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Defining Plant Ecological Specialists and Generalists: Building a Framework for Identification and Classification

By

Alex Kirsch

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Defining Ecological Specialists and Generalists: Building a Framework for Identification and Classification

Alex N. Kirsch

This thesis has been examined and approved by the following members of the student's committee.

Advisor

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Abstract

Defining Plant Ecological Specialists and Generalists: Building a Framework for Identification and Classification

This thesis is submitted in two chapters. The first chapter contains background research, literature review, relevant information, and justification for the primary study. The second chapter is written as a standalone paper for submission to *Ecology and Evolution*, that can be read and interpreted independently of the first chapter. Specialization is a widespread but highly ambiguous and context dependent ecological concept. Here, we construct a framework to assess specialization using an objective, metric-based approach, utilizing study system *Quercus*. To create Metric-Based Specialization rankings, metrics related to specialization were gathered for 141 *Quercus* species (Number of distinct inhabited ecoregions, extent of occurrence, plasticity of functional leaf traits, presence of domatia, number of notable documented interspecies interactions), and a model selection process was utilized to determine which were the best predictors of species threat level as determined by the International Union for Conservation of Nature (IUCN Red List) and the results of a specialization survey, where experts familiar with *Quercus* were asked to score species based on their level of ecological specialization. Alignments between Metric-Based Specialization Rankings, *Quercus* experts, and IUCN data show that specialization studies can be standardized, allowing for easier meta-analyses and comparisons across studies. Rankings, and the metrics they are comprised of, were analyzed for evolutionary trends using Phylogenetic Generalized Least Squares, Ancestral Character State Reconstruction, and PhylogeneticEM, for shift detection. Occurrence data were mapped to assess geographic distributions of species. Clustering analyses were performed on mean Metric-Based Specialization Rank and the number of distinct species by ecoregion for the continental United States, Mexico, and Central America, showing clear delineations in Rank across regions. Species tend to rank as more specialized in climates with extreme water availability or precipitation seasonality such as the tip of Florida, California, and Southern Mexico, while Eastern North American species are largely generalist. While the metrics that Metric-Based Rankings are comprised of show evolutionary relationships, sorting by region and environmental factors such as precipitation seasonality (Bioclimatic Variable 15) shows a stronger influence on specialization. Novel use of leaf functional traits extracted from herbarium specimens is shown to be of value to large-scale investigations of a clade. Metric-based systems are shown to be useful tools for bulk identification of at-risk species, and experts are shown to be reliable when evaluating the specialization level of a species, though it seems they largely rely upon native ranges for their determinations. We find that specialization acts as an emergent property of a species' native region, and similar ranking systems should be utilized to better identify and preserve threatened species, especially under conditions of accelerated climate change.

Chapter 1: Background information and Study Justification

Basics of Ecological Specialization and Generalization

All species are thought to exhibit characteristics that would classify them as being a generalist (G) or specialist (S). Many agree that classifying organisms using these designations can reveal useful trends in natural systems (Marvier et al., 2004; Devictor et al., 2008; Büchi and Vuilleumier, 2014;; Poisot et al., 2011; Wilson and Hayek, 2015; Sverdrup-Thygeson et al., 2017; Ramiadantsoa et al., 2018; Fussell et al., 2019; Reed and Tosh, 2019; Zettlemoyer et al., 2019), but there is not well defined consensus on what these designations mean when scrutinized. Broadly speaking, generalist species are species that can tolerate and utilize a relatively wide variety of environments and resources. They achieve this quality by having widely applicable features that are not constrained to a narrow function. This focus does come at a cost, however. In environments where specialists are present, generalists will likely be outcompeted. Ecological specialist species are thought to be opposite of generalists. They possess narrowly evolved features and qualities that optimize their fitness in their local environment, at the cost of vulnerability to disturbance and a high dependence on their niche being available. Specialists arise through natural selection acting on a population that has existed under the same conditions and in the presence of the same community for great lengths of time, over many generations. Generalists are thought to have the advantage when environments change, however (Marvier et al. 2004). The lack of extreme specialization in generalists may mean they can survive by utilizing a wider variety of resources, and a wider variety of conditions. When large shifts in ecosystems come about rapidly, it is likely specialist species are unable to cope with the change and die out or are outcompeted as the ecological role they have evolved for is no longer present.

Problems with Specialization and Generalization in Practice

Generalization and specialization are broad concepts, with many underlying factors contributing to each of them. While it may be easier to think of any given species as being one or the other, it is far more likely that generalization and specialization form a spectrum, with most species falling somewhere in the middle of the extremes; a quality that may be valuable for classifying species.

While G/S classifications may yield powerful opportunities to understand the natural world, their current usage is problematic. Many studies aim to utilize G and S designations to expose patterns, model theoretical communities, and compare species that are closely related, but use differing approaches (Martinů et al. 2015; Fussell et al. 2019; Reed and Tosh 2019). These include but are not limited to computational models and simulations, comparative and metric-based approaches, and classical approaches that aim to classify species in a conceptual manner. The main issue with this is a lack of standardization; most of these studies are not able to be compared with one another, as G and S designations are assigned on an author-to-author basis (Poisot et al. 2011).

Take for example wild ginger (*Asarum canadense L.)*. This plant thrives in shaded, upland forest environments. This species could be described as being an upland forest specialist; specifically adapted to the upland forest environment. However, it could also be categorized as a generalist species that inhabits many kinds of distinctly different upland forests, depending on how narrowly you divide the ecoregions and habitats it resides in. It is exactly this type of variable designation that makes the usage of the designations themselves problematic. While there is *broad* consensus on what constitutes a generalist or specialist, these terms can be ambiguous. This is in part because the degree in which a species is a G or S forms a spectrum that is not binary, dependent on the scale, or biological level that is being considered. However, the variables that lead to a species' designation as a G or S are also observable, and therefore quantifiable. This should allow for the development of an objective, quality-based ranking system. The creation and utilization of this system is covered in Chapter 2 of this document: Building a Framework for Identification and Classification.

Proposed Defining Characteristics of Plant Specialists

Identifying the qualities of specialists is not a new interest; previous studies have aimed to do this, as the potential uses have been recognized. These studies also recognize that specialization works differently for plant life than animal life (Devictor et al. 2010; Poisot et al. 2011; Forister et al. 2012; Sheth et al. 2019). Many scientists have recognized the connection between specialization and a species extinction risk, so improving methods related to the identification of specialists is often seen as a useful way to improve conservation efforts. Additionally, as life strategies, specialization and generalization at the species level may be closely tied to a multitude of other trends. By being better able to rank specialization, these related patterns will be more easily exposed, which may then be used to reinforce the identification system. For example, if a good system of classifying specialists is created, and it is then found that specialists plants all tend to share some common feature or quality, that common feature can then be used as a defining characteristic itself, further improving the system. Factors that are currently understood to be important are outlined below, and bulleted points define how certain characteristics will be represented in the framework.

Habitat Restriction

-Geographic range

- Extent of occurrence

-Community types

- Ecoregion maps imposed onto sample data (Level III)*

-Microsite conditions (Evapotranspiration, Soil pH, Light Levels, Bioclimatic variables)

Specialized Anatomy and Plasticity

-Anatomical tools for reproduction (burrs, samaras, vegetative growth, etc.)

-Defensive structures (Thorns, toxins, touch sensitive leaves, hairs, etc.)

- Domatia presence

-Mechanisms to cope with water stress (Surface area/biomass ratio, lobing, pubescence)

- Plasticity of functional leaf traits

-Regrowth and disturbance adaptations

Interspecies Interactions

-Codependence with other species (For growth, protection, root efficiency)

    - Number of notable interspecies interactions on GloBI (Global Biotic Interactions database)

-Relying on seed distributors (Acorn woodpeckers, rodents)

-Mimicry

-Herbivore density, defensive traits

*Ecoregions as designated by the EPA; these are areas of similar ecosystems. The higher the level, the higher the resolution of the divisions. Maps are publicly available.

What makes a plant specialist or generalist?

Specialists are organisms that are highly evolved for their environmental niche (Forister et al. 2012). They boast specialized traits that allow them to have robust fitness in their environment when utilizing their specialized strategies. They also often inhabit more extreme environments to limit the competition they face even further. For example, plants in the *Droseraceae* family are highly specialized for both insect carnivory to thrive in nutrient poor environments, and to endure the highly acidic conditions that come with them (Bourgeois et al. 2019). While members of the *Droseraceae* family can impressively survive in these harsh conditions, they have become so specialized that they are unable to return to ancestrally inhabited environments.

Habitat Restriction

Unambiguously specialist species are useful in exposing the underlying characteristics of specialization. In the *Droseraceae* example, selection has created populations endemic to uncommon conditions. Throughout literature, most agree that being restricted to conditions that only make up small percentages of land makes you a specialist, so habitat restriction is likely a determining and defining characteristic of specialist plant species. Within this one characteristic there are many factors to consider, such as a population's acceptable range of pH's, temperature ranges, soil compositions, rainfall patterns, and so on. The smaller the range for each of these traits a population can inhabit, the more of a specialist the species must be. For this framework, Ecoregions will be used to represent these factors; inhabiting many different ecoregions is indicative of generalization, while only appearing in a small number of them reflects specialization.

Specialized Anatomy

Apart from the types of habitats and geographic areas a species inhabits, and its susceptibility to changes, there are many anatomical factors that are indicative of specialization; though specialized traits are not always indicative of extreme specialization. Some features are specialized, but still useful in many different scenarios, such as extreme height, or anti-herbivory mechanisms like spines. Spines, for instance, may repel many different herbivores, provide structural support, and may serve as shelter for mutualistic species.

Another anatomically related feature many families of plants possess is specialized seed dispersal methods that only operate under certain conditions, such as wind or water dispersal (Salazar-Tortosa et al. 2019). While these natural mechanisms are widespread, adapting reproductive strategies that rely on them is a specialized strategy. Within the plant kingdom are thousands of specialized mechanisms, each with its own limited use outside of its evolved purpose (Hofhuis et al. 2016; Flores-Abreu et al. 2019; Volkov 2019; Zirondi et al. 2019). In some species patterns of specialization have resulted in very extreme life cycle requirements, such as serotiny, or hibernation periods that occur even if individuals are removed from climates with cold periods. As specialization is further exacerbated, such features can compound. For instance, species that exhibit serotiny may also possess lignotubers. These are belowground growths that act as fire resistant food stores, another feature that can help a plant recover after intense drought, fires, or browsing (Noble 2001). The degree of specification in these mechanisms is directly indicative of specialization. Some mechanisms, such as vertebrate based seed dispersal, are not only examples of specialized anatomy, but of another specialization determining characteristic; interspecies interactions.

Interspecies Interactions

Plants create a wide variety of secondary compounds, fruiting bodies, and mechanical features to utilize animal species in their environment. This allows plants to take advantage of the mobility of animals and greatly increase their ranges, but can lead to a fostered dependence upon seed dispersing species. As a given plant species become increasingly coevolved to specific pollinators and distributors, it becomes more specialized. These relationships can vastly increase the fitness of a species, but can also make them even more vulnerable to change as they are affected by changes to their partners as well. Another example is domatia; some plants produce structures known as domatia, which are specifically produced to house symbiotic arthropods (Agrawal and Karban 1997). Arthropods receive shelter, and will consume insect herbivores that would otherwise consume the host. If the arthropods able to colonize the plants were to disappear from the pair's native environment, the plants would suffer a significant drop in fitness, as their anti-herbivory mechanism has disappeared, and their domatia would become vestigial.

Perhaps the most prominent partnership that plants possess is their association with mycorrhiza; symbiotic fungi that provide a multitude of benefits to the host plant from their roots. (Chen et al. 2005; Roy et al. 2017; Duc and Posta 2018). Upper estimates claim mycorrhizal associations can be observed in 90% of all the worlds plant species, and the fitness advantage they provide to the host makes it clear why this might be. By either associating to the outside of the plant's roots, or boring directly into the host's roots, mycorrhiza greatly increase nutrient acquisition. Other major benefits include pathogen resistance or immunity, and a vastly increased surface area for absorption compared to the root system on its own.

Plants can also rely on other species in an indirect way through mimicry. Mimicry is a phenomenon that is observed when a species evolves the resemblance of another species or other environmental feature. Selective pressures, such as herbivores in the case of plants, then have trouble distinguishing between the mimic and the original feature or species. This results in the mimic gaining any benefits that the original species might have in these interactions, without evolving the tools and mechanisms that led to the resulting behaviors in the first place (Pannell and Farmer 2016). When plants have adapted a strategy that relies upon the presence of another species, they ultimately become dependent on them, even if they are exploiting them rather than having a mutualistic relationship. Take for instance, *Boquila trifoliolata.* This plant is a woody vine that climbs up trees, using their trunks to support themselves. They have also evolved leaves that look indistinguishable from those of their host tree’s, effectively protecting the

Boquila from herbivory (Gianoli and Carrasco-Urra 2014). The vine is extremely dependent upon the trees it mimics, and would likely be highly threatened in the event that the trees were removed from the vines' environment.

