elastica*	pinnate	tropical rain forest	tree	
Moraceae	*Ficus*	*obtusifolia*	pinnate	tropical rain forest	tree	
Moraceae	*Ficus*	*racemosa*	pinnate	tropical forest	tree	C3
Moraceae	*Ficus*	*scobina*	pinnate	tropical forest	tree	C3
Myoporaceae	*Myoporum*	*sandwicense*	pinnate	temperate rain forest	tree	C3
Myricaceae	*Comptonia*	*peregrina*	pinnate	temperate forest	tree	C3
Myrsinaceae	*Maesa*	*japonica*	pinnate	temperate forest	shrub	C3
Myrsinaceae	*Myrsine*	*lessertiana*	pinnate	temperate rain forest	tree	C3
Myrtaceae	*Calytrix*	*flavescens*	pinnate	wetland	shrub	C3
Myrtaceae	*Campomanesia*	*aromatica*	pinnate	tropical forest	tree	C3
Myrtaceae	*Corymbia*	*gummifera*	pinnate	wetland	tree	C3
Myrtaceae	*Eucalyptus*	*haemostoma*	pinnate	wetland	tree	C3
Myrtaceae	*Leptospermum*	*polygalifolium*	pinnate	temperate forest	shrub	C3
Myrtaceae	*Leptospermum*	*trinervium*	pinnate	wetland	shrub	C3
Myrtaceae	*Melaleuca*	*leucadendra*	parallel	tropical forest	tree	C3
Myrtaceae	*Melaleuca*	*viridiflora*	parallel	tropical forest	tree	C3
Myrtaceae	*Metrosideros*	*polymorpha*	pinnate	temperate rain forest	tree	C3
Myrtaceae	*Myrceugenia*	*planipes*	pinnate	temperate forest	tree	C3
Myrtaceae	*Regelia*	*ciliata*	pinnate	wetland	shrub	C3
Myrtaceae	*Scholtzia*	*involucrata*	pinnate	wetland	shrub	C3
Myrtaceae	*Syncarpia*	*glomulifera*	pinnate	temperate forest	tree	C3
Myrtaceae	*Syzygium*	*houttuynii*	pinnate	temperate rain forest	tree	C3
Myrtaceae	*Syzygium*	*suborbiculare*	pinnate	tropical forest	tree	C3
Myrtaceae	*Verticordia*	*nitens*	pinnate	wetland	shrub	C3
Myrtaceae	*Xanthostemon*	*paradoxus*	pinnate	tropical forest	tree	C3
Nyctaginaceae	*Neea*	*obovata*	pinnate	tropical rain forest	tree	C3
Oleaceae	*Fraxinus*	*americana*	pinnate	temperate forest	tree	C3
Oleaceae	*Fraxinus*	*angustifolia*	pinnate	wetland	tree	C3
Oleaceae	*Fraxinus*	*mandshurica*	pinnate	temperate forest	tree	C3
Oleaceae	*Phillyrea*	*latifolia*	pinnate	wetland	shrub	C3
Onagraceae	*Camissonia*	*brevipes*	pinnate	desert	herb	
Onagraceae	*Camissonia*	*claviformis*	pinnate	desert	herb	
Pandanaceae	*Freycinetia*	*arborea*	parallel	temperate rain forest	herb	C3
Papaveraceae	*Sanguinaria*	*canadensis*	palmate	temperate forest	herb	C3
Piperaceae	*Piper*	*aequale*	pinnate	tropical rain forest	shrub	C3
Piperaceae	*Piper*	*amalago*	pinnate	tropical rain forest	shrub	C3
Piperaceae	*Piper*	*auritum*	pinnate	tropical rain forest	shrub	C3
Piperaceae	*Piper*	*hispidum*	pinnate	tropical rain forest	shrub	C3
Piperaceae	*Piper*	*umbellatum*	palmate	tropical rain forest	shrub	C3
Plantaginaceae	*Plantago*	*lanceolata*	parallel	grassland/meadow	herb	C3
Poaceae	*Aegilops*	*geniculata*	parallel	grassland/meadow	grass	C3
Poaceae	*Agropyron*	*repens*	parallel	temperate forest	grass	C3
Poaceae	*Agrostis*	*scabra*	parallel	temperate forest	grass	C3
Poaceae	*Andropogon*	*gerardi*	parallel	temperate forest	grass	C4
Poaceae	*Avenula*	*bromoides*	parallel	grassland/meadow	grass	C3
Poaceae	*Avenula*	*crassifolia*	parallel	wetland	grass	C3
Poaceae	*Bouteloua*	*curtipendula*	parallel	temperate forest	grass	C4
Poaceae	*Brachypodium*	*distachyon*	parallel	grassland/meadow	grass	C3
Poaceae	*Brachypodium*	*phoenicoides*	parallel	grassland/meadow	grass	C3
Poaceae	*Bromus*	*erectus*	parallel	grassland/meadow	grass	C3
Poaceae	*Bromus*	*inermis*	parallel	temperate forest	grass	C3
Poaceae	*Bromus*	*lanceolatus*	parallel	grassland/meadow	grass	C3
Poaceae	*Calamagrostis*	*canadensis*	parallel	tundra	grass	
Poaceae	*Calamovilfa*	*longifolia*	parallel	temperate forest	grass	C4
Poaceae	*Dactylis*	*glomerata*	parallel	grassland/meadow	grass	C3

Poaceae	*Hierochloe*	*alpina*	parallel	tundra	grass	
Poaceae	*Koeleria*	*cristata*	parallel	temperate forest	grass	C3
Poaceae	*Panicum*	*capillare*	parallel	temperate forest	grass	C4
Poaceae	*Panicum*	*virgatum*	parallel	temperate forest	grass	C4
Poaceae	*Phleum*	*pratense*	parallel	grassland/meadow	grass	C3
Poaceae	*Poa*	*pratensis*	parallel	temperate forest	grass	C3
Poaceae	*Sorghastrum*	*nutans*	parallel	temperate forest	grass	C4
Poaceae	*Stipa*	*spartea*	parallel	temperate forest	grass	C3
Polygonaceae	*Oxyria*	*digyna*	palmate	alpine	herb	C3
Polygonaceae	*Polygonum*	*bistorta*	pinnate	tundra	herb	C3
Polygonaceae	*Polygonum*	*pensylvanicum*	pinnate	grassland/meadow	herb	
Polygonaceae	*Polygonum*	*viviparum*	pinnate	alpine	herb	C3
Polygonaceae	*Rheum*	*acuminatum*	palmate	alpine	herb	
Polygonaceae	*Rheum*	*nobile*	palmate	alpine	herb	
Primulaceae	*Primula*	*elatior*	pinnate	alpine	herb	C3
Proteaceae	*Banksia*	*attenuata*	pinnate	wetland	tree	C3
Proteaceae	*Banksia*	*menziesii*	pinnate	wetland	tree	C3
Proteaceae	*Embothrium*	*coccineum*	pinnate	temperate forest	tree	C3
Proteaceae	*Gevuina*	*avellana*	pinnate	temperate forest	tree	C3
Proteaceae	*Grevillea*	*buxifolia*	pinnate	wetland	shrub	C3
Proteaceae	*Grevillea*	*speciosa*	pinnate	wetland	shrub	C3
Proteaceae	*Hakea*	*dactyloides*	parallel	wetland	shrub	C3
Proteaceae	*Hakea*	*tephrosperma*	pinnate	wetland	shrub	C3
Proteaceae	*Hakea*	*teretifolia*	pinnate	wetland	shrub	C3
Proteaceae	*Lambertia*	*formosa*	pinnate	wetland	shrub	C3
Proteaceae	*Lomatia*	*hirsuta*	pinnate	temperate forest	tree	C3
Proteaceae	*Lomatia*	*silaifolia*	pinnate	temperate forest	shrub	C3
Proteaceae	*Protea*	*acaulos*	pinnate	temperate forest	shrub	
Proteaceae	*Protea*	*neriifolia*	pinnate	temperate forest	shrub	
Proteaceae	*Protea*	*nitida*	pinnate	temperate forest	shrub	
Proteaceae	*Protea*	*repens*	pinnate	temperate forest	shrub	
Proteaceae	*Stirlingia*	*latifolia*	palmate	wetland	shrub	C3
Proteaceae	*Xylomelum*	*pyriforme*	pinnate	temperate forest	tree	C3
Ranunculaceae	*Anemone*	*cylindrica*	palmate	temperate forest	herb	C3
Ranunculaceae	*Helleborus*	*foetidus*	pinnate	wetland	shrub	C3
Ranunculaceae	*Helleborus*	*lividus*	pinnate	wetland	shrub	C3
Ranunculaceae	*Paeonia*	*cambessedesii*	pinnate	wetland	herb	C3
Rhamnaceae	*Ceanothus*	*cuneatus*	pinnate	wetland	shrub	C3
Rhamnaceae	*Ceanothus*	*oliganthus*	pinnate	wetland	shrub	C3
Rhamnaceae	*Pomaderris*	*ferruginea*	pinnate	temperate forest	shrub	C3
Rhamnaceae	*Rhamnus*	*alaternus*	pinnate	wetland	shrub	C3
Rhamnaceae	*Rhamnus*	*californica*	pinnate	wetland	shrub	C3
Rhamnaceae	*Rhamnus*	*cathartica*	pinnate	temperate forest	shrub	C3
Rhamnaceae	*Rhamnus*	*crocea*	pinnate	wetland	shrub	C3
Rhamnaceae	*Rhamnus*	*ludovici-salvatoris*	pinnate	wetland	shrub	C3
Rosaceae	*Amelanchier*	*alnifolia*	pinnate	wetland	tree	C3
Rosaceae	*Aronia*	*melanocarpa*	pinnate	temperate forest	shrub	C3
Rosaceae	*Cercocarpus*	*betuloides*	pinnate	wetland	shrub	C3
Rosaceae	*Crataegus*	*monogyna*	pinnate	grassland/meadow	shrub	C3
Rosaceae	*Dryas*	*integrifolia*	pinnate	tundra	shrub	
Rosaceae	*Geum*	*montanum*	palmate	alpine	herb	C3
Rosaceae	*Geum*	*reptans*	palmate	alpine	herb	C3
Rosaceae	*Geum*	*rivale*	palmate	alpine	herb	C3
Rosaceae	*Heteromeles*	*arbutifolia*	pinnate	wetland	shrub	C3
Rosaceae	*Holodiscus*	*discolor*	pinnate	wetland	shrub	C3
Rosaceae	*Photinia*	*davidiana*	pinnate	temperate rain forest	tree	C3
Rosaceae	*Potentilla*	*anserina*	pinnate	alpine	herb	C3
Rosaceae	*Potentilla*	*arguta*	pinnate	temperate forest	herb	C3
Rosaceae	*Potentilla*	*crantzii*	palmate	alpine	herb	C3
Rosaceae	*Potentilla*	*peduncularis*	pinnate	alpine	herb	
Rosaceae	*Prunus*	*ilicifolia*	pinnate	wetland	shrub	C3
Rosaceae	*Prunus*	*mahaleb*	pinnate	grassland/meadow	shrub	C3
Rosaceae	*Prunus*	*sargentii*	pinnate	temperate forest	tree	C3
Rosaceae	*Prunus*	*serotina*	pinnate	temperate forest	tree	C3
Rosaceae	*Prunus*	*spinosa*	pinnate	grassland/meadow	shrub	C3
Rosaceae	*Prunus*	*ssiori*	pinnate	temperate forest	tree	C3
Rosaceae	*Pyrus*	*bourgaeana*	pinnate	wetland	shrub	
Rosaceae	*Rosa*	*micrantha*	pinnate	grassland/meadow	shrub	C3
Rosaceae	*Rubus*	*chamaemorus*	palmate	tundra	herb	