From all of this we determine the dependence of plants on partner species must be a determining characteristic of any specialist population. (Salazar-Tortosa et al. 2019)

What makes a Plant Generalist?

As two ends of the same spectrum, generalization and specialization are thought to share many of the same qualities, but in different capacities. The same traits that are critical to specialization determination are often equally important to the determination of generalization, but generalists express these traits at opposite extremes. For instance, habitat restriction; this is also a determining factor of generalization. Generalists are able to withstand a wide variety of habitats compared to specialized groups, as they are less adapted to one particular set of conditions (Devictor et al. 2008); Whereas specialized groups are often affected quite heavily and negatively by disturbances, such as those caused by human activity. Generalist groups can often go unaffected, and even benefit from these events (Marvier et al. 2004). The ability of generalist groups to withstand disturbances can make them successful invaders in the wake of habitat change.

The pattern of generalist populations having characteristics opposite specialist groups holds true for all the previously identified determining characteristics for specialists. Generalists lack highly specialized anatomy, are not constrained by relationships with other species, and are usually not highly threatened under natural conditions. As a foil to specialization, generalists create the other end of the generalization-specialization spectrum.

Phenotypic Plasticity

One thing that generalists may possess that specialists lack is the physiological plasticity that allows them to cope with change. This quality would also help explain why generalists have an easier time invading new environments (Sheth et al. 2019). Plasticity is an organism's ability to alter its phenotype in response to the environment; the ability to initiate a large morphological response to the environment could very much be related to generalists' ability to "adapt faster" (Ackerly et al. 2000). Previous work has shown that *Quercus* species exhibit plasticity in their response to water stress. *Quercus* species will produce a variety of osmolytes in order to adjust their water potential, and their responses are shown to differ across evolutionary groups (***Appendix: Part Three,*** *Contrasting Oaks Responses to Water Stress – Osmolyte Profiling Across Species*) This ability is conferred to the organisms that exhibit it via their genome, and as such, plasticity is a heritable trait that may be more common in generalist species. It is likely this characteristic plays a large part in generalists' ability to accommodate ranges across their range (Dong et al. 2020). Plasticity itself is a metric that can be related to other physiological phenomenon, such as with leaf abscission in oaks (Hernández-Calderón et al. 2013; Firmat et al. 2017). This can be parameterized for the purposes of this project by utilizing observed variation with herbarium specimens for species with multiple representative samples.

$$\textbf{Eq 1. Plasticity} = \frac{(\text{Trait Max} - \text{Trait Min})}{(\text{Trait Max} + \text{Trait Min})}$$

Related but non-defining variables for Plant Specialists and Generalists

Population Size and Range Fluctuations

Range fluctuations can be driven by a variety of influences, that can be ecologically or evolutionarily based, and can have differing implications for the G/S ecology of the relevant species. For instance, if a specialized population starts rapidly increasing its range, is this because the environment it is highly fit for has expanded, or because it has broadened its niche and become more of a generalist? Without even *more* related environmental data, it would be impossible to make this distinction. It would be far more sensible to utilize this framework with respect to the present-day conditions of a species or population. Rankings generated by this framework would ultimately expire once species had evolved significant differences from when they were calculated do to this, however. The problem of range shifts having different underlying causes also reveals another factor that's related to the G/S spectrum but is not viable for determination; phylogenetic relationships.

Phylogenetic Relationships

When one considers the phylogenetic arrangement of generalist and specialist species, some questions and hypotheses come to mind. Logically, one might assume that specialist species would be closely related to other specialist species, and generalists to generalists. Parsimony holds true for many trends in the biological world. Features are likely to have evolved the least number of times within a clade, as it's more reasonable that members of the clade all inherited the trait rather than evolved it separately (Hang et al. 2007).

G/S designations introduce a strange problem regarding inheritance, as they are just as much strategies as they are tangible evolutionary features. Specialists, with their extreme adaptations, suited for highly narrow scenarios, must have evolved their traits from simpler forms. Simpler, less specified forms that belonged to ancestral generalists. For example, the carnivorous members of the *Droseraceae* family could not have evolved their dew-covered leaves if not for the existence of a simple leaf prior. Rather than traits being inherited as speciation occurs like the traditional functional traits, is specialization a strategy that emerges from well-established generalists (Poisot et al. 2011)? At what rate do specialists beget specialists, and generalists begat specialists? Could timeframes related to specialization patterns be identified? Are these rates affected by various selection pressures?

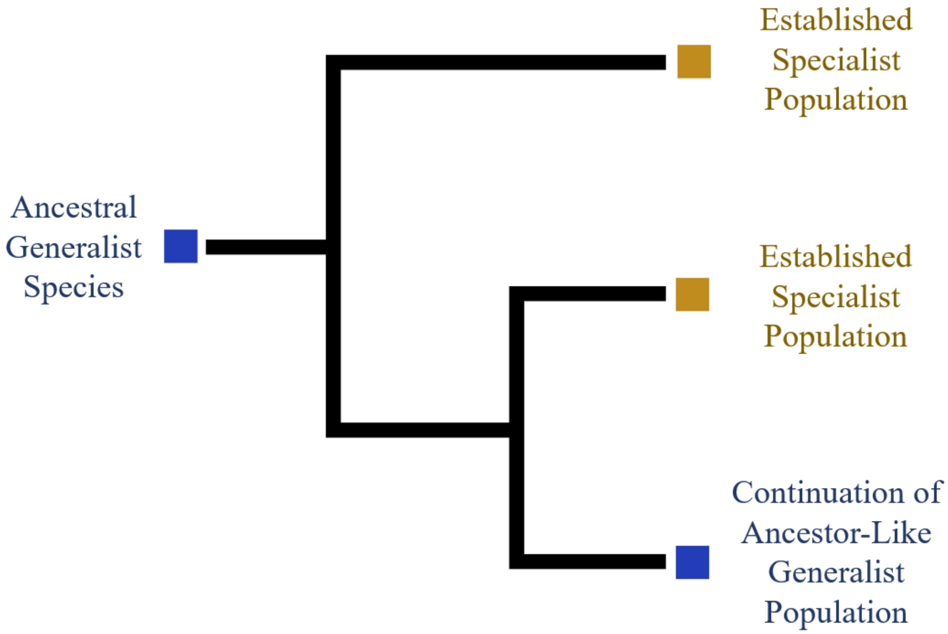

Figure 1. A possible phylogenetic tree representing specialist evolution. Rather than being a conserved trait, specialization may emerge when generalist populations become established in consistent environmental conditions.

Resource Utilization

Certainly, the narrowness of a species' resource usage is a factor in its specialization. In plant systems, this metric is co-occurring with other factors, so we recognize but exclude it. It is often cited in studies that focus on specialization in animals (Poisot et al 2011; Ramiadantsoa et al 2018; Fussell et al. 2019). However, the variety of resource usage is directly tied to two other factors that are already being considered for G/S designation regarding plants, those being habitat restriction and specialized anatomy. The resources an organism can utilize are directly tied to its anatomical features and the environment(s) in which it resides. As such, including resource utilization in G/S calculations would be factoring in what is essentially the same variable twice.

Attempts at Generalist/Specialist Modeling

Using G/S models to define species and predict changes in the environment is not a novel concept. Many research teams have attempted to use these classifications to find patterns, correlations, build ecosystem models, and identify community assembly tendencies among other things (Zahler and Khan 2003; Attum et al. 2006; Devictor et al. 2008; Cavender-Bares et al. 2009; Skopec et al. 2015; Wilson and Hayek 2015; Ramiadantsoa et al. 2018; Zettlemoyer et al.

2019). Researchers have worked from both ends of the problem to try and utilize G/S trends; some teams work on species they have identified as G/S, while others use a theoretical and computational approach where generalist and specialist groups are represented by variables in simulations (Ramiadantsoa et al. 2018).

Using Quercus Species to Model Specialization

In this study (Chapter 2), species in the *Quercus* genus will be used to construct a specialization framework. *Quercus*, more commonly known as oaks, encompasses approximately 455 species of trees (Nixon 1997). These species have spread globally, with representatives inhabiting the Americas, Asia, Europe, and Northern Africa (Hipp et al. 2018). These species can potentially be a useful genus for creating a specialization framework for multiple reasons, including large available data sets, recent publications (Hipp et al. 2018), and an expansive range that occupies multiple continents (Cavender-Bares 2019).

The ranges that oak species occupy span multiple continents. *Quercus* species inhabit a large variety of environment and habitats, meaning that throughout the family different features must have been evolved in order to cope with the stresses that each environment exhibits. They have also diversified into five major sections (large clades), with over 50 million years of separation (Hipp et al. 2018; Kremer and Hipp 2019). This means that the genera likely contains specialized species, as well as species that are more representative of a common ancestor, akin to *Figure 1* above. This will allow for the testing of hypotheses related to how specialization arises. Does specialization arise through inheritance or environmental factors? Do Specialists only give rise to more specialists, or do specialist populations sometimes re-generalize?

Some Hypothesized Quercus Specialists and Generalists

<u>Generalists</u>

-*Quercus michauxii* – has a large extent of occupancy, spans many ecoregions (IUCN)

-*Quercus bicolor* – same as *Q. michauxii* (Clark 1965)

-*Quercus pubescens* – suited to many different microclimates, over large regions of Europe and Asia (Flora Europaea; *Quercus pubescens*)

-*Quercus stellata* – capable of hybridization with many other white oaks (Nixon 1997)

-*Quercus macrocarpa* – has shown plasticity by adopting masting (USDA Forest Service; *Quercus macrocarpa*)

-*Quercus rubra* – has a large North American range and has shown to be successfully cultivated in Europe (European Forest Genetic Resources Programme; *Quercus rubra*)

<u>Specialists</u>

-*Quercus rugosa* – range is closely bound to highland regions of Mexico and the North American South (USDA, Missouri Botanical Garden)

-*Quercus palmeri* – has a shrub growth form, often prioritizes clonal growth (Nixon 1997)

-*Quercus graciliformis* – critically endangered, has an incredibly restricted range, adapted to highly specific microclimate (IUCN)

-*Quercus toumeyi* – highly restricted range, data deficient (IUCN)

-*Quercus douglasii* – extremely drought tolerant, has many relationships with gall wasps (USDA)

-*Quercus brandegeei* – rare, endangered oak of Mexico with a small range threatened by habitat loss (IUCN)

Questions related to specialization in *Quercus*, and the creation of a ranking framework are covered an answered in Chapter 2.

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Chapter 2 - Building a Framework for Identification and Classification

Introduction

Ecological specialization is a widespread concept in biological fields and studies (*Table 1;* Berenbaum 1996). This concept is valuable because specialist species have been shown to be at greater risk of extinction, making their identification critical to conservation efforts (Clavel and Devictor 2011; Poisot et al. 2011; Colles and Prinzing 2009; Dudley et al. 2019). Some systems show no correlations between specialization and threat to a species, but this is often cited as a failure of characterizing specialization due to the variety of concepts it is comprised of (Vasquez and Simberloff 2002). Usage of the relevant terminology has become problematic due to ambiguity and variance, making comparison across studies difficult, and the ideas highly context dependent (Devictor et al. 2010). Other studies have shown that standardizing terminology and definitions is of value (Futuyma and Moreno 1988; Avolio et al 2018).

Attempts to classify, define, and index specialization are numerous and add to the increasing number of definitions assigned to the idea (Ferry-Graham, Bolnick & Wainwright 2002). Within these definitions and systems, it is often not made clear what biological level is being referenced. Species may be considered specialists relative to their clade, but generalists compared to other genera (Devictor et al. 2010). Issues are further compounded by the fact that many of these studies are purely theoretical, and do not attempt to apply concepts to a specific system. Do experts assess generalized and specialized species in a consistent manner? If they do, can we infer which traits experts are utilizing to make these designations? The factors that are cited as determining specialization and generalization are often consistent across related literature when they are mentioned (*Table 1*). These factors are often clear to see when looking at **unambiguously specialist** species.

Table 1. Summary of which factors related to specialization appear in corresponding literature concerned with ecological specialization.

	Appearance of Determining Factors of Specialization in Literature			
	Habitat	**Threat Level**	**Anatomical Features**	**Interspecies Interactions**
Futuyma and Moreno (1988)	**X**	**X**	**X**	**X**
Zahler and Khan (2003)	**X**	**X**	**X**	**X**
Marvier (2004)	**X**	**X**		
Sorensen (2005)			**X**	
Attum (2006)	**X**	**X**	**X**	
Devictor (2008)	**X**	**X**	**X**	
Shipley (2009)	**X**		**X**	
Poisot et al. (2011)	**X**		**X**	**X**
Buchi & Vuilleumier (2014)	**X**		**X**	**X**
Martinu (2015)	**X**		**X**	
Skopec (2015)	**X**		**X**	
Sverdrup-Thygeson (2017)	**X**	**X**	**X**	
Londero (2017)		**X**		
Ramiadantsoa (2018)	**X**	**X**		**X**
Reed and Tosh (2019)	**X**	**X**	**X**	
Zettlemoyer (2019)	**X**	**X**	**X**	

Table 2. Glossary

Unambiguous Specialist	Species with one or more of; a restricted range/low tolerance in habitat diversity, anatomy with narrow usage, a high reliance on or evolution against interspecies interactions, are highly threatened.
Ambiguous Specialist	Species that express some level of the same qualities as unambiguous specialists, but at a more intermediate level, making their designation as specialized subjective.
Generalist	A species with one or more of; a large relative range of tolerable habitats, high phenotypic plasticity, varied resource usage, a high tolerance to disturbance/ability to capitalize on disturbance.
Ecoregion	A major ecosystem defined by distinctive geography, that receives uniform solar radiation and moisture.
Evolutionary Peak	A conceptual tool for understanding how evolved a species is for its strategy; being higher up a peak on the evolutionary landscape represents a species that is more specialized into its niche.
IUCN Designation	How threatened a species is, as evaluated by the IUCN Red List of species. I.e., 'Critically Endangered', 'Least Concern', 'Near Threatened'.
Biological Level	The taxonomic rank being considered. I.e., species to species, genus to genus, order to order.

Consider family *Droseraceae* (Linnaeus, sundews) (Rivadavia et al. 2003; Rodondi et al. 2004; Sudandarini et al. 2007; Gonella et al. 2016). Members are carnivorous, herbaceous plants endemic to bog-like conditions, boasting specialized traits that grant them robust fitness (Thum 1989; Volkova et al 2010). Members of the *Droseraceae* family utilize insect carnivory to thrive in nutrient poor, acidic environments (Jones et al. 2016; Bourgeois et al. 2019). Their environmental preference is so pronounced that increased Nitrogen content is toxic to the point of lethality (Redbo-Tortensson 1994). *Drosera* leaves are also unambiguously specialized, having been modified into an adhesive snare that envelops prey immobilized in the dew-like secretion as a means of prey capture, circumventing nitrogen and phosphorous deficiencies (Thum 1986; Millet and Waldron 2003; Ellison and Gotelli 2009; Naidoo and Heneidak 2013). Unambiguous specialists such as these are valuable for characterizing specialization, as their extreme state makes it clear what factors influence the designation; narrow habitat preference, highly modified anatomy with potentially narrow use, and a high dependance on other members of the same ecological network. They also demonstrate how a species can become restricted to its evolutionary peak, becoming so specialized that evolution into new environmental niches becomes difficult (Wright 1932).

Problems arise when assigning specialist and generalist designations without making the related biological context clear, and when considering species that are *not* blatantly specialized. Ambiguous specialists are exactly that; species that could be considered either a specialist or generalist, depending on how you frame your justification. Consider *Asarum canadense* (Clayton, wild ginger). This plant occupies many kinds of upland forests, but generally will not be found in any habitat that would not be considered some type of upland forest. Is wild ginger a specialist, highly evolved for upland forests, or a generalist that can inhabit multiple habitats with an upland forests' conditions? We can't say without context. This species may have a very narrow set of ecological conditions that it can utilize *relative to its clade,* but might seem quite far reaching compared to *other genera.* In reality, it could be both, and it is more pragmatic to consider specialization and generalization as a spectrum where most species fall somewhere in the middle.

Here we propose a simple and practically applicable system for characterizing specialization within a clade, where specialization and generalization form a spectrum, rather than act as binary designations, sensu Ainsworth and Drake 2020. *Quercus* species are ranked for their level of ecological specialization, and these characterizations are also used to answer questions surrounding specialization. Does colloquial usage of related terminology in the field correlate with metric-based rankings of specialization? What kind of emergence pattern does specialization follow across the *Quercus* genus, if any pattern at all? We hypothesize a pattern similar to that proposed by Holt 2009; “an ancestor with a generalist niche may have spawned specialized descendants”. Or in other words, populations of generalists give rise to specialized descendants once they have expanded into new territories, with relatively different conditions from those the ancestral generalists evolved in, and speciated. We also aim to test if theories suggesting specialists are more threatened by disturbance will be supported by a metric-based system.

Comparisons to Grime’s CSR Triangle

One of the many proposed systems of classifying ecological strategies is Grime’s Competition-Stress-Ruderal (CSR) triangle (Grime 1977). In this system, a triangle represents a species’ strategy, with its position in this triangle representing the trade-offs it has made regarding its ecological strategy. Each axis of the triangle represents one of the three areas where species are forced to make trade-offs, and species that fall close to the tips are exhibiting the extreme form of the related strategy. These three aspects that force trade-offs are the ‘CSR’ of the triangle; C (competition) being how invested a species is into biological competition in less stressful environments, S (stress) being how much a species has invested into tolerating stressful environments, and R (ruderal), how rapidly a species regenerates and propagates in order to cope with environmental disturbances. Calculating ecological strategies using the CSR framework has been shown to be both feasible and of value to related fields (Pierce et al. 2017).

Another way one could interpret Grime’s triangle is that species along the edges and tips of the triangle are more specialized, and species that are more central are more generalized. How our system relates to the CSR triangle is through dimensionality; while position on the triangle is a two-dimensional metric, our system produces one dimensional rankings that make comparative systematics more accessible and practical.

Study System

To demonstrate how a species' degree of specialization can be characterized, here the *Quercus* genus (Manos 2016; Cavender-Bares et al. 2017; Kremer and Hipp 2019) is utilized. *Quercus* represents an ideal system to study specialization (Cavender-Bares 2019), a model clade for integrating ecology and evolution) - the genus *Quercus* is comprised of approximately 455 species of trees (Nixon 1993), some of which boast a stark cosmopolitan distribution while others are found only in very narrow ranges (Manos 2016; Cavender-Bares et al. 2017; Kremer and Hipp 2019). Representatives of *Quercus* tolerate a diverse range of environmental conditions. Species inhabit regions of extreme drought and high-water availability, and areas with mild to severe winters. Oaks are also adaptable when it comes to soil condition, making use of soils that are acidic, alkaline, loamy, sandy, and of high clay content. This large variation in habitat preference, as well as the bulk of existing species, means that this genus likely contains species of varying degrees of specialization. Without differentiation it is unlikely that *Quercus* would be so widespread and prominent, being ecologically dominant in North America and regions of Europe, as well as having representatives in Central America, Asia, and Africa (Cavender-Bares et al. 2018).

This prominence also contributes to making *Quercus* an attractive study system. This genus has a high biological relevance, which makes research done on its species valuable to conservation efforts. Specialist species are thought to be more highly threatened by disturbance and anthropogenic effects (Townsend and Harper 2003), though this is somewhat of a point of contention (Colles et al. 2009; Monks & Burrows 2014). Perhaps more important than this however is that this makes data related to these species abundant, readily available, and diverse (Cavender-Bares et al. 2004; Hipp and Pearse 2012; Cavender-Bares & Kaproth 2016; Kremer 2016; Moreira et al. 2020; Moreira and Abdala-Roberts 2020).

Methods

Ranking Process

To objectively assess specialization, we developed a quantitative ranking system comprised of four metrics representative of it (*Table 3)*, similarly to trait-based approaches used by Ainsworth and Drake 2020 and Morelli et al. 2019. These traits were chosen through a combination of *a priori* research and AICc model selection. Species were assigned points toward specialization based on their value in each metric compared to all other *Quercus* species in this study, and their scores for each metrics were summed to create the final Metric-Based Specialization Ranking (Ranking Generation, *Figure 1).* Some metrics that were collected were not utilized in ranking, having been omitted due to the model selection process outlined below. Rankings were produced for 141 species of oaks; a similar system could be utilized for most clade level investigations. Rankings were tested against IUCN Red List data to look for correlation between specialized and threatened species. Rankings were similarly compared to the results of a specialization survey, where experts familiar with *Quercus* were asked to rank the specialization of species (Model Validation, *Figure 1*).

Table 3. Ranking metrics, how their relationship to specialization-generalization is interpreted, and the source of the related data. All traits below other than <u>*Domatia Prescence*</u>*,* <u>*Leaf Venation*</u>*, and* <u>*Perimeter per unit Leaf Area*</u> *were included in the final AICc Metric-Based Rankings.*

Metric	**Interpretation for Specialization**	**Data Source (and Method)**
Extent of Occurrence (EOO)	The more specialized a species is, the smaller its extent of occurrence should be (specialists have smaller ranges)	Hipp et al. 2018 (ArcMap)
Number of Distinct Inhabited Ecoregions (DE)	The more specialized a species is, the less distinct ecoregions it should inhabit (specialists have more restricted habitats)	Generated using Hipp et al. 2018 samples with Level III ecoregions of North America (ArcMap)
Plasticity; (Petiole Length, Leaf Length, Leaf Lobedness, Specific Leaf Area, <u>Perimeter per unit Leaf Area,</u> <u>Venation</u>,)	More specialized species should be less plastic (lower plasticity is associated with a higher vulnerability to disturbance)	Calculated from Kaproth et al. 2020 (Appendix, Formula 2)
Number of Notable Documented Interspecies Interactions	More specialized species should have a higher number of notable species interactions (specialized species are thought to rely on interspecies relationships)	Global Biotic Interactions Tool 2021
<u>Domatia Prescence</u>	Having domatia is interpreted as making a species more specialized (Domatia represent specialized anatomy in oaks)	Various sources; Kaproth et al. 2020 data archive, SEINet, digitized herbaria, Oaks of the World, and iNaturalist.

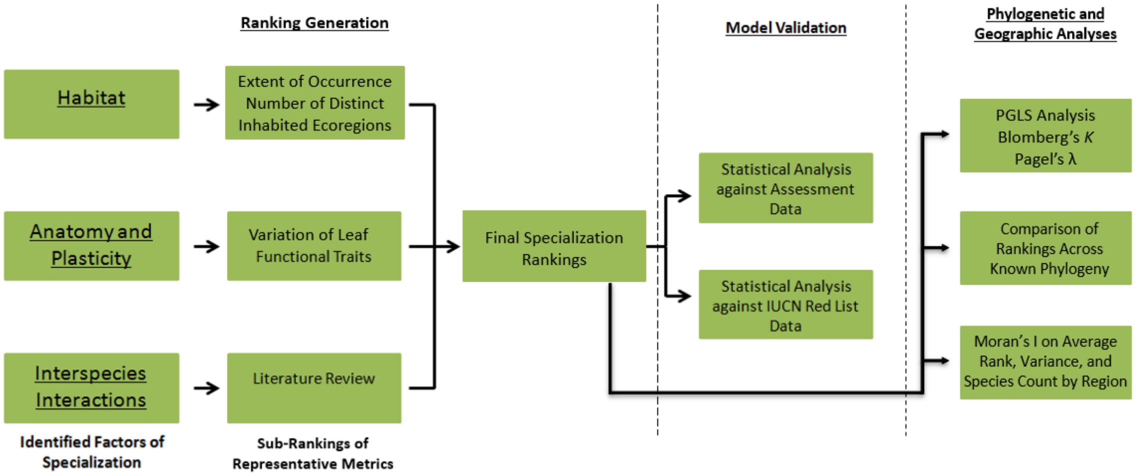

Figure 1. Flowchart of overall methodology. Specialization rankings were generated, validated against two control data sets, and then used for various phylogenetic and geographic analyses.

Metric-Based Specialization Rankings and Percentile Scoring

Metric-Based Specialization Rankings are metric-based, numeric values, with higher values representing higher specialization, and lower values representing higher generalization. Depending on where a species' metric value falls within the range for all species for that metric, it is assigned points towards its final specialization ranking. E.g., species with small ranges relative to other *Quercus* members get more points towards specialization. This was repeated for every metric for all 141 species. The totals of a species' metric scores were combined to produce its final ranking, with each representing 25% of the total. Species that were data deficient for a metric had the weighting of their available metrics adjusted to compensate. For example, a species with only three of the four metrics available would have each metric make up 33% of the total instead. This was done to keep data deficient species on the same scale as fully represented species. How each metric was obtained, the reasoning for its inclusion, and its associated calculations are outlined below.