Rosaceae	*Rubus*	*corylifolius*	pinnate	wetland	vine	C3
Rosaceae	*Rubus*	*hispidus*	pinnate	temperate forest	tree	C3
Rosaceae	*Sanguisorba*	*minor*	pinnate	grassland/meadow	herb	C3
Rosaceae	*Sorbus*	*alnifolia*	pinnate	temperate forest	tree	C3
Rosaceae	*Sorbus*	*commixta*	pinnate	temperate forest	tree	C3
Rosaceae	*Spiraea*	*alba*	pinnate	temperate forest	shrub	C3
Rosaceae	*Spiraea*	*tomentosa*	pinnate	temperate forest	shrub	C3
Rubiaceae	*Pittoniotis*	*trichantha*	pinnate	tropical rain forest	tree	
Rubiaceae	*Coprosma*	*ochracea*	pinnate	temperate rain forest	tree	C3
Rubiaceae	*Psychotria*	*limonensis*	pinnate	tropical rain forest	shrub	
Rubiaceae	*Psychotria*	*marginata*	pinnate	tropical rain forest	shrub	
Rubiaceae	*Retiniphyllum*	*truncatum*	pinnate	tropical rain forest	tree	C3
Rubiaceae	*Rubia*	*peregrina*	pinnate	grassland/meadow	shrub	C3
Rutaceae	*Cneorum*	*tricoccon*	pinnate	wetland	shrub	C3
Rutaceae	*Correa*	*reflexa*	pinnate	temperate forest	shrub	C3
Rutaceae	*Philotheca*	*difformis*	pinnate	wetland	shrub	C3
Salicaceae	*Populus*	*deltoides*	pinnate	temperate forest	tree	C3
Salicaceae	*Populus*	*fremontii*	pinnate	wetland	tree	C3
Salicaceae	*Populus*	*grandidentata*	pinnate	temperate forest	tree	C3
Salicaceae	*Populus*	*maximowiczii*	pinnate	temperate forest	tree	C3
Salicaceae	*Populus*	*sieboldii*	pinnate	temperate forest	tree	C3
Salicaceae	*Populus*	*tremuloides*	pinnate	temperate forest	tree	C3
Salicaceae	*Salix*	*fragilis*	pinnate	temperate forest	tree	C3
Salicaceae	*Salix*	*glauca*	pinnate	temperate forest	tree	C3
Salicaceae	*Salix*	*hultenii*	pinnate	temperate forest	tree	C3
Salicaceae	*Salix*	*planifolia*	pinnate	temperate forest	tree	C3
Salicaceae	*Salix*	*pulchra*	pinnate	tundra	shrub	
Salicaceae	*Salix*	*reticulata*	pinnate	tundra	shrub	C3
Santalaceae	*Santalum*	*acuminatum*	pinnate	wetland	tree	C3
Sapindaceae	*Acer*	*campestre*	palmate	wetland	tree	C3
Sapindaceae	*Acer*	*mono*	palmate	temperate forest	tree	C3
Sapindaceae	*Acer*	*monspessulanum*	palmate	grassland/meadow	tree	C3
Sapindaceae	*Acer*	*palmatum*	palmate	temperate forest	tree	C3
Sapindaceae	*Acer*	*pensylvanicum*	palmate	temperate forest	tree	C3
Sapindaceae	*Acer*	*platanoides*	palmate	temperate forest	tree	C3
Sapindaceae	*Acer*	*pseudoplatanus*	palmate	temperate forest	tree	C3
Sapindaceae	*Acer*	*rubrum*	palmate	temperate forest	tree	C3
Sapindaceae	*Acer*	*saccharum*	palmate	temperate forest	tree	C3
Sapindaceae	*Dodonaea*	*viscosa*	pinnate	wetland	shrub	C3
Scrophulariaceae	*Mimulus*	*aurantiacus*	pinnate	wetland	shrub	C3
Scrophulariaceae	*Penstemon*	*grandiflorus*	pinnate	temperate forest	herb	C3
Scrophulariaceae	*Picrorhiza*	*scrophulariiflora*	pinnate	alpine	herb	
Scrophulariaceae	*Verbascum*	*sinuatum*	pinnate	wetland	herb	C3
Solanaceae	*Datura*	*stramonium*	pinnate	wetland	herb	C3
Solanaceae	*Solanum*	*ferocissimum*	pinnate	wetland	shrub	C3
Styracaceae	*Styrax*	*camporum*	pinnate	tropical forest	tree	C3
Theaceae	*Camellia*	*japonica*	pinnate	temperate forest	tree	C3
Theaceae	*Cleyera*	*japonica*	pinnate	temperate forest	tree	C3
Thymelaeaceae	*Dirca*	*occidentalis*	pinnate	wetland	shrub	C3
Thymelaeaceae	*Pimelea*	*linifolia*	pinnate	wetland	shrub	C3
Thymelaeaceae	*Pimelea*	*microcephala*	pinnate	wetland	shrub	C3
Ulmaceae	*Celtis*	*occidentalis*	pinnate	temperate forest	tree	C3
Ulmaceae	*Trema*	*tomentosa*	pinnate	tropical rain forest	tree	
Ulmaceae	*Ulmus*	*americana*	pinnate	temperate forest	tree	C3
Ulmaceae	*Ulmus*	*davidiana*	pinnate	temperate forest	tree	C3
Ulmaceae	*Ulmus*	*laciniata*	pinnate	temperate forest	tree	C3
Urticaceae	*Cecropia*	*ficifolia*	palmate	tropical rain forest	tree	C3
Urticaceae	*Cecropia*	*longipes*	palmate	tropical rain forest	tree	
Urticaceae	*Urera*	*caracasana*	pinnate	tropical rain forest	tree	
Urticaceae	*Urtica*	*atrovirens*	palmate	wetland	herb	C3
Verbenaceae	*Vitex*	*agnus-castus*	pinnate	wetland	shrub	C3
Violaceae	*Viola*	*jaubertiana*	palmate	wetland	herb	C3
Winteraceae	*Drimys*	*piperita*	pinnate	tropical rain forest	shrub	C3
Winteraceae	*Drimys*	*winteri*	pinnate	temperate forest	tree	C3

Appendix 1.3: Secondary vein type, biome, and growth form for each of the species used in this study, ordered by family. Biome and growth form data were compiled from Wright et al. (2004).

Family	**Genus**	**Species**	**Secondary vein type**	**Biome**	**Growth Form**
Aextoxicaceae	*Aextoxicon*	*punctatum*	closed	temperate forest	tree
Amaranthaceae	*Atriplex*	*stipitata*	open	wetland	shrub
Anacardiaceae	*Anacardium*	*excelsum*	intermediate	tropical rain forest	tree
Anacardiaceae	*Astronium*	*graveolens*	intermediate	tropical forest	tree
Anacardiaceae	*Buchanania*	*lanzan*	open	tropical forest	tree
Anacardiaceae	*Buchanania*	*obovata*	closed	tropical forest	tree
Anacardiaceae	*Spondias*	*radlkoferi*	open	tropical rain forest	tree
Anacardiaceae	*Toxicodendron*	*diversiloba*	open	wetland	shrub
Annonaceae	*Annona*	*spraguei*	intermediate	tropical rain forest	tree
Annonaceae	*Guatteria*	*dumetorum*	closed	tropical rain forest	tree
Apiaceae	*Carum*	*carvi*	open	alpine	herb
Apiaceae	*Daucus*	*carota*	open	grassland/meadow	herb
Apiaceae	*Eryngium*	*campestre*	open	grassland/meadow	herb
Apiaceae	*Ligusticum*	*mutellina*	open	alpine	herb
Apiaceae	*Niphogeton*	*dissecta*	open	alpine	herb
Apiaceae	*Oreomyrrhis*	*andicola*	open	alpine	herb
Apiaceae	*Peucedanum*	*multivittatum*	open	boreal	herb
Apocynaceae	*Aspidosperma*	*megalocarpon*	closed	tropical rain forest	tree
Apocynaceae	*Wrightia*	*pubescens*	intermediate	tropical forest	tree
Aquifoliaceae	*Ilex*	*verticillata*	intermediate	temperate forest	shrub
Araliaceae	*Astrotricha*	*floccosa*	closed	temperate forest	shrub
Araliaceae	*Oreopanax*	*xalapensis*	intermediate	tropical forest	tree
Asclepiadaceae	*Asclepias*	*syriaca*	closed	temperate forest	herb
Asteraceae	*Achillea*	*erba*	open	alpine	herb
Asteraceae	*Achillea*	*millefolium*	open	alpine	herb
Asteraceae	*Ambrosia*	*artemisiifolia*	open	temperate forest	herb
Asteraceae	*Baccharis*	*pilularis*	open	wetland	shrub
Asteraceae	*Chrysanthemum*	*leucanthemum*	open	alpine	herb
Asteraceae	*Echinacea*	*purpurea*	open	temperate forest	herb
Asteraceae	*Echinops*	*ritro*	open	grassland/meadow	herb
Asteraceae	*Eupatorium*	*rugosum*	open	temperate forest	herb
Asteraceae	*Helianthus*	*microcephalus*	closed	temperate forest	herb
Asteraceae	*Petasites*	*frigidus*	open	tundra	herb
Asteraceae	*Silphium*	*integrifolium*	intermediate	temperate forest	herb
Asteraceae	*Silphium*	*terebinthinaceum*	intermediate	temperate forest	herb
Asteraceae	*Solidago*	*rigida*	open	temperate forest	herb
Asteraceae	*Taraxacum*	*alpinum*	open	alpine	herb
Asteraceae	*Taraxacum*	*officinale*	open	alpine	herb
Berberidaceae	*Berberis*	*trifoliolata*	open	wetland	shrub
Berberidaceae	*Podophyllum*	*peltatum*	open	temperate forest	herb
Betulaceae	*Alnus*	*crispa*	open	tundra	shrub
Betulaceae	*Alnus*	*hirsuta*	open	temperate forest	tree
Betulaceae	*Alnus*	*incana*	open	tundra	tree
Betulaceae	*Betula*	*ermanii*	open	temperate forest	tree
Betulaceae	*Betula*	*glandulosa*	open	tundra	shrub
Betulaceae	*Betula*	*nana*	open	tundra	shrub
Betulaceae	*Betula*	*nigra*	open	temperate forest	tree
Betulaceae	*Betula*	*papyrifera*	open	tundra	tree