Model Selection

To independently generate traits for Metric-Based Specialization rankings, an initial literature review and *a priori* metric selection process were utilized. This yielded more metrics than those that were included in the final rankings of specialization (*Table 3*). Akaike Information Criterion (AICc) was used to compare models for predicting both IUCN red list designation, and average expert survey score. Metrics that appeared in one of the most optimized predictive models for these two data sets were used in ranking generation. All gathered metrics were utilized except for Plasticity of Perimeter per unit Leaf Area, Plasticity of Leaf Venation, and Domatia Prescence (*Table 3*). More on domatia is available in the appendix: part one. The resulting model has metrics that represent ecological, physiological, and geographical data. A stronger predictive model with a ~3% lower AICc value is possible, but this model contains only plasticity metrics (Plasticity of Leaf Lobedness, Plasticity of Specific Leaf Area, Plasticity of Petiole Length) and is therefore no longer representative of overall specialization. This may also suggest that plasticity is the largest determining factor of specialization or generalization. Without the inclusion of other data types, we lose the ability to make inferences about patterns in specialization and scientific assessment of the concept. These results were validated through phylogenetic generalized least squares (PGLS) models in R version 4.0.4 (R Core Development Team 2019) with packages APE v.5.4.1 (Paradis & Schliep 2018), MAGRITTR v.2.0.1 (Bache & Wickham 2014), NLME v.3.1.152 (Pinheiro et al. 2020), and PHYTOOLS v.0.7.80 (Revell 2012) in comparison with AICc selection outputs from JMP (Version 15.1.0). The phylogeny utilized for the PGLS analyses was a trimmed version of the

Hipp 2018 phylogeny, with data deficient species being dropped from the tree. The resulting phylogeny had 91 species at the tips.

Specialization Survey

To create a comparative dataset of *Quercus* specialization, experts familiar with *Quercus* species were asked rank species based on how specialized they felt they were, and to define specialization and generalization to aid in synthesizing less ambiguous meanings for the terms (***Appendix Part 1, Survey Sample***). Metric-based data was compared to these results to gain insights on the consistency of specialization evaluation from experts familiar with the species studied. These results were also analyzed to determine what factors experts were using in their designations of specialization (or lack thereof). Surveys were sent via email to 42 respondents across multiple regions, representative of the locales the relevant *Quercus* species are native to. Twenty-six respondents completed the survey for an average of 3.8 responses per species. Survey respondents showed a variety of backgrounds and occupations, with many coming from academia, arboretums, herbariums, and other groups that work closely with *Quercus* species. Survey respondents were identified through relevant literature and online sources connected to the aforementioned institutions.

-Extent of Occurrence

A species' extent of occurrence (EOO) is defined as the area contained within the shortest continuous imaginary boundary that can be drawn to encompass all the known and inferred sites of present occurrence of a taxon, excluding obvious cases of vagrancy (Guidelines for assessing the conservation status of native species, Environment and Biodiversity Conservation Act of 1999). EOO was calculated from 150,886 *Quercus* samples from Hipp et al. 2018. Values were calculated using ArcMap following the guidelines set forth by the Threatened Species Scientific Committee; a diagram is provided in the appendix (***Appendix Part 1, Figure A***). The top 20th percentile species were assessed as having max EOO scoring due to the logarithmic distribution of values (*Figure 2)*. These 29 species were assigned zero points towards specialization from EOO; the remaining scores were calculated using ***Formula 1*** (***Appendix Part 1***).

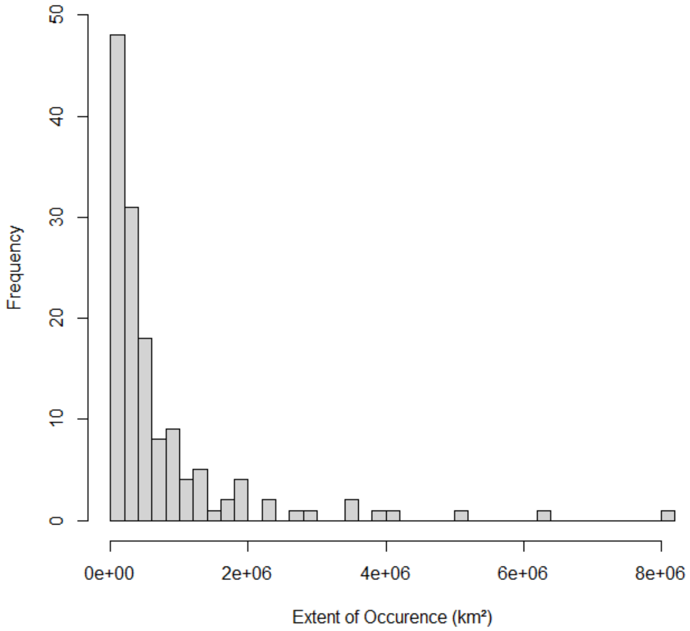

Figure 2. Histogram of Extent of Occurrence (EOO) of 141 Quercus species. 92.2% of these species have an Extent of Occurrence below 2,000,000km².

-Number of Distinct Inhabited Ecoregions

Ecoregions are geographic areas where ecosystems and environmental resources are generally similar (Omernik 1987). Ecoregions come at different levels of resolution, with higher levels having more subdivisions. Level I divides North America into 15 ecoregions, while level III defines 182. Central and South America, as well as the Caribbean, are broken into 12 regions at level I, and 121 regions at level III. Level III is used throughout this study, as the level of detail is the highest without favoring certain regions over others. Additionally, at lower levels, many *Quercus* species would only inhabit one ecoregion even when their range sizes differ greatly, which would make using it as a differentiating metric unfeasible. The methods used to define ecoregions are given in Omernik (1995, 2004), and Omernik and Griffith (2014). Here, the number of ecoregions a species occurs in is used as a measure of niche breadth and a species' ability to utilize a variety of resources and conditions.

The Hipp et al. 2018 dataset includes 877±2385 (SD) unique presence records per species for each of 137 species. Thirteen species had 10 or fewer records, while 98 had at least 50 (Hipp et al. 2018). ArcMap was used to map level III ecoregions with presence records overlain. This was used to produce a dataset of what ecoregion each sample occurred in. Processing in R produced a count of distinct ecoregions inhabited per species (DEL3, distinct ecoregions at level III). Inhabiting a lower number of ecoregions was interpreted as meaning a species is more specialized. Scoring for DEL3 also done using ***Formula 1***. DEL3 ranged from a high of 59 distinct regions (*Q. rubra*) to a low of 1 (10 species).

-Plasticity

Plasticity is often cited as a useful quality for species persisting in unpredictable environments and invaders alike (Marvier 2004). Specialized species are thought to lack plasticity at both an individual and evolutionary level, which likely contributes to their susceptibility to disturbance. Here, our approach utilizes novel use of herbarium data to investigate patterns in a large clade. We represent plasticity as variation in four functional leaf traits, calculated from samples used in Kaproth et al. 2020 with ***Formula 2***. Species had an average of 14±12 (SD) individuals, with 115 of 136 species having more than 3 samples. These individuals are a mix of field samples and items from herbarium collections. Plasticity traits tend to correlate highly with other plasticity traits (***Appendix Part 1, Figure B***), or in other words, species that are plastic for one leaf trait are usually plastic for many traits.

-Interspecies Interactions

Many species globally are specialized by virtue of ecological and evolutionary relationships with other species. This includes both mutualists and symbiotes as well as species highly adapted to defend against other species. Here, specialization via interspecies interactions is represented by the presence and quantity of known interactions on the Global Biotic Interactions tool. More on this tool and its usage is included in the appendix.

Model Validation and Testing Consistency of Ecological Concepts

To validate the model, associations among the three models of species designation were tested (*Figure 3*): Metric-Based Specialization Rankings and IUCN designations, specialization rankings and survey results, and survey results and IUCN designations. This also allowed us to detect any potential inconsistencies between metric-based rankings, scientific discourse, and conservation efforts.

Phylogenetic Methods

To investigate the relationship between evolutionary history and specialization, metrics related to the concept of specialization were tested across the *Quercus* phylogeny for Blomberg's K (to analyze differences in specialization across clades) and Pagel's Lambda (to test covariance across the tips of the phylogeny). The metrics utilized were Extent of Occurrence (EOO), Number of Distinct Inhabited ecoregions at ecoregion level III (DEL3), Plasticity of four functional leaf traits (Petiole Length, Leaf Length, Leaf Lobedness, Specific Leaf Area), and the

Number of Notable Documented Interspecies Interactions (*Table 3*). Tests for phylogenetic signal were performed using the R package PHYLOSIGNAL v.1.3 (Keck et al. 2016). R was used to generate all phylogenetic figures (***Appendix Part 2***). An Ancestral Character State Reconstruction was also performed using the ape package, to infer ancestral conditions of specialization using our Metric-Based Specialization Rankings for modern species, and to further explore evolutionary trends (Irisarri and Zardoya 2013) (*Figure 5*). We also tested for phylogenetic shifts in Metric-Based Specialization Rankings and individual ranking metrics by evaluating the relative support for alternative Ornstein-Uhlenbeck (O-U) models. This process models transitions in trait values as responses to shifting selective regimes (Bastide et al. 2018; McCormack et al. 2020). The analysis was performed using an Expectation Maximization (EM) search algorithm (Bastide et al 2018) over the space of 10 transitions, with the O-U model for independent traits. Phylogenetic shift testing was performed using the R package PHYLOGENETICEM v.1.4.0 (Bastide et al. 2017).

Geographic Methods

Clustering of specialization, variance of specialization by ecoregion, and the number of distinct species per ecoregion were plotted in ArcMap (10.8.1) and analyzed using a Moran's I (*Figures 6, 7)*. The mean Metric-Based Specialization Ranking in each region was determined as the mean specialization value of distinct species that appeared in said region; multiple occurrences of the same species did not count multiple times for these calculations. I.e., a region with hundreds of individuals of one particular species wouldn't have that species' specialization ranking factored into the average more than one time. PGLS analyses were also performed between Metric-Based Specialization Rankings and two metrics related to water availability (*Figure 8*). Species mean environmental traits for precipitation seasonality (bioclimatic variable

15; Hijmans et al., 2005) and $I_m GS$ (growing season Moisture Index, Kaproth and Cavender-Bares 2016). These analyses were performed using the Generalized Least Squares: Fit Linear Model Using Generalized Least Squares function (gls) of the NLME package in R, using a Maximum-Likelihood method.

Results

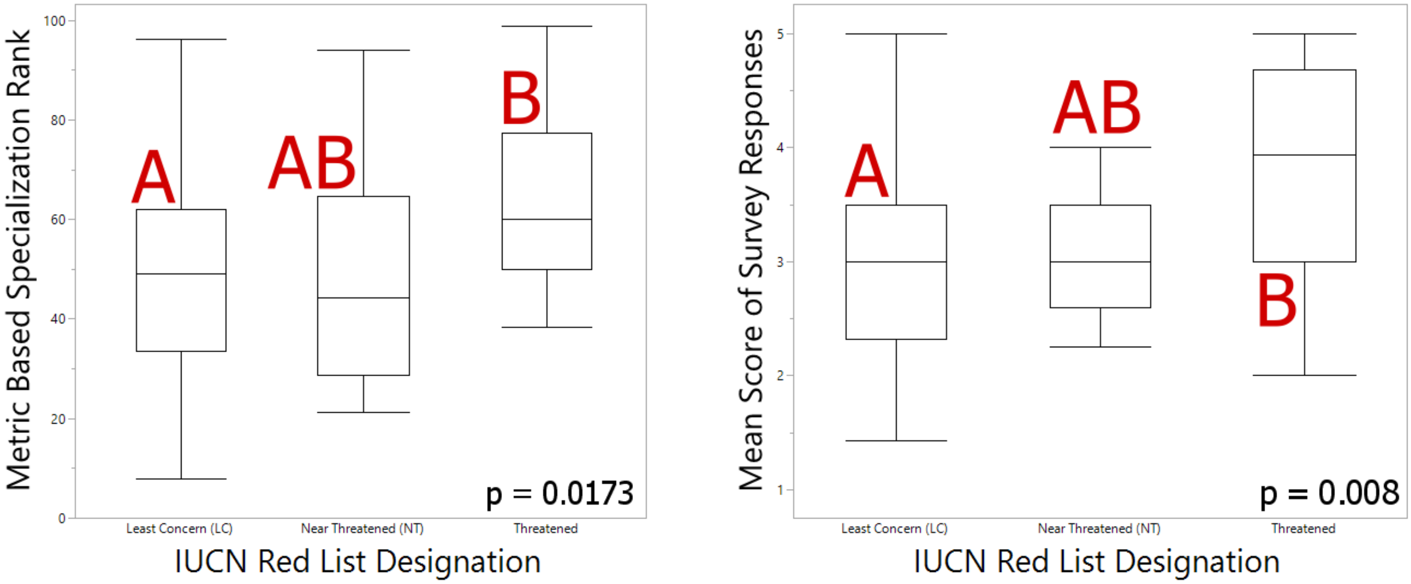

Figure 3. Results of one-way ANOVA tests between IUCN red list designations, Metric-Based Specialization Rankings, and survey scores. Displayed with Tukey-Kramer connecting letters reports beside the corresponding boxplots.