Betulaceae	*Betula*	*pendula*	open	temperate forest	tree
Betulaceae	*Betula*	*populifolia*	open	temperate forest	tree
Betulaceae	*Betula*	*pumila*	open	temperate forest	tree
Betulaceae	*Carpinus*	*betulus*	open	temperate forest	tree
Betulaceae	*Carpinus*	*caroliniana*	open	tropical forest	tree
Betulaceae	*Ostrya*	*japonica*	open	temperate forest	tree
Bignoniaceae	*Jacaranda*	*copaia*	intermediate	tropical rain forest	tree
Bignoniaceae	*Mansoa*	*verrucifera*	closed	tropical rain forest	vine
Bignoniaceae	*Tabebuia*	*chrysantha*	intermediate	tropical forest	tree
Bignoniaceae	*Tabebuia*	*rosea*	closed	tropical rain forest	tree
Boraginaceae	*Cordia*	*alliodora*	closed	tropical rain forest	tree
Boraginaceae	*Eriodictyon*	*californicum*	open	wetland	shrub
Brassicaceae	*Capparis*	*flexuosa*	intermediate	tropical forest	tree
Brassicaceae	*Capparis*	*pachaca*	closed	wetland	tree
Brassicaceae	*Capparis*	*verrucosa*	intermediate	tropical rain forest	shrub
Brassicaceae	*Dentaria*	*laciniata*	open	temperate forest	herb
Brassicaceae	*Morisonia*	*americana*	intermediate	tropical rain forest	tree
Burseraceae	*Bursera*	*simaruba*	closed	tropical forest	tree
Burseraceae	*Protium*	*tenuifolium*	closed	tropical rain forest	tree
Burseraceae	*Tetragastris*	*panamensis*	closed	tropical rain forest	tree
Burseraceae	*Trattinickia*	*aspera*	closed	tropical rain forest	tree
Caprifoliaceae	*Lonicera*	*bella*	intermediate	temperate forest	shrub
Caprifoliaceae	*Sambucus*	*mexicana*	open	wetland	shrub
Caprifoliaceae	*Viburnum*	*cassinoides*	intermediate	temperate forest	shrub
Caprifoliaceae	*Viburnum*	*tinus*	closed	grassland/meadow	shrub
Cercidiphyllaceae	*Cercidiphyllum*	*japonicum*	intermediate	temperate forest	tree
Chloranthaceae	*Hedyosmum*	*maxicanum*	intermediate	tropical forest	tree
Chrysobalanaceae	*Hirtella*	*triandra*	closed	tropical rain forest	tree
Chrysobalanaceae	*Licania*	*arborea*	intermediate	tropical forest	tree
Chrysobalanaceae	*Licania*	*heteromorpha*	intermediate	tropical rain forest	tree
Clethraceae	*Clethra*	*alnifolia*	open	wetland	shrub
Clethraceae	*Clethra*	*mexicana*	intermediate	tropical forest	tree
Clusiaceae	*Caraipa*	*heterocarpa*	intermediate	tropical rain forest	tree
Clusiaceae	*Vismia*	*japurensis*	intermediate	tropical rain forest	tree
Clusiaceae	*Vismia*	*lauriformis*	closed	tropical rain forest	tree
Cochlospermaceae	*Cochlospermum*	*vitifolium*	open	wetland	tree
Combretaceae	*Terminalia*	*ferdinandiana*	intermediate	tropical forest	tree
Cornaceae	*Cornus*	*contraversa*	intermediate	temperate forest	tree
Cornaceae	*Cornus*	*florida*	intermediate	temperate forest	tree
Cornaceae	*Cornus*	*racemosa*	intermediate	temperate forest	shrub
Cunoniaceae	*Eucryphia*	*cordifolia*	closed	temperate forest	tree
Cyrillaceae	*Cyrilla*	*racemiflora*	closed	wetland	shrub
Diapensiaceae	*Galax*	*aphylla*	open	temperate forest	herb
Dipterocarpaceae	*Shorea*	*robusta*	intermediate	tropical forest	tree
Ebenaceae	*Diospyros*	*melanoxylon*	intermediate	tropical forest	tree
Ebenaceae	*Diospyros*	*texana*	intermediate	wetland	shrub
Elaeagnaceae	*Eleagnus*	*angustifolia*	intermediate	wetland	tree
Ericaceae	*Arbutus*	*andrachne*	intermediate	wetland	shrub
Ericaceae	*Arbutus*	*menziesii*	intermediate	wetland	shrub
Ericaceae	*Arbutus*	*unedo*	closed	wetland	shrub
Ericaceae	*Arctostaphylos*	*alpina*	intermediate	tundra	shrub
Ericaceae	*Arctostaphylos*	*tomentosa*	closed	wetland	shrub
Ericaceae	*Arctostaphylos*	*uva-ursi*	intermediate	temperate forest	shrub
Ericaceae	*Gaylussacia*	*baccata*	open	temperate forest	shrub
Ericaceae	*Kalmia*	*angustifolia*	closed	temperate forest	tree

Ericaceae	*Kalmia*	*latifolia*	intermediate	temperate forest	tree
Ericaceae	*Leucothoe*	*racemosa*	intermediate	wetland	shrub
Ericaceae	*Lyonia*	*lucida*	open	wetland	shrub
Ericaceae	*Rhododendron*	*lapponicum*	intermediate	tundra	shrub
Ericaceae	*Rhododendron*	*maximum*	intermediate	temperate forest	tree
Ericaceae	*Vaccinium*	*arboreum*	closed	temperate forest	shrub
Ericaceae	*Vaccinium*	*corymbosum*	intermediate	temperate forest	shrub
Ericaceae	*Vaccinium*	*myrtilloides*	intermediate	temperate forest	shrub
Ericaceae	*Vaccinium*	*myrtillus*	intermediate	tundra	shrub
Ericaceae	*Vaccinium*	*uliginosum*	closed	tundra	shrub
Ericaceae	*Vaccinium*	*vitis-idaea*	intermediate	tundra	shrub
Erythroxylaceae	*Erythroxylum*	*densum*	open	tropical forest	tree
Euphorbiaceae	*Elateriospermum*	*tapos*	closed	tropical rain forest	tree
Euphorbiaceae	*Hyeronima*	*alchornoides*	intermediate	tropical rain forest	tree
Euphorbiaceae	*Macaranga*	*lowii*	closed	tropical rain forest	tree
Euphorbiaceae	*Manihot*	*esculenta*	intermediate	tropical rain forest	shrub
Euphorbiaceae	*Micrandra*	*sprucei*	closed	tropical rain forest	tree
Euphorbiaceae	*Sapium*	*aucuparium*	intermediate	tropical rain forest	tree
Fabaceae	*Acacia*	*auriculiformis*	closed	tropical forest	tree
Fabaceae	*Acacia*	*doratoxylon*	closed	wetland	shrub
Fabaceae	*Acacia*	*oswaldii*	closed	wetland	shrub
Fabaceae	*Andira*	*inermis*	closed	tropical forest	tree
Fabaceae	*Baptisia*	*leucophaea*	intermediate	temperate forest	herb
Fabaceae	*Bauhinia*	*megalandra*	open	tropical forest	tree
Fabaceae	*Butea*	*monosperma*	intermediate	tropical forest	tree
Fabaceae	*Caesalpinia*	*granadillo*	open	wetland	tree
Fabaceae	*Cassia*	*grandis*	closed	tropical forest	tree
Fabaceae	*Ceratonia*	*siliqua*	intermediate	wetland	shrub
Fabaceae	*Chamaedaphne*	*calyculata*	intermediate	temperate forest	shrub
Fabaceae	*Desmodium*	*canadense*	open	temperate forest	herb
Fabaceae	*Eperua*	*purpurea*	closed	tropical rain forest	tree
Fabaceae	*Hedysarum*	*alpinum*	closed	tundra	herb
Fabaceae	*Lespedeza*	*capitata*	closed	temperate forest	herb
Fabaceae	*Maackia*	*amurensis*	intermediate	temperate forest	tree
Fabaceae	*Medicago*	*minima*	open	grassland/meadow	herb
Fabaceae	*Pithecellobium*	*dulce*	intermediate	tropical rain forest	tree
Fabaceae	*Prioria*	*copaifera*	closed	tropical rain forest	tree
Fabaceae	*Robinia*	*pseudoacacia*	open	temperate forest	tree
Fabaceae	*Swartzia*	*simplex*	closed	tropical rain forest	tree
Fabaceae	*Tachigali*	*versicolor*	closed	tropical rain forest	tree
Fabaceae	*Trifolium*	*repens*	open	alpine	herb
Fagaceae	*Fagus*	*crenata*	open	temperate forest	tree
Fagaceae	*Fagus*	*sylvatica*	open	temperate forest	tree
Fagaceae	*Nothofagus*	*betuloides*	intermediate	temperate forest	tree
Fagaceae	*Nothofagus*	*dombeyi*	open	temperate forest	tree
Fagaceae	*Nothofagus*	*nitida*	open	temperate forest	tree
Fagaceae	*Quercus*	*acuta*	intermediate	temperate forest	tree
Fagaceae	*Quercus*	*austrina*	open	temperate forest	tree
Fagaceae	*Quercus*	*chapmanii*	intermediate	temperate forest	tree
Fagaceae	*Quercus*	*coccifera*	intermediate	wetland	shrub
Fagaceae	*Quercus*	*coccinea*	open	temperate forest	tree
Fagaceae	*Quercus*	*ellipsoidalis*	open	temperate forest	tree
Fagaceae	*Quercus*	*faginea*	open	wetland	tree
Fagaceae	*Quercus*	*falcata*	open	temperate forest	tree
Fagaceae	*Quercus*	*geminata*	open	temperate forest	tree