Correlations between Metric-Based Specialization Rankings, Specialization Survey Results, and IUCN Red List Designations

Metric-Based Specialization Rankings, the results of the specialization survey, and IUCN red list designations all significantly and positively correlate with one another. A paired *t-test* between survey data and ranking data also reveals that while the two datasets correlate, experts tend to rank species about 14% higher, or more specialized on average ($p > t = <0.0001$, Mean Difference = 13.6, t-Ratio = 7.54). Expert survey responses aligned most with Extent of Occurrence ($p = 0.0005$, $R^2 = 0.10$). The survey data and IUCN designations tested significantly with a one-way ANOVA ($p = 0.0089$), and a Tukey-Kramer connecting letters report revealed that while species of least concern (LC) differed significantly from those that were threatened (Any designation more severe than Near Threatened, NT), species that were near threatened could not be determined to significantly differ from either the species of least concern, or those that were threatened. Compared to species of least concern, experts scored near threatened species as 1% less specialized on average, and threatened species 29.1% more specialized on average. When comparing the Metric-Based Specialization Rankings and the IUCN data, also with a one-way ANOVA, the relationship was also significant, and the connecting letters report exhibited the same pattern ($p = 0.0373$); these results are shown graphically in ***Appendix Figure D and E***. When compared to species of least concern, near threatened species were ranked as 3.3% more specialized on average, and threatened species were ranked 35.5% more specialized on average. While there was some overlap between the metrics utilized by the IUCN red listing process and the Metric-Based Specialization Rankings, namely in Extent of Occurrence, AICc model selection revealed that overlapping metrics were not favored as predictive variables.

Table 4. Phylogenetic Signal of Specialization Ranks and each factor of specialization individually (Blomberg et al.'s K and Pagel's Lambda). Total plasticity is the sum of plasticity in the six traits that were measured for plasticity; values for individual leaf traits are in their own table in the appendix (***Appendix Part 1, Table 1***). Asterisks* denote significance. Methodology for these tests can be found under the Phylogenetic Methods subheading in the Methods section. Phylogenetic Shifts were only detected for species extent of occurrence (***Appendix Part 1, Figure C***).

Character	***K***	**p**	**λ**	**p**
Specialization Rank	0.132514	0.013*	0.472912	<0.001*
Extent of Occurrence	0.14894	0.070	0.412588	<0.001*
Distinct Ecoregions at Level III	0.204776	0.001*	0.832833	<0.001*
Total Domatia Score	0.129575	0.017*	0.421461	<0.001*
Number of Interspecies Interactions	0.0675066	0.962	0.000067	1
Total Plasticity	0.175	0.003*	0.279	0.002*

Phylogenetic Signals

Tests for phylogenetic signal (Blomberg's K and Pagel's Lambda) yielded significant results for all the metrics considered for Metric-Based Specialization Rankings, with the exceptions of Number of Notable Documented Interspecies Interactions, which did not test significantly for either of the two signals, and Blomberg's K for EOO. The metric-based rankings themselves also tested significantly for both signals. Overall, values of K and lambda suggest moderate phylogenetic influence, and high signal for covariance across tips.

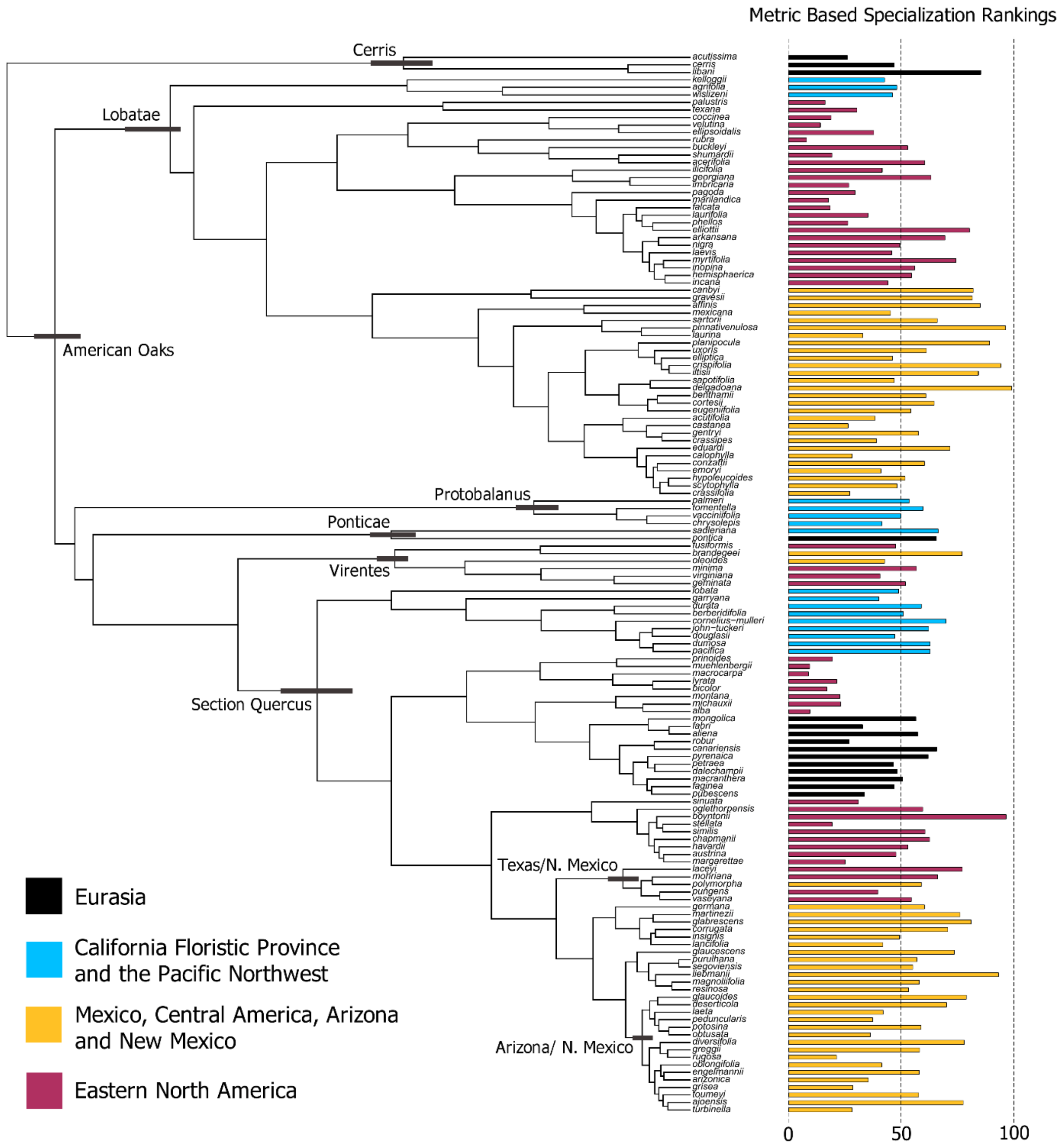

Figure 4. Specialization rank and region of *Quercus* species across *Quercus* phylogeny (141 species). Major groups are defined at their respective nodes, and the length of the grey bars indicate the relative uncertainty in dating. Longer bars at the tips of the tree represent higher specialization (above 50), while short bars indicate more generalized species (below 50). Color of the bar corresponds to the region a species is native to.

Metric-Based Specialization Rankings, the Quercus Phylogeny, and Native Regions

Within the *Quercus* phylogeny, there are a few visible patterns with respect to Metric-Based Specialization Rankings and the region a species is native to. Perhaps the most striking of which is exhibited by the clade of Eastern North American (ENA) species within Section Quercus spanning *Q. prinoides* to *Q. alba*. This clade contains some of the most generalized species in the study, with rankings ranging from 8.85 (*Q. macrocarpa*) to 23.0 (*Q. michauxii*), with a mean ranking of 16.2 ± 2.2. Sister taxa of this clade that inhabit the California Floristic Province and Pacific Northwest (CFPN) show a slight bias towards specialization, with a mean ranking of 55.9 ± 3.2. Sister taxa native to Eurasia lack a clear bias towards specialization or generalization, with a mean ranking of 47.8 ±3.8. Overall, the rankings of natives of the CFPN and Eurasia tend towards the midpoint of 50. *Quercus libani* (Eurasian native, ranking of 85.2) is the sole deviant from the pattern seen in species of the CFPN and Eurasia. This is likely due to data deficiency however, as *Q. libani* only had representative metrics for EOO and DEL3; additionally, *Q. libani* is a species of least concern (LC) as determined by the IUCN red list of species, further supporting that the anomalous ranking is explained by data deficiency. Of the 104 species with all metrics available, *Q. myrtifolia* scored the highest, at 74.3 points, and *Q. rubra* scored the lowest, at 7.99 points.

Eastern North American natives that are more closely related to natives of Mexico, Central America, Arizona and New Mexico (MCAN), are more prone to higher specialization (*Q. elliottii - 80.1, Q. boyntonii – 96.5, Q. laceyi – 77.1*). Natives of MCAN show the greatest propensity for specialization, with a mean ranking of 58.7±2.6 . Even the most highly generalized representative from this group, *Q. rugosa*, scored 21.2; over twice as high as some of the most generalized representatives of Eastern North America. Despite this, the MCAN region also contains many generalists as well, albeit a large majority of which are not as highly generalized as those seen in ENA. Twenty-two of 59 species from the MCAN region are generalized, or 37.3% of MCAN natives.

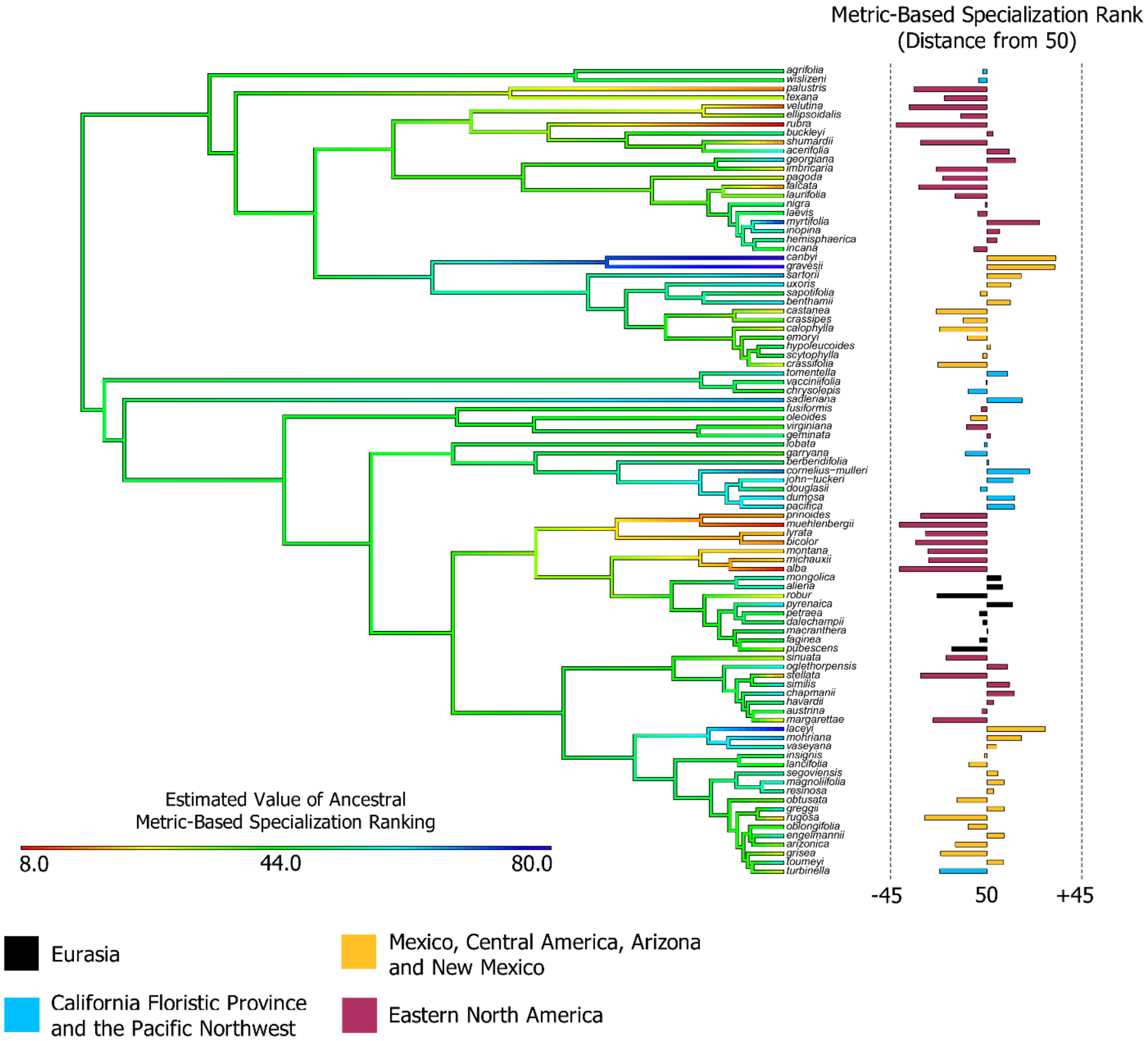

Figure 5. Ancestral state reconstruction of Metric-Based Specialization Rank across *Quercus* phylogeny (91 species). Bars at the tips represent Metric-Based Specialization Rank as a distance from 50 (the midpoint of possible rankings). Bars that extend right are species ranked as specialized, while bars extending left are more generalized; Bars are color coded according to region. The branches of the tree are color coded by the estimated Metric-Based Specialization Ranking of ancestors.

Ancestral Character State Reconstruction

Across the *Quercus* phylogeny, there are some clades of highly generalized or specialized species. The most generalized groups are seen in Eastern North American (ENA) species, such as the species from *Q. prinoides* through *Q. alba* (*Quercus* subsections *Prinoideae and Albae* (Trel.) A. Camus), and *Q. palustris* though *Q. laurifolia* (*Quercus* subsections *Phellos, Coccineae,* and *Palustres*). The most specialized species are almost exclusively natives of Mexico, Central America, Arizona and New Mexico (MCAN), such as *Q. laceyi* (rank of 77.1), *Q. mohriana* (rank of 66.1), and *Q. canbyi* through *Q. uxoris* (ranks from 61.8 to 81.9, Lobatae and *Quercus* subsection Erythromexicana). Some highly specialized species that are not native to the MCAN region include *Q. myrtifolia*, an Eastern North American native (rank of 74.3), and *Q. cornelius-mulleri* through *Q. pacifica* (*Quercus* subsects *Dumosae* and *Prinoideae*), natives of the California Floristic Province and the Pacific Northwest (CFPN) (ranks from 62.8 to 69.8). The overall pattern in the entire measured clade is one of moderate generalization, with most ancestors beyond one node being estimated to have a specialization ranking of about 44.

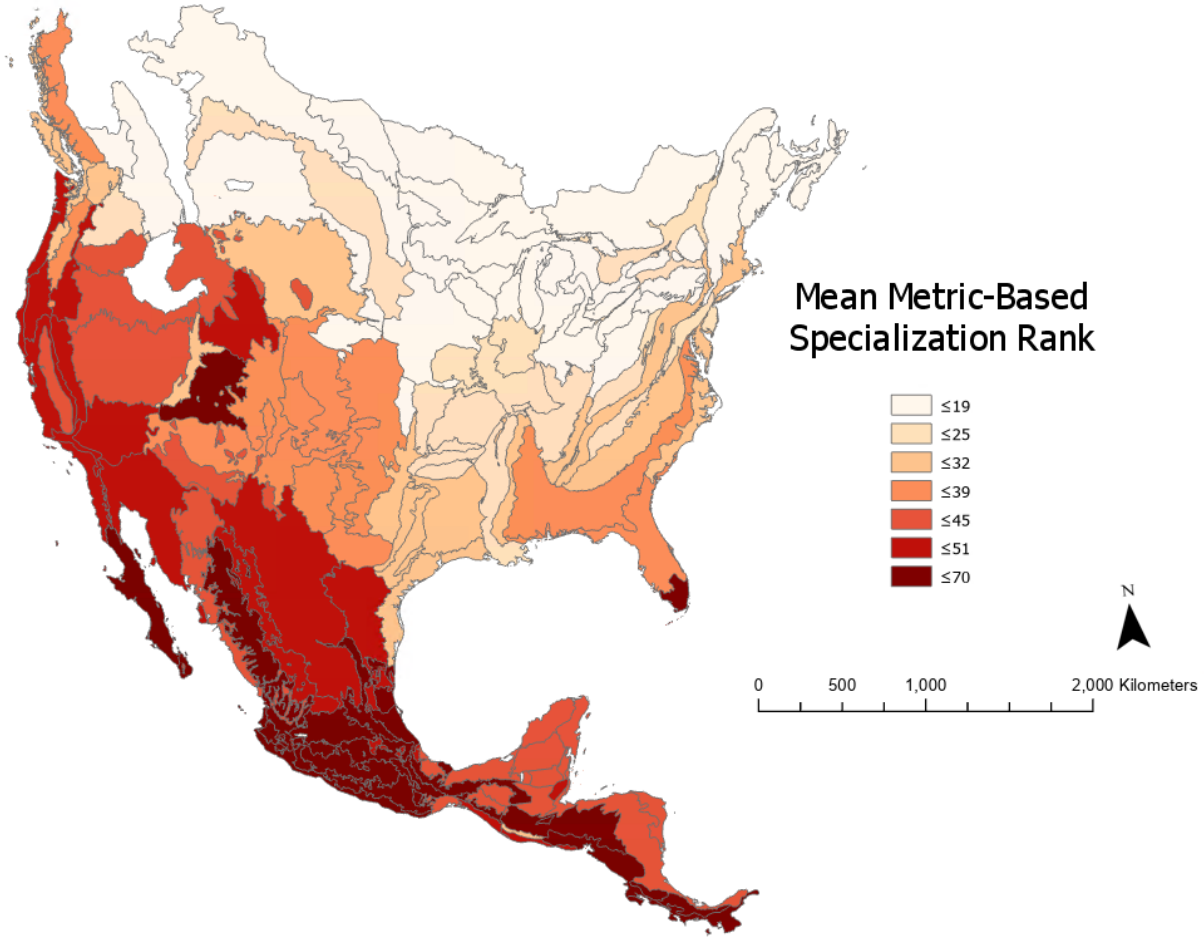

Figure 6. Mean Metric-Based Specialization Rank of species within each ecoregion across the continental United States through Mexico and Central America.

Mean Metric-Based Specialization Rankings by Ecoregion

The mean specialization ranks of ecoregions are significantly clustered (Moran's I, p <0.0001), with a pattern of increasing specialization in regions at lower latitudes. Examples can be seen in the Southern Florida Coastal Plain, the U.S. west coast, and regions spanning central and northern Mexico. North of the United States Northern Border, mean specialization is always generalist dominated; mean specialization rank at latitudes this far north surpasses 25 in only one region, the Pacific and Nass Ranges of the Canadian western border (mean rank < 39).

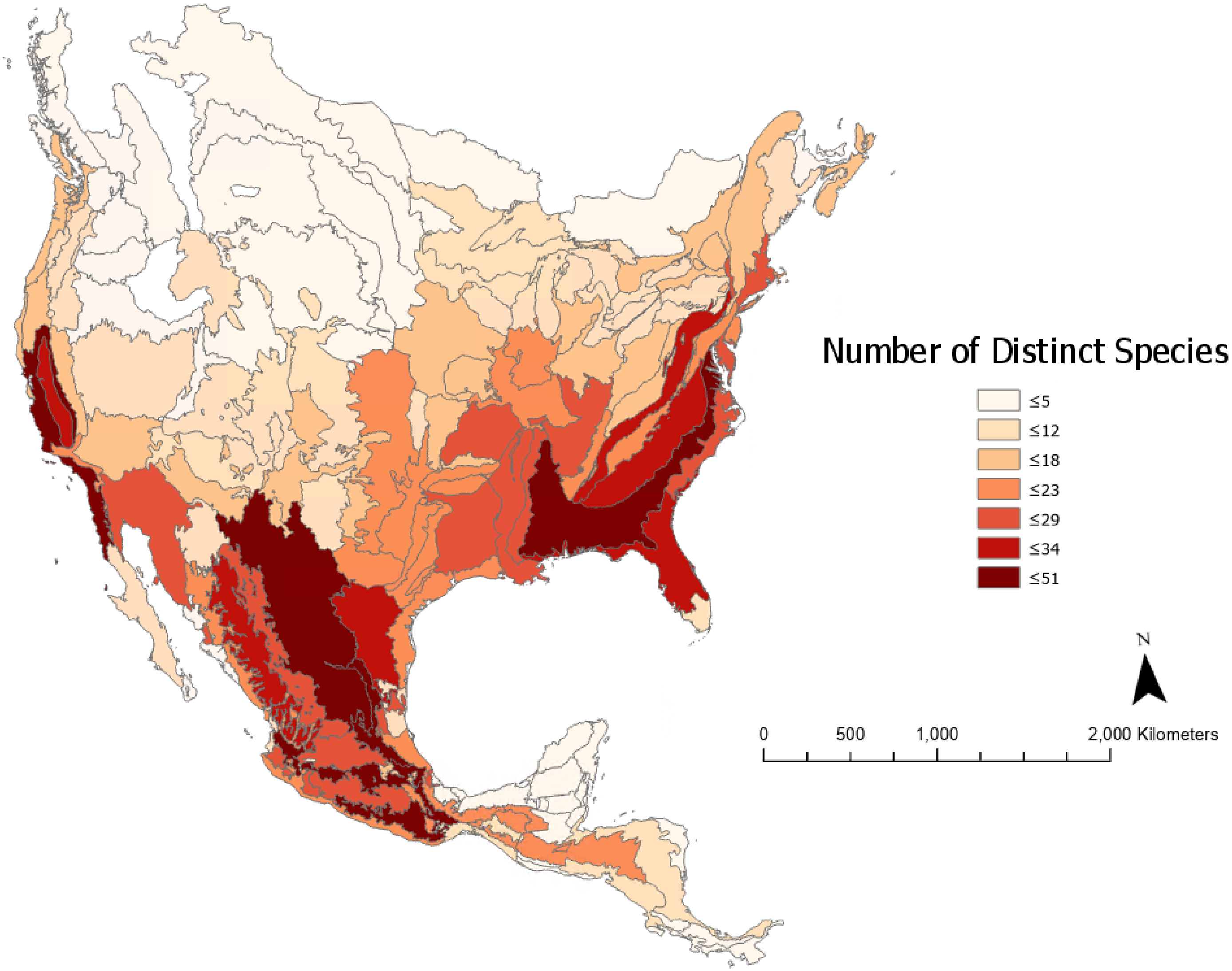

Figure 7. The number of distinct species inhabiting the ecoregions of the United States, Mexico, and Central America.

Quercus Species Diversity by Ecoregion

Quercus species diversity is highest in the southeast United States, the United States west coast and large ranges in Mexico; the number of distinct species is significantly clustered (Moran's I, p<0.0001; Figure 7). Species diversity also tends to be higher in transitional regions that exist between specialist and generalist dominated regions, that likely contain areas that can accommodate both strategies, such as the Southeastern Plains. This region spans states bordering the gulf such as Mississippi, Alabama, and Florida, up through much of the U.S. east coast to states like Virginia and North Carolina. Ecoregions in the Intermountain West show low species diversity, with most ecoregions containing less than 12 distinct species.

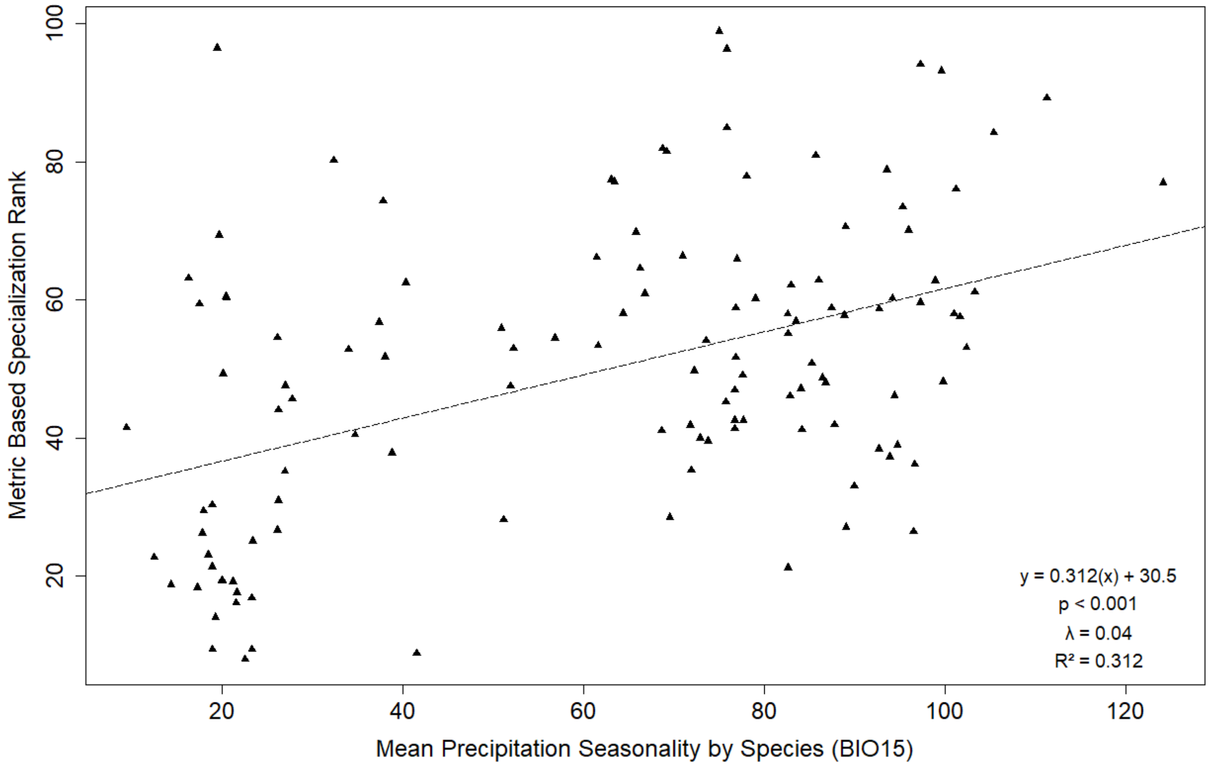

Figure 8. Scatterplot of Metric-Based Specialization Rankings versus Mean Precipitation Seasonality by Species (Bioclimatic Variable 15).