Fagaceae	*Quercus*	*hemisphaerica*	open	temperate forest	tree
Fagaceae	*Quercus*	*ilex*	intermediate	grassland/meadow	tree
Fagaceae	*Quercus*	*incana*	intermediate	temperate forest	tree
Fagaceae	*Quercus*	*laevis*	intermediate	temperate forest	tree
Fagaceae	*Quercus*	*laurifolia*	intermediate	temperate forest	tree
Fagaceae	*Quercus*	*macrocarpa*	open	temperate forest	tree
Fagaceae	*Quercus*	*margaretta*	intermediate	temperate forest	tree
Fagaceae	*Quercus*	*michauxii*	open	temperate forest	tree
Fagaceae	*Quercus*	*minima*	open	temperate forest	tree
Fagaceae	*Quercus*	*mongolica*	open	temperate forest	tree
Fagaceae	*Quercus*	*myrtifolia*	intermediate	temperate forest	tree
Fagaceae	*Quercus*	*nigra*	intermediate	temperate forest	tree
Fagaceae	*Quercus*	*pubescens*	open	grassland/meadow	tree
Fagaceae	*Quercus*	*pyrenaica*	open	wetland	tree
Fagaceae	*Quercus*	*robur*	intermediate	temperate forest	tree
Fagaceae	*Quercus*	*rubra*	open	temperate forest	tree
Fagaceae	*Quercus*	*salicina*	intermediate	temperate forest	tree
Fagaceae	*Quercus*	*shumardii*	open	temperate forest	tree
Fagaceae	*Quercus*	*stellata*	intermediate	temperate forest	tree
Fagaceae	*Quercus*	*suber*	open	wetland	tree
Fagaceae	*Quercus*	*turbinella*	open	wetland	tree
Fagaceae	*Quercus*	*virginiana*	intermediate	temperate forest	tree
Fagaceae	*Quercus*	*xalapensis*	open	tropical forest	tree
Goupiaceae	*Goupia*	*glabra*	intermediate	tropical rain forest	tree
Grossulariaceae	*Ribes*	*californicum*	open	wetland	shrub
Grossulariaceae	*Ribes*	*uva-crispa*	open	wetland	shrub
Hamamelidaceae	*Liquidambar*	*styraciflua*	intermediate	tropical forest	tree
Illiciaceae	*Illicium*	*anisatum*	intermediate	temperate forest	tree
Iteaceae	*Itea*	*virginica*	intermediate	wetland	shrub
Juglandaceae	*Carya*	*ovata*	open	temperate forest	tree
Juglandaceae	*Juglans*	*nigra*	intermediate	temperate forest	tree
Lamiaceae	*Calamintha*	*nepeta*	intermediate	grassland/meadow	shrub
Lamiaceae	*Lepechinia*	*calycina*	open	wetland	shrub
Lamiaceae	*Teucrium*	*chamaedrys*	open	grassland/meadow	shrub
Lauraceae	*Cinnamomum*	*japonicum*	intermediate	temperate forest	tree
Lauraceae	*Cryptocarya*	*alba*	intermediate	temperate forest	tree
Lauraceae	*Machilus*	*thunbergii*	intermediate	temperate forest	tree
Lauraceae	*Neolitsea*	*sericea*	closed	temperate forest	tree
Lauraceae	*Ocotea*	*costulata*	intermediate	tropical rain forest	tree
Lauraceae	*Persea*	*borbonia*	closed	temperate forest	shrub
Lauraceae	*Persea*	*lingue*	intermediate	temperate forest	tree
Lecythidaceae	*Planchonia*	*careya*	closed	tropical forest	tree
Magnoliaceae	*Liriodendron*	*tulipifera*	open	temperate forest	tree
Magnoliaceae	*Magnolia*	*obovata*	closed	temperate forest	tree
Magnoliaceae	*Magnolia*	*schiedeana*	closed	tropical forest	tree
Malvaceae	*Apeiba*	*aspera (=membran*	closed	tropical rain forest	tree
Malvaceae	*Apeiba*	*tibourbou*	closed	tropical rain forest	tree
Malvaceae	*Brachychiton*	*populneus*	open	wetland	tree
Malvaceae	*Lasiopetalum*	*ferrugineum*	closed	temperate forest	shrub
Malvaceae	*Luehea*	*seemannii*	open	tropical rain forest	tree
Malvaceae	*Quararibea*	*asterolepis*	closed	tropical rain forest	tree
Malvaceae	*Tilia*	*cordata*	open	temperate forest	tree
Marantaceae	*Calathea*	*inocephala*	intermediate	tropical rain forest	herb
Marantaceae	*Pleiostachya*	*pruinosa*	intermediate	tropical rain forest	herb
Melanthiaceae	*Trillium*	*grandiflora*	open	temperate forest	herb

Melastomataceae	*Bellucia*	*grossularioides*	closed	tropical rain forest	tree
Melastomataceae	*Clidemia*	*sericea*	closed	tropical rain forest	tree
Melastomataceae	*Miconia*	*argentea*	closed	tropical rain forest	tree
Melastomataceae	*Miconia*	*dispar*	closed	tropical rain forest	tree
Meliaceae	*Guarea*	*glabra*	intermediate	tropical rain forest	tree
Meliaceae	*Synoum*	*glandulosum*	intermediate	temperate forest	tree
Meliaceae	*Trichilia*	*cipo*	closed	tropical rain forest	tree
Moraceae	*Castilla*	*elastica*	closed	tropical rain forest	tree
Moraceae	*Ficus*	*racemosa*	closed	tropical forest	tree
Moraceae	*Ficus*	*scobina*	closed	tropical forest	tree
Moraceae	*Poulsenia*	*armata*	closed	tropical rain forest	tree
Myrsinaceae	*Maesa*	*japonica*	open	temperate forest	shrub
Myrsinaceae	*Myrsine*	*seguinii*	intermediate	temperate forest	tree
Myrtaceae	*Amomyrtus*	*luma*	closed	temperate forest	tree
Myrtaceae	*Corymbia*	*gummifera*	open	wetland	tree
Myrtaceae	*Eucalyptus*	*haemostoma*	closed	wetland	tree
Myrtaceae	*Melaleuca*	*leucadendra*	closed	tropical forest	tree
Myrtaceae	*Melaleuca*	*viridiflora*	intermediate	tropical forest	tree
Myrtaceae	*Myrceugenia*	*planipes*	closed	temperate forest	tree
Myrtaceae	*Psidium*	*guajava*	intermediate	tropical forest	tree
Myrtaceae	*Syncarpia*	*glomulifera*	intermediate	temperate forest	tree
Myrtaceae	*Syzygium*	*cumini*	closed	tropical forest	tree
Myrtaceae	*Syzygium*	*suborbiculare*	closed	tropical forest	tree
Myrtaceae	*Xanthostemon*	*paradoxus*	intermediate	tropical forest	tree
Nyctaginaceae	*Neea*	*obovata*	intermediate	tropical rain forest	tree
Oleaceae	*Fraxinus*	*americana*	intermediate	temperate forest	tree
Oleaceae	*Fraxinus*	*mandshurica*	intermediate	temperate forest	tree
Oleaceae	*Olea*	*europaea*	intermediate	wetland	shrub
Onagraceae	*Epilobium*	*angustifolium*	closed	tundra	herb
Papaveraceae	*Sanguinaria*	*canadensis*	open	temperate forest	herb
Piperaceae	*Piper*	*aequale*	closed	tropical rain forest	shrub
Piperaceae	*Piper*	*amalago*	closed	tropical rain forest	shrub
Piperaceae	*Piper*	*auritum*	closed	tropical rain forest	shrub
Piperaceae	*Piper*	*hispidum*	closed	tropical rain forest	shrub
Piperaceae	*Piper*	*peltatum*	intermediate	tropical rain forest	shrub
Piperaceae	*Piper*	*umbellatum*	intermediate	tropical rain forest	shrub
Polygalaceae	*Xanthophyllum*	*stipitatum*	intermediate	tropical rain forest	tree
Polygonaceae	*Oxyria*	*digyna*	open	alpine	herb
Polygonaceae	*Polygonum*	*bistorta*	intermediate	tundra	herb
Polygonaceae	*Polygonum*	*viviparum*	intermediate	alpine	herb
Primulaceae	*Primula*	*elatior*	open	alpine	herb
Proteaceae	*Embothrium*	*coccineum*	closed	temperate forest	tree
Proteaceae	*Gevuina*	*avellana*	closed	temperate forest	tree
Proteaceae	*Grevillea*	*buxifolia*	intermediate	wetland	shrub
Proteaceae	*Grevillea*	*speciosa*	closed	wetland	shrub
Proteaceae	*Hakea*	*dactyloides*	intermediate	wetland	shrub
Proteaceae	*Lomatia*	*hirsuta*	intermediate	temperate forest	tree
Proteaceae	*Xylomelum*	*pyriforme*	closed	temperate forest	tree
Ranunculaceae	*Anemone*	*cylindrica*	open	temperate forest	herb
Ranunculaceae	*Caltha*	*palustris*	open	alpine	herb
Rhamnaceae	*Ceanothus*	*cuneatus*	intermediate	wetland	shrub
Rhamnaceae	*Ceanothus*	*oliganthus*	intermediate	wetland	shrub
Rhamnaceae	*Condalia*	*hookeri*	closed	wetland	shrub
Rhamnaceae	*Pomaderris*	*ferruginea*	closed	temperate forest	shrub
Rhamnaceae	*Rhamnus*	*californica*	intermediate	wetland	shrub