Specialization and Precipitation Seasonality

To determine which environmental factors are related to specialization, the relationships between Metric-Based Specialization Rankings and the mean precipitation seasonality by species, and Metric-Based Specialization Rankings and Index of Moisture were analyzed. These factors were chosen as they have been shown to be ecologically relevant in prior work (McCormack et al. 2020). On average, a species with a mean precipitation seasonality of 10 would likely have half the specialization score of a species with a seasonality of 120; as seasonality increases, so do metric based rankings of specialization. Metric-Based Specialization Rankings and I_mGS (growing season Moisture Index, *sensu* Kaproth and Cavender-Bares 2016) are not significantly correlated, however.

Discussion

Viability of Metric-Based Ranking Systems

Specialization in *Quercus* presents interesting insights into specialization as a whole. The first of which is that creating a practical, objective ranking system of specialization is indeed possible. The ability to assess specialization in bulk could be of great use in studies concerning themselves with a large number of related taxa (Reece et al. 2013; Mounce et al. 2018; Catenazzi and von May 2021). The resulting rankings provide a good basis of comparison for specialization, and by extension threat level, which has utility for conservationists and scientists alike (Todd and Burgman 1998; Reece et al. 2013; Schröter et al. 2017). The resulting Metric-Based Rankings can also be assumed to be accurate designations of specialization, given significant correlations to IUCN red listing designations and scientific assessment (*Figure 3).* Additionally, results tell us that experts are, on average, good at picking out specialized and generalized species, even if they tend to rate species as slightly more specialized than a metric-based system would suggest (*Figure 3)*. Correlation between survey responses and Extent of Occurrence indicate that experts may be using range sizes as a proxy for specialization.

Of the 11 species with only two available metrics to be considered, two scored in the seventies, four in the eighties, and five in the nineties, indicating some bias in our model towards data deficient species. Results also suggest some correlation between data deficiency and threat level, so the omission of species lacking some metrics is not necessarily ideal; of the 11 species ranked highly due to missing data, four are threatened in some capacity, including the only critically endangered species in this study, *Q. boyntonii*. 36% of the 11 highly ranked data deficient species were threatened, while only 10.6% of the 141 species ranked were threatened. Deficiencies in data may be indicative of threat level for a variety of reasons (Todd and Burgman 1998; Howard and Bickford 2014). Species lacking information tend to be less widespread, understudied, and are potentially harder to access. There is some work that has found contrary patterns in other groups, however this may be attributed to differences between genera (Luiz et al. 2016).

Given the patterns regarding data deficiencies described above, our framework suggests more data deficient species should be prioritized in conservation efforts, as they are more likely specialized compared to their well represented relatives. Results in *Figure 3* also suggest that scientific literature that contains specialists and generalist species (as decided by the authors) may be reliable regarding these designations, even if there is a lack of methodology or biological context provided. Results would suggest some level of unity amongst scientific designation of specialization, and suggest that there are potentially common things experts are identifying and using in their designations, such as range size. In cases with few species, assigning specialization rankings can be accomplished through expert evaluation of the study system. However, a metric-based approach may provide an advantage when the number of species of interest gets increasingly large. Regardless of the approach utilized, both systems are potentially useful means of identifying threatened species (*Figure 3*).

Evolutionary and Geographic Patterns of Specialization in Quercus

Specialization, and the metrics used herein to represent it, yield insightful phylogenetic and geographic trends. Every trait used to calculate specialization rankings, with the sole exception of the number of interspecies interactions were recorded for a species, tested significantly for both Blomberg's K and Pagel's Lambda (λ) (*Table 4*). Overall, values for K and λ indicate a relatively low phylogenetic signal in specialization and its representative metrics. λ values range from between 0.27 and 0.83, and suggest that while phylogenetic relationships play a role in the determination of specialization, species are being influenced by other factors apart from relatedness (Pearse et al. 2012; Li et al. 2016). Low *K* values ranging from 0.1 to 0.2 indicate that differences between clades are not as pronounced as would be expected under Brownian Motion (Lessard-Therrien et al. 2014; Li et al. 2016).

Interestingly, the number of distinct ecoregions a species inhabits shows the highest phylogenetic influence (λ 0.833), surpassing even plasticity traits, which are likely genetically controlled to some degree. This is potentially explained by more closely related species inhabiting similar ranges of comparable size (Manos and Hipp 2020). This is supported at large scales by Cavender-Bares 2019, but is contrary to the pattern found at small scales in the same publication. Biodiversity richness patterns could also be explained by differences in environmental heterogeneity between Eastern North America and Mexico/Central America. The region a species occupies would appear to be just as if not more influential on overall specialization than phylogenetic relationships (*Figure 6, Figure 7*), similar to thinking in publications such as Guttová et al. 2019, where specialization is assumed to be entirely a product of the environment. The mean specialization ranks of ecoregions are also significantly clustered (Moran's I, $p < 0.0001$) (*Figure 6*). These results support our initial hypothesis that expanding generalist populations radiate into new regions, and specialize into open niches unoccupied in those regions, similar to the process of allopatric speciation (Nosil and Rundle 2009). Regions with high concentrations of specialists have increasingly extreme water availability compared to the distributions of ancestral North American oaks, namely those in Eastern North America. Mexico, Central America, the U.S. West Coast and the U.S. Southeast all tend to specialize the local oaks more heavily than Eastern North America, likely due to a mix of harsh conditions that prove challenging for more generalized species; namely the extremes of water availability (Ramírez et al. 2020). This is supported by results shown in *Figure 8*; specialization is significantly correlated to water seasonality, with little influence from phylogenetic relationships ($\lambda = 0.04$).

Overall, specialization and generalization appear to be heavily controlled by the geographic region a species is native to, and as such specialization tends to act as more of an emergent property of a place rather than a more typical inherited trait (Küttner et al. 2014). This is not too surprising, as specialization falls somewhere between an ecological strategy and a relative physiological state. It is useful to note however that there are differences between Mexico/Central America (MCAN) and Eastern North America (ENA) regarding ecoregions; Mexico is more heavily dissected at all ecoregion levels, while the differences in ENA are drastic between levels. This may have led to differential ranking potential for ENA species, as they have disproportionately more regions available as the ecoregion level increases. The MCAN region contains a relatively large number of species, and while many of them are highly specialized (36 species), this region also contains generalists as well (22 species). Appearance of generalists in MCAN could possibly be attributed to the fact that this region contains a great variety of environmental conditions, that are driving speciation in an equally diverse manner.

Despite specialization appearing to be dictated largely by geographic influences, phylogenetic relationships also clearly play a part in specialization, as evidenced by our findings and other literature (Cooper and Lenski 2000; Ballabeni et al. 2003). Within clades and sections of *Quercus*, trends of specialization often tend to be preserved and clustered, as evidenced by *Figure 4.* This could potentially be due in part to the fact that more related species sometimes tend to be closer together geographically, as shown by other studies (Beaudrot et al. 2014; Hipp et al. 2018); but this may not always be the case for *Quercus* species (Cavender-Bares et al. 2018). It has also been shown that coexistence between specialists and generalists within a group can be restricted (Egas et al. 2004), which may help to explain species whose Metric-Based Specialization Rankings are contrary to their sister taxa.

Overall, *Quercus* species tend to come from generalized ancestors. Results of Ancestral Character State Reconstruction (*Figure* 5) suggest that clades of highly generalized or specialized species arise from ancestral oak populations that maintain a moderate level of generalization. These data support our initial hypothesis that generalist ancestors give rise to specialist species. It may be the case that the level of specialization an evolutionary line exhibits can only change so quickly, and may be limited by time; these time scales have been the point of interest for other studies (Cantalapiedra et al. 2011; Crouch and Ricklefs 2019). Species ranked contrary to their close relatives may have undergone allopatric speciation across vast distances that span different types of regions, resulting in environmental pressures that selected for a different strategy (Aquilar-Romero et al. 2017). This may explain large cases of variation in specialization rankings across regions, such as those seen in section Lobatae and section Quercus. Variation may also be explained as the result of species attempting to avoid niche overlap or competition (Cavender-Bares et al. 2018). Further analyses of geographic patterns and evolutionary timescale may yield useful insights into how specialization emerges, and the rate at which is does so. Additional research should also be directed into how water stress seasonality (i.e. BIO15) may drive specialization and what may occur to endemic species under climate change (Hanson and Weltzin 2000).

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Appendix: Part One

Survey Sample

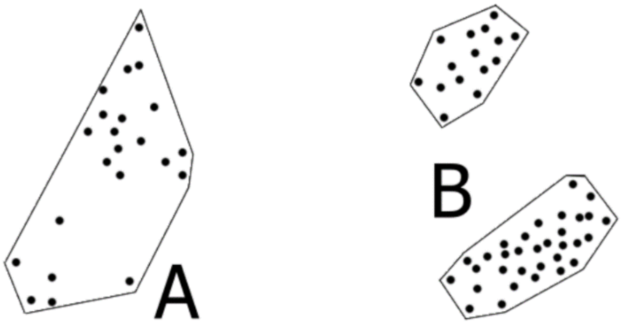
Survey respondents were asked both to define ecological specialization and generalization, and to rank individual species on their level of ecological specialization. Questions one and two asked respondents “How would you define a plant that is an ecological specialist?” and “How would you define a plant that is an ecological generalist?”. All remaining questions asked respondents to rank a species based on its level of ecological specialization, with a five being the most specialized an oak can be and a one being the most generalized (these directions were provided at the head of the survey). Each question also provided a link to the corresponding species’ page on the Oaks of the World website, so that they could clarify which species they were being asked about, as some species have been subject to taxonomic changes. An example is provided below.

Example Question:

Quercus lyrata

http://oaks.of.the.world.free.fr/quercus_lyrata.htm

○ 5
○ 4
○ 3
○ 2
○ 1
○ No Familiarity



Appendix Figure A. Examples of how Extent of Occurrence was drawn and calculated in ArcMap for two species, A and B. Using multiple polygons for a species was reserved for only a small number of instances where the space between polygons was more than likely uninhabitable for that species, as was the case with some Mexican taxa native to high altitude mountain ranges.

*Explanation of **Formula 1** (Percentile Scoring) and Example*

Formula 1.

1. $(Species\ EOO/\ 957{,}618\ \text{km}^2) = A$
2. 1 - A = B
3. B * 25 = # Points Assigned

Step one of formula 1 represents where a species falls in the context of all EOO values ($957{,}618\text{km}^2$ is the highest EOO before the 20th percentile cutoff, and is used as the max range). Step two inverts this, since higher EOO's represent lower specialization. In step 3, the value from step 2 is multiplied by total available points for this metric (25), resulting in the number of points the species gets for this metric. An example is provided below using *Quercus lancifolia (EOO = 343,905km²).*

1. $342{,}905\text{km}^2/957{,}618\text{km}^2 = 0.359$
2. 1 – 0.359 = 0.641
3. 0.641 * 25 = 16.02 Points awarded for EOO

*Plasticity Traits and **Formula 2***

Functional traits measured and used for plasticity were Petiole Length, Leaf Length, Leaf Lobedness, and Specific Leaf Area (Cornelissen et al. 2003). These particular 4 metrics were chosen due to both model selection, and their impact on an individual's ability to inhabit certain regions. They were not the only traits measured, however (*Table 3*). Minimums and maximums of these traits across individuals of a given species were used in ***Formula 2*** to calculate plasticity.

Formula 2

$$\frac{(Max\ of\ Trait - Min\ of\ Trait)}{(Max\ of\ Trait + Min\ of\ Trait)}$$

Appendix Figure B. Regression Matrix showing correlations between leaf functional traits (Generated in JMP version 15.1.0, Analyze, Multivariate). Leaf functional traits show a high degree of correlation between one another. Species with a high or low plasticity for one leaf trait tend to exhibit a similar state for all of them. With a mean correlation coefficient of 0.55, plasticity in functional leaf traits is positively correlated for every combination of the six traits collected for this study. The AICc model selection process retained four of the six available functional leaf traits when predicting IUCN Red List designation and the average response score from the specialization survey (*Table 3*), suggesting that including multiple measures of plasticity in similar studies may bolster predictive power. As a trait thought to be tied to generalization, metrics of plasticity have additional value in representing this concept apart from improving predictive models.

Global Biotic Interactions (GloBI)

GloBI is an open access search tool/conglomerate database that contains 10,006,690 (7,077,559) interaction records that span 727,371 taxa as of November 6th, 2021. These interactions can be sorted by the type of interaction, and by taxa the user is interested in. Records were filtered for each species, with types of interactions not relevant to *Quercus* omitted. The included interaction types were the following:

Commensalist, Dispersal Vector, Ecologically Related to, Flowers Visited by, Mutualist of, Pollinator, and Symbiont.