Rhamnaceae	*Rhamnus*	*cathartica*	intermediate	temperate forest	shrub
Rhamnaceae	*Rhamnus*	*crocea*	intermediate	wetland	shrub
Rosaceae	*Aronia*	*melanocarpa*	intermediate	temperate forest	shrub
Rosaceae	*Cercocarpus*	*betuloides*	intermediate	wetland	shrub
Rosaceae	*Crataegus*	*monogyna*	open	grassland/meadow	shrub
Rosaceae	*Dryas*	*octopetala*	open	tundra	shrub
Rosaceae	*Geum*	*montanum*	open	alpine	herb
Rosaceae	*Geum*	*reptans*	open	alpine	herb
Rosaceae	*Geum*	*rivale*	open	alpine	herb
Rosaceae	*Heteromeles*	*arbutifolia*	intermediate	wetland	shrub
Rosaceae	*Holodiscus*	*discolor*	open	wetland	shrub
Rosaceae	*Potentilla*	*anserina*	open	alpine	herb
Rosaceae	*Potentilla*	*crantzii*	open	alpine	herb
Rosaceae	*Potentilla*	*fruticosa*	open	tundra	herb
Rosaceae	*Potentilla*	*hyparctica*	open	tundra	herb
Rosaceae	*Potentilla*	*matsumurae*	open	boreal	herb
Rosaceae	*Prunus*	*mahaleb*	closed	grassland/meadow	shrub
Rosaceae	*Prunus*	*serotina*	intermediate	temperate forest	tree
Rosaceae	*Prunus*	*spinosa*	intermediate	grassland/meadow	shrub
Rosaceae	*Pyrus*	*bourgaeana*	intermediate	wetland	shrub
Rosaceae	*Rosa*	*micrantha*	open	grassland/meadow	shrub
Rosaceae	*Rubus*	*chamaemorus*	open	tundra	herb
Rosaceae	*Rubus*	*corylifolius*	open	wetland	vine
Rosaceae	*Rubus*	*hispidus*	intermediate	temperate forest	tree
Rosaceae	*Sanguisorba*	*minor*	open	grassland/meadow	herb
Rosaceae	*Sibbaldia*	*procumbens*	open	tundra	herb
Rosaceae	*Sorbus*	*commixta*	open	temperate forest	tree
Rosaceae	*Spiraea*	*alba*	open	temperate forest	shrub
Rosaceae	*Spiraea*	*tomentosa*	open	temperate forest	shrub
Rubiaceae	*Adina*	*cordifolia*	closed	tropical forest	tree
Rubiaceae	*Alseis*	*blackiana*	closed	tropical rain forest	tree
Rubiaceae	*Antirrhoea (=Pitton*	*trichantha*	closed	tropical rain forest	tree
Rubiaceae	*Diplospora*	*malaccensis*	closed	tropical rain forest	tree
Rubiaceae	*Macrocnemum*	*glabrescens*	closed	tropical rain forest	tree
Rubiaceae	*Psychotria*	*limonensis*	closed	tropical rain forest	shrub
Rubiaceae	*Psychotria*	*marginata*	intermediate	tropical rain forest	shrub
Rubiaceae	*Retiniphyllum*	*truncatum*	closed	tropical rain forest	tree
Rubiaceae	*Rubia*	*peregrina*	open	grassland/meadow	shrub
Rutaceae	*Correa*	*reflexa*	intermediate	temperate forest	shrub
Rutaceae	*Zanthoxylum*	*ekmanii*	open	tropical rain forest	tree
Rutaceae	*Zanthoxylum*	*panamense*	intermediate	tropical rain forest	tree
Salicaceae	*Casearia*	*arborea*	intermediate	tropical rain forest	tree
Salicaceae	*Homalium*	*dictyoneurum*	intermediate	tropical rain forest	tree
Salicaceae	*Populus*	*balsamifera*	open	tundra	tree
Salicaceae	*Populus*	*deltoides*	open	temperate forest	tree
Salicaceae	*Populus*	*fremontii*	open	wetland	tree
Salicaceae	*Populus*	*maximowiczii*	open	temperate forest	tree
Salicaceae	*Populus*	*sieboldii*	open	temperate forest	tree
Salicaceae	*Populus*	*tremuloides*	open	temperate forest	tree
Salicaceae	*Salix*	*alaxensis*	intermediate	tundra	shrub
Salicaceae	*Salix*	*arctica*	intermediate	tundra	shrub
Salicaceae	*Salix*	*fragilis*	intermediate	temperate forest	tree
Salicaceae	*Salix*	*glauca*	open	temperate forest	tree
Salicaceae	*Salix*	*herbacea*	open	tundra	shrub
Salicaceae	*Salix*	*myrsinites*	intermediate	tundra	shrub

Salicaceae	*Salix*	*planifolia*	intermediate	temperate forest	tree
Salicaceae	*Salix*	*polaris*	closed	tundra	shrub
Salicaceae	*Salix*	*pulchra*	intermediate	tundra	shrub
Salicaceae	*Salix*	*reticulata*	open	tundra	shrub
Salicaceae	*Zuelania*	*guidonia*	intermediate	tropical rain forest	tree
Santalaceae	*Santalum*	*acuminatum*	intermediate	wetland	tree
Sapindaceae	*Acer*	*mono*	open	temperate forest	tree
Sapindaceae	*Acer*	*monspessulanum*	intermediate	grassland/meadow	tree
Sapindaceae	*Acer*	*platanoides*	open	temperate forest	tree
Sapindaceae	*Acer*	*pseudoplatanus*	open	temperate forest	tree
Sapindaceae	*Acer*	*rubrum*	open	temperate forest	tree
Sapindaceae	*Acer*	*saccharum*	open	temperate forest	tree
Sapindaceae	*Cupania*	*rufescens*	open	tropical rain forest	tree
Sapindaceae	*Cupania*	*sylvatica*	closed	tropical rain forest	tree
Sapindaceae	*Dodonaea*	*viscosa*	closed	wetland	shrub
Sapindaceae	*Sapindus*	*saponaria*	intermediate	tropical forest	tree
Sapotaceae	*Chrysophyllum*	*argenteum*	closed	tropical rain forest	tree
Scrophulariaceae	*Mimulus*	*aurantiacus*	closed	wetland	shrub
Scrophulariaceae	*Pedicularis*	*lapponica*	open	tundra	herb
Simaroubaceae	*Simarouba*	*amara*	closed	tropical rain forest	tree
Simaroubaceae	*Simarouba*	*glauca*	closed	tropical forest	tree
Solanaceae	*Solanum*	*ferocissimum*	intermediate	wetland	shrub
Staphyleaceae	*Turpinia*	*insignis*	intermediate	tropical forest	tree
Theaceae	*Camellia*	*japonica*	closed	temperate forest	tree
Theaceae	*Cleyera*	*japonica*	closed	temperate forest	tree
Theaceae	*Eurya*	*japonica*	closed	temperate forest	tree
Theophrastaceae	*Jacquinia*	*revoluta*	intermediate	wetland	tree
Thymelaeaceae	*Dirca*	*occidentalis*	open	wetland	shrub
Ulmaceae	*Celtis*	*occidentalis*	closed	temperate forest	tree
Ulmaceae	*Trema*	*micrantha*	intermediate	tropical rain forest	tree
Ulmaceae	*Ulmus*	*americana*	open	temperate forest	tree
Ulmaceae	*Ulmus*	*laciniata*	open	temperate forest	tree
Urticaceae	*Cecropia*	*ficifolia*	closed	tropical rain forest	tree
Urticaceae	*Cecropia*	*insignis*	closed	tropical rain forest	tree
Urticaceae	*Cecropia*	*longipes*	intermediate	tropical rain forest	tree
Urticaceae	*Cecropia*	*obtusifolia*	closed	tropical rain forest	tree
Urticaceae	*Urera*	*caracasana*	open	tropical rain forest	tree
Winteraceae	*Drimys*	*winteri*	intermediate	temperate forest	tree

Appendix 2.1. Primer sequences for the rbcL_atpB spacer and ndhF.

Primer name	**Sequence (5’- 3’)**	**Length (bp)**
atpB1	TGT CCG ATA GGA AGT TGA CG	20
rbcL	ATA TGC TTT AGT TCT GTT TGT	21
nhdF972f	GTC TCA ATT GGG TTA TAT GAT G	22
ndhF1955r3	CGA TTA TAT GCC CAA TTG TAT A	22

Appendix 2.2: Species means and SE for all traits.
N is the number of leaves sampled for each trait/species combination.

	Dioscorea alata	*Dioscorea batatas*	*Dioscorea bulbifera*	*Dioscorea cotinifolia*	*Dioscorea dodecaneura*	*Dioscorea dumetorum*	*Dioscorea macroura*	*Dioscorea nipponica*	*Dioscorea pentaphylla*	*Dioscorea sylvatica*	*Dioscorea trifida*	*Dioscorea villosa*
Lamina area (cm^2)	145.88±25.48	22.51±3.19	221.01±6.39	49.91±2.22	159.75±14.46	212.84±25.68	395.84±69.38	37.94±5.59	155.57±9.41	29.25±2.7	257.97±20.1	18.86±3.2
N	2	2	3	3	3	3	2	2	2	2	2	3
Lamina dry mass (g)	0.531±0.098	0.075±0.026	0.76±0.067	0.184±0.004	0.404±0.085	0.68±0.046	1.29±0.397	0.116±0.019	0.518±0.132	0.098±0.003	0.896±0.152	0.052±0.009
N	2	2	3	3	4	3	2	2	2	2	2	3
LMA ($g\ m^{-2}$)	36.31±0.35	32.09±7.14	34.27±2.02	38.49±1.91	27.5±3.95	32.34±1.58	31.8±4.46	30.6±0.46	32.89±6.47	33.56±1.96	34.47±3.19	27.72±0.44
N	2	2	3	3	3	3	2	2	2	2	2	3
Petiole length (cm)	7.55±0.98	2.92±0.16	9.65±0.31	4.71±0.2	6.07±0.56	10.26±1.11	13.65±0.16	4.76±0.84	5.44±0.62	4.31±0.61	9.82±1.05	2.96±0.24
N	2	2	3	2	4	3	2	2	2	2	2	3
(mm^2)	14.12±1.4	3.54±0.38	17.33±2.2	4.91±0.35	21.23±2.03	14.42±1.84	29.7±7.35	1.53±0.11	17.98±2.3	2.84±0.08	19.88±1.24	1.22±0.13
N	2	2	3	2	4	3	2	2	2	2	2	3
Primary vein density ($cm\ cm^{-2}$)	0.84±0.049	1.61±0.094	0.66±0.018	0.76±0.003	0.72±0.052	0.44±0.057	0.45±0.04	1.13±0.042	0.39±0.016	1.33±0.005	0.53±0.002	2.18±0.317
N	2	2	3	2	4	3	2	2	2	2	2	3
Minor vein density ($mm\ mm^{-2}$)	10.61±0.55	9.79±0.56	13.48±2.38	6.93±0	9.17±0.53	9.93±0.51	8.02±0.16	11.49±0.22	9.94±0.85	5.76±0.25	12.29±0.61	11.38±0.45
N	3	3	2	1	2	3	3	3	2	3	2	3
%N	3.41±0.395	4.22±0.29	3.95±0.179	4.24±0.093	4.78±0.219	4.5±0.062	4.45±0.081	2.99±0.204	3.45±0.983	2.97±0.266	4.32±0.338	3.13±0.298
N	2	2	3	2	4	2	2	2	2	2	2	3
%C	43.94±0.929	43.41±0.086	46.83±1.25	41.88±1.128	43.91±0.459	44.84±0.004	44.19±1.053	44.32±0.494	40.35±0.037	46.2±0.591	45.88±0.953	45.05±0.542
N	2	2	3	2	4	2	2	2	2	2	2	3
$\delta^{13}C$	-24.03±0.51	-28.2±1.08	-25.26±0.27	-27.67±0.14	-27.33±0.56	-24.67±0.36	-25.98±0.06	-26.03±0.08	-25.29±0.52	-27.08±0.36	-25.89±0.13	-27.78±0.68
N	2	2	3	2	4	3	2	2	2	2	2	3
Stomatal density (mm^{-2})	194.4±18.22	100±4.81	172.9±6.25	97.2±5.56	141.7±17.35	196.9±20.97	119.4±10.02	302.8±32.03	286.1±32.75	66.7±4.81	227.8±36.11	144.4±2.78
N	3	3	4	3	3	3	3	3	3	3	3	3
Guard cell length (mm)	0.024±0.000295	0.029±0.000698	0.027±0.000488	0.031±0.000248	0.022±0.000654	0.018±0.000728	0.022±0.000714	0.02±0.000704	0.026±0.000157	0.03±0.000528	0.028±0.000354	0.026±0.003157
N	3	3	4	3	3	3	3	3	3	3	4	3
Stomatal index	0.229±0.01	0.209±0.014	0.189±0.01	0.219±0.005	0.136±0.009	0.205±0.035	0.121±0.015	0.246±0.02	0.369±0.035	0.243±0.029	0.257±0.06	0.196±0.017
N	3	3	4	3	3	3	3	3	3	3	3	3
Stomatal pore index	0.112±0.01	0.083±0.001	0.127±0.007	0.09±0.005	0.07±0.012	0.062±0.009	0.058±0.002	0.125±0.017	0.198±0.03	0.058±0.003	0.179±0.033	0.098±0.033
N	3	3	4	3	3	3	3	3	3	3	3	3
Ψ_{min} (Mpa)	0.71±0.045	0.96±0.085	0.47±0.03	0.7±0.029	1.05±0.068	1.11±0.034	0.19±0.083	1.53±0.027	0.69±0.058	1.03±0.038	0.48±0.043	1.34±0.026
N	4	4	4	4	4	4	3	4	4	3	4	4
Mean $K_{t-mr} \times 10^3$ (mmol m Mpa^{-1}	17.13±3.9	1.24±0.04	10.36±1.06	1.93±0.33	2.13±0.5	15.95±6.53	15.52±15.11	1.46±1.04	3±2.93	0.37±0.19	14.79±3.81	0.23±0.09
N	2	2	2	2	2	2	2	2	2	2	2	2
(mm)	0.038±0.0024	0.019±0.0004	0.035±0.003	0.022±0.0009	0.022±0.0024	0.033±0.0068	0.041±0.0005	0.018±0.0048	0.02±0.0091	0.015±0.0006	0.032±0.001	0.015±0.002
N	2	2	2	2	2	2	2	2	2	2	2	2
(mm)	0.074±0.006	0.064±0.008	0.057±0.004	0.054±0.007	0.044±0.007	0.047±0.016	0.049±0.01	0.024±0	0.064±0.006	0.049±0.006	0.072±0.009	0.038±0.01
N	2	2	2	2	2	2	2	2	2	2	2	2
(mm)	0.09±0.021	0.085±0.008	0.069±0.005	0.044±0.002	0.081±0.024	0.063±0.002	0.083±0.02	0.056±0.017	0.066±0.01	0.111±0.027	0.074±0.015	0.036±0.004
N	2	2	2	2	2	2	2	2	2	2	2	2
Lamina thickness (mm)	0.269±0.01	0.211±0.022	0.18±0.005	0.164±0.005	0.177±0.028	0.151±0.024	0.171±0.038	0.097±0.015	0.17±0.058	0.216±0.041	0.184±0.027	0.113±0.022
N	2	2	2	2	2	2	2	2	2	2	2	2
K_{leaf} mmol $Mpa^{-1}\ m^{-2}\ sec^{-1}$	2.64±0.58	2.42±0.03	4.77±0.04	2.92±1.02	3.77±0.61	2.73±1.18	5.26±1.36	1.99±0.49	na	2.78±0.71	4.41±0.99	3.13±1.13
N	3	2	2	2	3	2	2	2		2	2	2
A_{max} (μmol $CO_2\ m^{-2}\ s^{-1}$)	9.6±2	11.3±0.2	7.2±0.9	8.5±2.3	12.3±2	10±1.8	11.2±0.6	5.6±0.9	8.5±0.8	7.8±0.2	5.4±0.8	7.1±1.1
N	2	2	3	2	3	3	2	2	2	2	2	3
A_{max} (μmol $CO_2\ g^{-1}\ s^{-1}$)	0.265±0.056	0.372±0.09	0.214±0.036	0.226±0.073	0.465±0.102	0.312±0.06	0.356±0.031	0.183±0.027	0.274±0.077	0.233±0.008	0.16±0.038	0.256±0.038
N	2	2	3	2	3	3	2	2	2	2	2	3
gs at A^{max} (mol $H_2O\ m^{-2}\ s^{-1}$)	0.062±0.0004	0.21±0.076	0.058±0.017	0.052±0.006	0.108±0.017	0.101±0.02	0.055±0.011	0.032±0.003	0.08±0.002	0.052±0.005	0.05±0.023	
N	2	2	3	2	3	3	2	2	2	2	2	3
WUE_{inst} (μmol $CO^2\ mol^{-1}\ H_20$)	6.32±0.76	3.1±0.71	6.05±1.18	7.55±3.31	5.91±1.53	4.73±0.56	9.43±1.02	6.06±0.02	4.47±0.48	7.68±0.45	4.84±1.57	5.92±0.55
N	2	2	3	2	3	3	2	2	2	2	2	3

Appendix 2.3: Pair-wise phylogenetic generalized least squares correlations (below the diagonal) and standard correlations (above the diagonal) among all traits. Numbers in bold represent P<0.05 and underlined number represent 0.05<P<0.08.

Species	Lamina area (cm^2)	LMA (g/m^2)	A_{max} (µmol CO_2 g^{-1} s^{-1})	g_s at A_{max} (mol H_2O m^{-2} s^{-1})	WUEinst (µmol CO2 mol^{-1} $H_2$0)	%N	%C	δ^{13}C	Minor vein density (mm mm^{-2})
Lamina area (cm^2)	x	0.19	0.03	-0.11	0.19	0.57	0.05	**0.69**	0.24
LMA (g/m^2)	0.25	x	-0.26	-0.13	0.13	0.05	-0.14	0.34	-0.21
A_{max} (µmol CO_2 g^{-1} s^{-1})	-0.34	**-0.68**	x	**0.67**	-0.01	0.54	-0.43	-0.34	-0.42
g_s at A_{max} (mol H_2O m^{-2} s^{-1})	**-0.61**	**-0.71**	**0.72**	x	**-0.69**	0.46	-0.23	-0.28	0.01
WUEinst (µmol CO2 mol^{-1} $H_2$0)	0.53	0.35	-0.13	**-0.71**	x	-0.15	0.20	0.07	-0.46
%N	-0.01	-0.50	**0.60**	0.55	-0.32	x	-0.07	-0.04	0.03
%C	0.14	-0.06	-0.29	-0.11	0.21	-0.04	x	0.08	0.22
δ^{13}C	**0.79**	0.56	**-0.61**	**-0.72**	0.44	-0.52	0.07	x	0.38
Minor vein density (mm mm^{-2})	0.38	-0.05	-0.39	-0.03	-0.32	0.05	0.18	0.38	x
Stomatal density (mm^{-2})	**0.65**	0.33	**-0.60**	-0.54	-0.01	-0.27	-0.21	**0.58**	**0.65**
Stomatal index	-0.13	**0.56**	**-0.65**	-0.26	-0.33	**-0.61**	-0.29	0.33	0.16
Guard cell length (mm)	**-0.66**	0.01	0.08	0.37	-0.24	-0.02	0.09	**-0.63**	-0.21
Stomatal pore index	0.26	0.43	**-0.70**	-0.37	-0.21	-0.37	-0.19	0.45	**0.67**
Ψ_{min} (Mpa)	0.06	0.01	0.17	0.11	0.20	0.29	0.16	-0.34	-0.15
Palisade mesohphyll thickness (mm)	0.00	0.07	-0.01	0.20	<0.01	-0.09	0.13	-0.05	0.20
Spongy mesohphyll thickness (mm)	0.40	0.14	-0.27	-0.29	0.05	-0.18	-0.02	0.41	-0.03
Lamina thickness (mm)	0.18	0.23	-0.07	-0.02	0.15	-0.38	-0.06	0.28	0.08
Kleaf mmol Mpa^{-1} m-^{2} sec^{-1}	0.33	-0.35	0.22	0.13	0.31	0.31	0.45	-0.13	0.14
$K_{t\text{-}mr}$ x 10^3 (mmol m Mpa^{-1} sec^{-1})	**0.90**	0.51	-0.50	**-0.68**	0.39	-0.08	0.07	**0.87**	0.43
Mean maximum conduit diameter (mm)	**0.90**	0.42	-0.35	**-0.60**	**0.57**	-0.05	0.23	**0.80**	0.39

Stomatal density (mm-2)	Stomatal index	Guard cell length (mm)	Stomatal pore index	Ψ_{min} (Mpa)	Palisade mesohphyll thickness (mm)	Spongy mesohphyll thickness (mm)	Lamina thickness (mm)	Kleaf mmol Mpa^{-1} m^{-2} sec^{-1}	$K_{t\text{-}mr}$ x 10^3 (mmol m Mpa^{-1} sec^{-1})	Mean maximum conduit diameter (mm)
0.39	-0.26	-0.35	0.13	0.52	0.30	0.41	0.33	**0.70**	**0.89**	**0.86**
-0.10	0.40	0.45	0.22	0.49	0.47	0.11	0.45	-0.01	0.42	0.39
-0.44	-0.56	-0.16	**-0.60**	0.20	0.04	0.17	0.23	0.05	-0.06	0.02
-0.25	-0.16	0.02	-0.25	0.11	0.29	0.29	0.36	-0.08	-0.06	-0.08
-0.30	-0.42	-0.06	-0.37	0.15	-0.22	-0.14	-0.18	0.35	0.03	0.18
-0.10	-0.53	-0.14	-0.24	**0.58**	0.26	0.20	0.21	0.52	0.55	0.55
-0.17	-0.32	-0.07	-0.25	-0.12	-0.13	0.02	-0.28	0.36	0.06	0.18
0.63	0.22	-0.46	0.31	-0.01	0.12	0.32	0.24	0.12	**0.77**	**0.69**
0.75	0.11	-0.25	**0.63**	-0.23	0.08	-0.14	-0.11	0.13	0.34	0.30
x	0.39	-0.48	**0.72**	-0.30	-0.11	-0.06	-0.13	-0.07	0.42	0.26
0.48	x	0.33	**0.68**	-0.15	0.13	-0.06	0.15	-0.59	-0.15	-0.34
-0.64	0.12	x	0.27	0.54	**0.60**	-0.02	0.44	0.13	-0.35	-0.31
0.73	**0.71**	0.05	x	0.08	0.34	-0.10	0.19	0.04	0.16	0.02
-0.45	-0.30	**0.63**	-0.02	x	**0.74**	0.38	**0.68**	**0.70**	0.41	0.44
-0.22	0.02	**0.61**	0.27	**0.67**	x	0.50	**0.90**	0.40	0.36	0.38
0.40	0.14	-0.46	0.09	-0.19	-0.17	x	**0.71**	0.16	0.40	0.35
0.02	0.19	0.30	0.31	0.43	**0.83**	0.22	x	0.26	0.36	0.34
-0.31	**-0.68**	0.29	-0.13	**0.73**	0.56	-0.25	0.29	x	0.44	0.56
0.76	0.07	**-0.69**	0.36	-0.14	-0.07	0.45	0.13	<0.01	x	**0.96**
0.53	-0.18	**-0.56**	0.19	0.04	0.11	0.27	0.22	0.27	**0.93**	x

Appendix 3.1**.** Collection locations of *Dioscorea* plants used in the greenhouse study.

Collection Number	**Species**	**State**	**Latitude (decimal degrees)**	**Longitude (decimal degrees)**	**Elevation (m)**
RW111	*D. bartlettii*	Chiapas	-91.99633	17.40513	325
RW112	*D. bartlettii*	Chiapas	-91.99633	17.40513	325
RW110	*D. composita*	Chiapas	-91.99633	17.40513	325
RW117A	*D. composita*	Chiapas	-92.0646	17.23448	302
RW73	*D. convolvulacea*	Quintana Roo	-88.77978	18.48225	12
RW74	*D. convolvulacea*	Quintana Roo	-88.77978	18.48225	12
RW52	*D. floribunda*	Yucatan	-89.62618	21.11183	9
RW53	*D. floribunda*	Yucatan	-89.62618	21.11183	9
RW57	*D. floribunda*	Yucatan	-89.62253	21.11043	9
RW69	*D. floribunda*	Quintana Roo	-88.29452	19.9995	12
RW94	*D. floribunda*	Chiapas	-93.20383	16.83467	847
RW95	*D. floribunda*	Chiapas	-93.20383	16.83467	847
RW98	*D. floribunda*	Chiapas	-93.20643	16.84148	865
RW103	*D. floribunda*	Chiapas	-92.11795	16.96562	1050
RW114	*D. floribunda*	Chiapas	-92.0646	17.23448	302
RW116	*D. floribunda*	Chiapas	-92.0646	17.23448	302
RW101	*D. gomez-pompea*	Chiapas	-93.20467	16.84673	890
RW127	*D. guerrerensis*	Guerrero	-99.4424	17.48302	1123
RW139	*D. guerrerensis*	Guerrero	-99.60245	17.63827	1570
RW140	*D. guerrerensis*	Guerrero	-99.60245	17.63827	1570
RW141	*D. guerrerensis*	Guerrero	-99.60245	17.63827	1570
RW131	*D. igualamontana*	Guerrero	-99.4424	17.48302	1123
RW132	*D. igualamontana*	Guerrero	-99.4424	17.48302	1123
RW70	*D. matagalpensis*	Quintana Roo	-88.779783	18.48225	12
RW71	*D. matagalpensis*	Quintana Roo	-88.779783	18.48225	12
RW106	*D. mexicana*	Chiapas	-92.1277	17.13895	848
RW113	*D. mexicana*	Chiapas	-92.0646	17.23448	302
RW149	*D. morelosana*	Morelos	-99.2737	19.03487	2525
RW145	*D. multinervis*	Mexico	-99.84158	19.39367	2598
RW161	*D. pumicicola*	Morelos	-99.12028	18.87148	1327
MAR s.n.	*D. remotiflora*	Morelos	-99.08	18.8659	1423
RW88	*D. sp1*	Chiapas	-93.18482	16.79458	721
RW89	*D. sp1*	Chiapas	-93.18482	16.79458	721
RW117	*D. sp3*	Guerrero	-99.52422	17.60788	1383
RW136	*D. sp6*	Guerrero	-99.4424	17.48302	1123
RW138	*D. sp6*	Guerrero	-99.4424	17.48302	1123
RW59	*D. spiculiflora*	Yucatan	-89.54817	21.08327	11
RW66	*D. spiculiflora*	Yucatan	-89.76667	20.375	21
RW72	*D. spiculiflora*	Yucatan	-88.54192	20.63508	15
RW76	*D. subtomentosa*	Chiapas	-93.2541	16.80245	914
RW83	*D. subtomentosa*	Chiapas	-96.30142	19.76012	974
RW86	*D. subtomentosa*	Chiapas	-96.30142	19.76012	974
RW79	*D. sumiderensis*	Chiapas	-93.2541	16.80245	914
RW80	*D. sumiderensis*	Chiapas	-93.2541	16.80245	914
RW155	*D. urceolata*	Morelos	-99.2737	19.03487	2525

Appendix 3.2**.** Mean values of all Worldclim climate parameters (Hijmans et al. 2005) for each species used in this study, plus the number of localities. The 20 species in bold are those included in the greenhouse study. The remaining species are those used for the 48-species comparison of climate niches to phylogeny.

Species	# Locations	Altitude (m)	BIO_1 Annual Mean Temperature (°C*10)	BIO_2 Mean Diurnal Range Mean of monthly (max temp - min temp) (°C*10)	BIO_3 Isothermality (BIO_2/BIO_7)* 100	BIO_4 Temperature Seasonality (standard deviation *100)	BIO_5 Max Temperature of Warmest Month (°C*10)	BIO_6 Min Temperature of Coldest Month (°C*10)	BIO_7 Temperature Annual Range (BIO_5-BIO_6) (°C*10)	BIO_8 Mean Temperature of Wettest Quarter (°C*10)	BIO_9 Mean Temperature of Driest Quarter (°C*10)	BIO_10 Mean Temperature of Warmest Quarter (°C*10)	BIO_11 Mean Temperature of Coldest Quarter (°C*10)	BIO_12 Annual Precipitation (mm)	BIO_14 Precipitation of Driest Month (mm)
D. bartlettii	138	402.478	240.797	104.514	65.152	1769.319	321.254	162.399	158.855	252.978	238.486	260.428	219.029	2238.971	47.123
D. composita	450	343.993	239.171	104.142	60.442	2182.4	327.429	156.656	170.773	254.751	235.22	263.824	212.291	2371.387	46.276
D. convolvulacea	581	833.537	221.122	118.663	66.217	1813.327	310.847	131.867	178.979	233.952	214.308	242.441	199.045	1684.525	23.279
D. cyphocarpa	12	1023	240.333	146.083	69.417	1514.417	346.75	137.25	209.5	244.583	235.5	261.25	223.083	1048.917	2
D. densiflora	175	403.023	240.251	110.377	64.903	1874.4	325.52	156.8	168.72	253.383	235.297	261.446	217.109	1850.989	34.229
D. fasciculocongesta	11	1378.091	178.091	116.182	61.727	2288.818	270.273	84.636	185.636	196.364	160.545	203.818	150.636	1686.273	42.364
D. floribunda	343	400.067	239.95	110.764	65.242	1859.254	324.641	156.093	168.548	253.359	233.408	260.755	217.023	1638.017	27.638
D. galeottiana	166	2077.157	165.62	153.59	68.06	1911.066	275.313	50.946	224.367	179.042	152.428	188.783	142.349	1004.452	8.398
D. gallegosii	29	1135.793	233.276	145.759	67.483	1669.897	342.517	128.103	214.414	238.414	227.586	256.345	214.345	935.379	2.241
D. gaumeri	40	98.625	251.4	111.55	63.925	2100.9	337.6	164.65	172.95	267.35	246.3	274.175	225.5	1163.775	25.6
D. gomez-pompea	24	465.167	247.875	130.208	70.167	1443.208	339.125	154.958	184.167	256.167	236.25	265.25	230.25	1723.208	12.708
D. guerrerensis	8	1425.875	217.5	136.375	67.625	1501	321.375	120.625	200.75	218.375	222.875	239.5	201.625	1140.25	3.375
D. igualamontana	4	939	246.25	139.75	71	1327.5	346.5	150.75	195.75	249.5	248.25	264.75	231.5	1167.75	1.25
D. jaliscana	65	1135.585	206.123	144.846	63.969	2553.846	315.462	89.862	225.6	231.246	201.385	236.108	175.215	1096.108	3.954
D. juxtlahuacensis	8	2093	164.625	138.5	75	1030.125	253	68.625	184.375	172.125	152.625	177.625	152.625	948.75	5.25
D. liebmannii	63	638.19	235.444	124.032	66.587	1804.841	325.73	140.968	184.762	248.492	227.841	256.937	213.349	1426.651	16.413
D. longirhiza	1	1636	208	131	70	1280	303	116	187	209	214	226	194	963	4
D. matagalpensis	95	411	238.558	108.874	68.453	1598.863	318.705	159.768	158.937	249.558	234.495	256.253	218.747	1653.937	28.389
D. matudae	7	1014.143	212	142.429	59.714	3268.857	329.857	93.429	236.429	239.429	183.857	248.429	172.429	1464.571	25.714
D. mcvaughnii	2	998.5	224.5	156	64	2484.5	342	101	241	250	220	252.5	194.5	999.5	4
D. mesoamericana	1	205	261	116	68	1325	344	175	169	269	246	277	246	1435	1
D. mexicana	212	454.321	239.66	103.17	69.099	1387.226	315.802	165.689	150.113	246.623	235.005	256.302	222.712	2217.179	34.118
D. millitaris	49	1462.449	188.898	139.796	64.755	2361.98	291.898	76.837	215.061	210.571	180.122	216.694	160.224	1162.143	6.408
D. minima	37	1950.432	171.459	149.622	65.081	2074.405	285.405	57.568	227.838	184.405	171.054	197.73	146.189	1022.946	6.351
D. morelosana	19	1373	217.368	155.316	68.579	1765.895	330.895	105.737	225.158	226	204.263	241.105	196.789	972.947	3.263
D. multinervis	39	2030.462	160.974	144.718	63.154	2465.487	273	46.051	226.949	181.41	159.205	190.615	130.974	1041.949	6.564
D. oaxacensis	14	1567.929	183.643	130.429	66.571	1848.357	280	84.857	195.143	197.714	166.571	206	161.286	1118.571	17.286
D. oreodoxa	3	601.333	241	134	71.333	1522.667	326.333	139.333	187	254.667	228.333	259	222	947.667	1.667
D. pallens	67	549.224	228.552	117.687	64.239	2162.254	319.134	136.866	182.269	246.09	214.388	252.313	201.716	1394.194	27.507
D. palmeri	23	179.435	256.826	128.522	71.652	1651.13	333.696	155.261	178.435	273.826	244.348	276.304	235.913	870	1
D. pilosiuscula	86	27.198	255.895	123.279	66.558	2075.581	347.581	163.942	183.64	273.314	244.244	277.988	229.849	1155.64	25.779
D. platycolpata	11	1364.182	215.455	143	67.364	1709.909	323.091	111.273	211.818	222.727	211.182	238.727	195.909	985.364	3.455
D. polygonoides	87	325.92	246.736	119.862	66.31	1892.782	336.218	156.655	179.563	261.069	238.207	268.276	223.46	1390.31	18.126
D. preslii	4	381.75	247.25	118.25	69.25	1568.75	329.75	159.5	170.25	259.5	237.75	266	228.25	1277.75	11.75
D. pringlei	10	1545.6	198	149.8	65.3	2012.3	312.1	83.9	228.2	210.6	195.6	223.2	173.7	1016.8	4.7
D. pumicicola	3	1451.333	212.667	167	70.333	1805	331	95.333	235.667	224.333	192	236.333	191	964.333	3
D. remotiflora	181	1049.475	220.74	149.028	65.724	2295.558	332.939	106.199	226.74	238.635	215.961	249.403	193.68	1070.459	3.956
D. sessiflora	8	1302.5	191.875	131.125	65	2346.625	282	81.875	200.125	217.625	185.375	218.5	163	1431.375	4
D. sp1	1	896	231	130	69	1661	323	135	188	243	217	250	207	984	6
D. sp3	1	1425	221	140	69	1330	323	122	201	222	212	239	204	847	2
D. sp6	1	1336	223	141	71	1249	321	123	198	225	226	240	206	918	2
D. spiculiflora	248	177.601	249.694	113.762	66.016	1903.786	336.702	165.073	171.629	263.427	242.222	270.843	225.988	1544.585	28.508
D. subtomentosa	95	676.495	239.726	130.874	68.737	1640.211	333.874	144.263	189.611	250.632	230.347	260.074	220.032	1086.474	4.863
D. sumiderensis	13	868.769	224.769	130.308	69.923	1547.231	317.692	132.385	185.308	234.385	215.846	242.769	205.846	1550.846	21.846
D. tubiperiantha	1	2240	157	149	67	1868	269	47	222	166	158	181	134	1124	8
D. ulinei	30	1676.533	186.1	148.933	67.567	1969.533	293.167	73.5	219.667	201.033	177.067	210.333	162.3	1042.467	5.4
D. urceolata	45	1905.089	179.8	146.889	67.689	1751.289	287.933	72.933	215	189.356	168.578	202.756	159.156	1221.289	9.778
D. urophylla	77	142.571	256.766	86.429	71.416	1022.922	322.104	199.039	123.065	257.805	257.117	269.948	244.636	2394.208	31.13

Species	**BIO_13 Precipitation of Wettest Month**	**BIO_15 Precipitation Seasonality**	**BIO_16 Precipitation of Wettest Quarter**	**BIO_17 Precipitation of Driest Quarter**	**BIO_18 Precipitation of Warmest Quarter**	**BIO_19 Precipitation of Coldest Quarter**
	(mm)	(coefficient of variation)	(mm)	(mm)	(mm)	(mm)
D. bartlettii	374.464	59.833	963.572	160.797	498.696	251.261
D. composita	422.32	68.358	1116.147	157.311	550.276	261.78
D. convolvulacea	316.797	81.293	841.232	82.482	412.981	163.071
D. cyphocarpa	223.917	102.833	647.667	14	218.833	22.667
D. densiflora	328.366	69.531	858.389	117.943	438.754	189.771
D. fasciculocongesta	308.909	68.273	805.727	137.091	430.818	148.273
D. floribunda	296.659	71.752	770.458	95.551	415.813	151.691
D. galeottiana	209.554	93.012	582.681	32.729	266.006	44
D. gallegosii	197.586	100.69	569.483	14.448	250.828	27.828
D. gaumeri	193.225	59.825	488.125	89.3	317.825	128.3
D. gomez-pompea	340	88.958	888.958	45.083	373.458	83.458
D. guerrerensis	241.625	101.25	702.875	18.875	273.5	32.625
D. igualamontana	256	105.75	734.5	12.25	231.5	25
D. jaliscana	277.846	106.708	747.123	19.154	564.015	49.015
D. juxtlahuacensis	199.125	93.875	531.625	22.75	318.625	26.125
D. liebmannii	285.032	89.857	753.381	61.492	412.381	128.968
D. longirhiza	202	98	584	21	236	43
D. matagalpensis	266.484	63.137	711.221	103.958	374.432	233.453
D. matudae	350.571	84.143	763	85.286	500	90.286
D. mcvaughnii	274.5	110	676	19.5	454.5	48.5
D. mesoamericana	339	103	834	8	446	26
D. mexicana	385.708	72.316	1005.458	125.014	472.052	370.354
D. millitaris	279.959	101.061	754.673	27.082	553.102	55.531
D. minima	232	97.946	633.162	26.162	301.892	46.432
D. morelosana	207.789	101.158	595.684	17.684	266.474	28
D. multinervis	239.821	95.564	646.513	30.59	420.513	71.795
D. oaxacensis	224.643	84.857	574.357	57.357	347.5	71.286
D. oreodoxa	220	104.667	635	8.333	479.667	31.333
D. pallens	253.687	71.657	668.746	90.985	394.866	110.269
D. palmeri	229.348	105.304	585.783	10.957	421.435	29.522
D. pilosiuscula	197.36	64.942	523.814	87.047	361.605	104.372
D. platycolpata	209.364	102	594.364	17.727	250.909	23.909
D. polygonoides	265.034	81.011	709.977	63.253	370.931	90.322
D. preslii	280.25	97.25	726.5	40.25	433	56.25
D. pringlei	221.9	99.4	617.4	22.1	354.7	35.7
D. pumicicola	200	102	593.667	17	276.333	23.667
D. remotiflora	253.702	103.829	684.983	19.298	410.47	47.823
D. sessiflora	363.5	112	1017.125	19	886.75	54.25
D. sp1	208	98	569	19	300	22
D. sp3	177	103	510	14	232	14
D. sp6	215	106	588	16	221	20
D. spiculiflora	269.19	66.839	706.19	99.234	372.919	184.903
D. subtomentosa	240.558	102.242	652.137	19.063	348.411	36.211
D. sumiderensis	293.846	81.538	760.846	74.462	406.538	113.308
D. tubiperiantha	240	92	655	38	267	52
D. ulinei	235.733	98.6	645.533	24	353.067	38.7
D. urceolata	250.2	95.267	711.244	39.089	315.356	49.311
D. urophylla	390.935	65.74	1018.558	123.143	422.844	614.403

Appendix 3.3. Mean values of leaf functional traits for each species.

Species	Lamina area (cm^2)	LMA ($g\ m^{-2}$)	L:W	Stomatal Density (mm^{-2})	Guard cell length (mm)	A_{max} (µmol $m^{-2}\ s^{-1}$)	g_s at A_{max} (mol $m^{-2}\ s^{-1}$)	WC at full turgor	g_{min} (mmol $m^{-2}\ sec^{-1}$)	Capacitance (Mpa^{-1})	Ψ_{min} (Mpa)
D. barlettii	72.072	52.91	1.870	98.61	0.026	5.253	0.027	0.799	2.013	0.093	-0.938
D. composita	140.345	39.27	1.756	361.11	0.020	6.347	0.047	0.755	0.634	0.039	-1.063
D. convolvulaceae	65.156	35.24	1.294	83.68	0.031	8.772	0.053	0.894	3.372	0.038	-0.721
D. floribunda	51.594	36.82	2.027	150.62	0.025	6.086	0.035	0.760	1.107	0.036	-1.060
D. gomez-pompei	99.236	37.27	1.095	289.58	0.024	4.238	0.019	0.758	1.103	0.053	-0.950
D. guererensis	64.695	41.87	1.068	202.01	0.027	8.82	0.046	0.835	5.417	0.088	-0.961
D. igualamontana	51.248	25.98	1.220	245.83	0.025	8.499	0.069	0.879	3.753	0.009	-0.600
D. matagalpensis	26.471	36.85	1.089	182.50	0.026	3.233	0.016	.	2.159	.	-0.425
D. mexicana	87.696	40.22	1.715	200.69	0.022	7.229	0.042	0.798	1.513	0.044	-0.917
D. morelosana	26.060	27.21	1.143	233.33	0.027	7.841	0.052	0.883	2.684	0.056	-0.836
D. multinervis	13.449	22.80	0.877	108.33	0.027	3.227	0.034	.	4.747	.	-1.150
D. pumicicola	61.522	44.59	1.378	161.11	0.026	8.373	0.057	0.821	2.407	0.105	-1.200
D. remotiflora	73.029	30.27	1.174	239.58	0.022	8.997	0.069	.	2.108	.	-1.075
D. sp1	98.978	35.94	1.379	134.17	0.029	10.683	0.114	0.826	3.213	0.025	-0.850
D. sp3	61.828	37.27	1.282	147.22	0.025	9.96	0.043	0.828	3.829	0.058	-0.967
D. sp6	49.673	31.07	1.270	225.00	0.025	12.74	0.091	0.869	3.253	0.069	-0.884
D. spiculiflora	74.487	43.63	1.254	188.89	0.025	4.59	0.025	0.797	2.802	0.056	-0.896
D. subtomentosa	78.285	27.23	1.246	171.53	0.024	3.46	0.018	0.837	1.058	0.01	-1.338
D. sumiderensis	21.432	28.48	1.054	188.89	0.023	9.704	0.060	0.903	4.326	0.029	-0.650
D. urceolata	9.856	24.88	1.018	227.78	0.023	5.09	0.031	.	3.185	.	-0.650