The number of known interactions for each species was recorded, and these were used with ***Formula 1***. The inversion step of ***Formula 1*** was *not* used for this factor, as more specialized interactions are assumed to reflect higher specialization.

Domatia

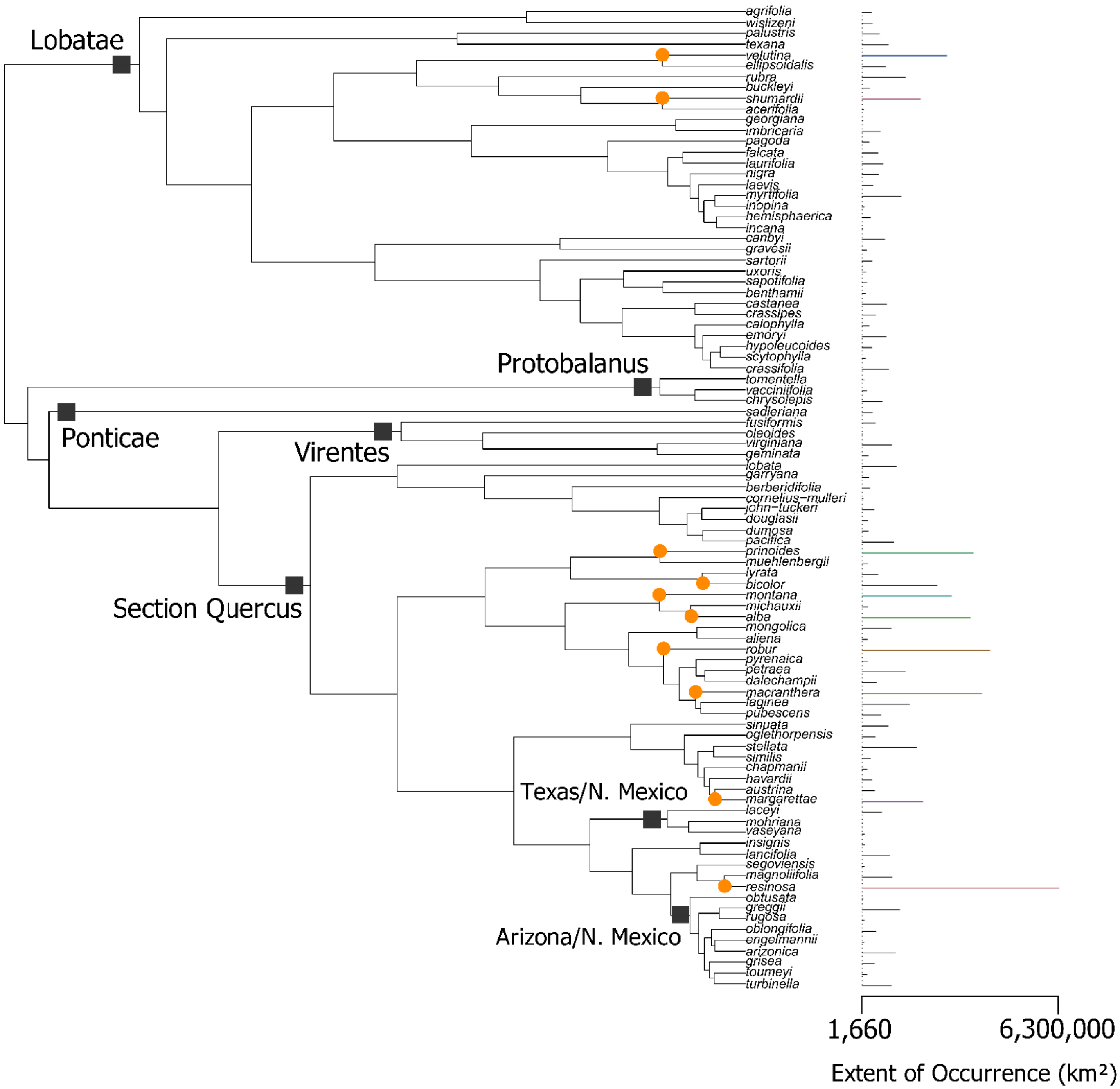
Anatomical features with narrow uses are a key aspect of specialized species. While *Quercus* is considered largely generalist at a broader biological level, domatia represent specialized anatomy that can assessed for the oaks. In *Quercus*, domatia are small chambers made of trichomes at the intersections along the mid-vein of the leaf. These are created to shelter beneficial arthropods that likely help reduce herbivory on the tree. Presence or absence of domatia may be interpreted as being indicative of interspecies specialization.

Domatia presence or absence was assessed for three individuals per species. Each of the three samples was denoted with a 0 (no domatia), 1 (hair present but likely non-functional), or 2 (functional domatia present). These were summed per species, and the totals were scored using ***Formula 1***, minus the inversion of step 3, as a higher domatia presence is interpreted as higher specialization.

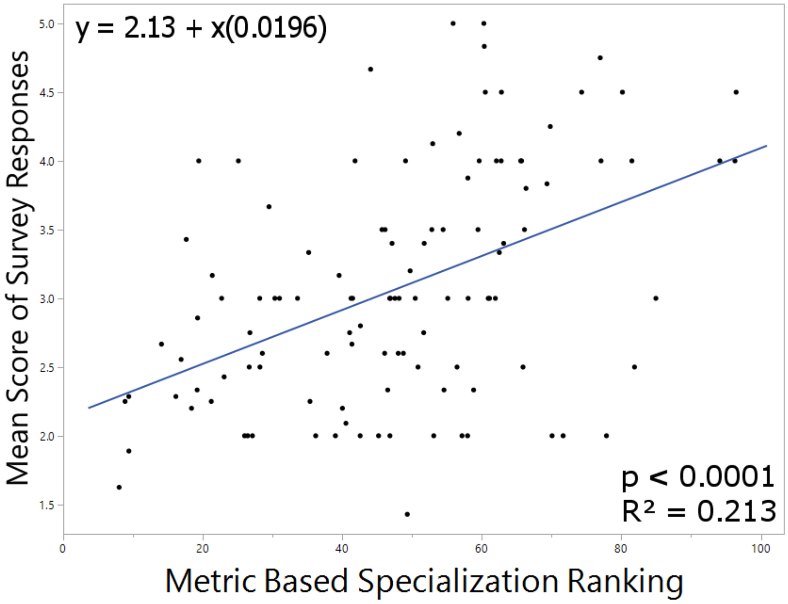
Appendix Table 1. Blomberg's K and Pagel's Lambda for Plasticity of Individual Leaf Traits using mean values per species across *Quercus* phylogeny

Plasticity of Leaf Trait	***K***	**p**	**λ**	**p**
Petiole Length	0.103068	0.857	0.0000681	1
Leaf Length	0.118428	0.4	0.0000681	1
Perimeter per unit Leaf Area	0.11096	0.637	0.0000681	1
Venation	0.102882	0.875	0.0000681	1
Leaf Lobedness	0.103159	0.863	0.0000681	1
Specific Leaf Area	0.11603	0.464	0.0000681	1

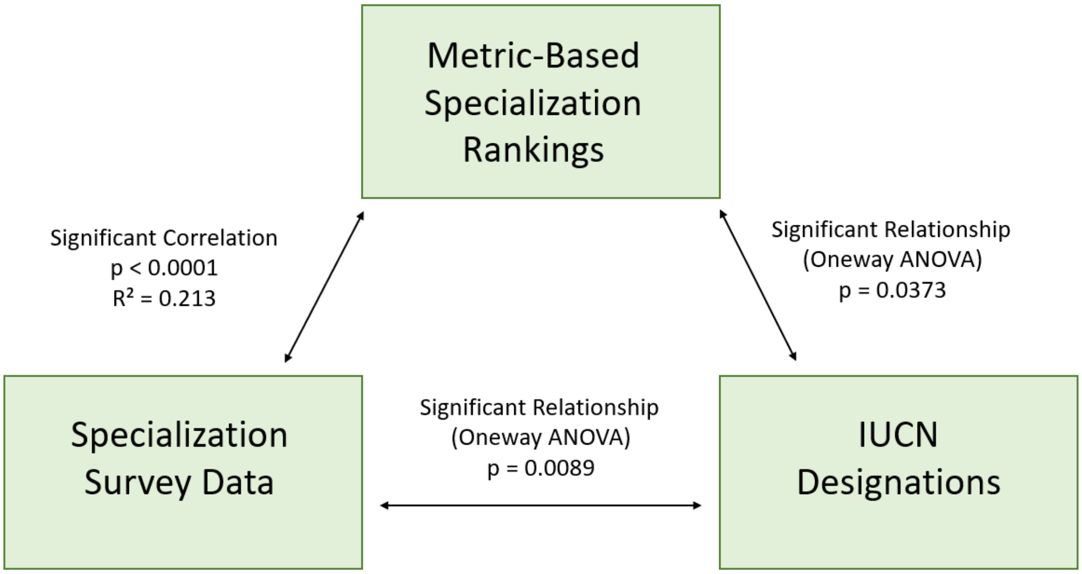
F



Appendix Figure C. Phylogenetic EM results for Species Extent of Occurrence. EOO (Km²) was the only trait of those considered for specialization that showed phylogenetic shifts. Shifts are represented by the orange circles.



Appendix Figure D. Correlation between mean score of survey responses and Metric-Based Specialization Rankings, displayed with p, R^2, and the equation of the line of best fit.



Appendix Figure E. Results of statistical analysis between Specialization Rankings, results of the specialization survey, and IUCN designations. Significance levels and the type of test performed are shown by the double-sided arrows between data. All groups significantly tested against the other two.

Appendix: Part Two – Data and Coding Scripts

***Appendix Table 2**. Subset of Quercus species ranking data used to parameterize metric generalist-specialist rankings (pre-model selection).*

Species	acerifolia	calophylla	lancifolia	mohriana	uxoris
Native Region (Main)	E	M	M	M	M
Native Region (Sub)				E	
Extent of Occurrence (km^2)	38725.81	715975.09	343905.79	433384.5	114800.89
Distinct Inhabited Ecoregions at Level III	3	20	12	11	4
Distinct Inhabited Ecoregions at Level II	2	17	9	7	3
Distinct Inhabited Ecoregions at Level I	1	8	4	4	2
Domatia Score Total	4	5	7	3	5
Number of Notable, Documented Interspecies Interactions (GloBi)	0	0	2	0	0
Plasticity of Petiole Length	0.30300807	0.178082192	0.625	0.574468	0.536679537
Plasticity of Leaf Length	0.359649123	0.095477387	0.597444089	0.474747	0.305882353
Plasticity of Perimeter per Unit Leaf Area	0.486989577	0.035800454	0.508829966	0.589311	0.317107169
Plasticity of Venation	0.774674719	0.85223613	0.503628138	0.792529	0.751401532
Plasticity of Leaf Lobedness	0.453223543	0.108213589	0.319516002	0.581022	0.174577189
Plasticity of Specific Leaf Area	0.597289489	0.081812091	0.427190396	0.723584	0.269431712
Metric-Based Specialization Rank (AICc)	60.33916445	28.15050487	41.81753187	66.10377	61.08799708
Average Score from Expert Survey	4.833333333	3	4	3.5	3
IUCN Status	EN	NT	LC	LC	LC
IUCN Status (Threatened, Near Threatened, Least Concern)	THR	NT	LC	LC	LC

Plotting Metric-Based Specialization Rankings as Color-Coded Bars at Tips of Phylogeny (R Script)

```
#Set Working Directory to Location of Data Files
setwd("C:/Users/Alex/Desktop/R")

###LIBRARIES###
library(phytools)
library(magrittr)

###READING IN DATA###
#Read in Metric Data from Excel (.csv)
traits <- read.csv("MainMetrics3821.csv", as.is=TRUE)
#Read in List of Species with Data Deficiencies
NADropList <- read.csv("NADropList.csv", as.is=TRUE)
#Read in the primary phylogenetic tree
tr <- read.tree("tr.singletons.GlobalOaks2019.tre")
#strip genera from each species binomial
tr$tip.label <- gsub('Quercus_', '', tr$tip.label, fixed = T)
#make a list of species in .tre
tr$tip.label <- sapply(strsplit(tr$tip.label, "|", fixed = T),
                       '[', 1) %>% make.unique
#Create tr.pruned; tree with only species in Specialization Study
tr.pruned <- drop.tip(tr, which(!tr$tip.label %in% traits$Species))
#Changes row numbers to species names
traits <- read.csv("MainMetrics3821.csv", as.is=TRUE, row.names = 1)


###PLOTTING###
```

```
#The lines below plot Metric-Based Specialization Rankings as color coded bars
#at the respective tips of the tree. The object 'Colors' is a vector of colors
#telling the plotting function what color each species' bar should be, that
#must be declared prior to plotting. Dashed lines and the text labels denoting
#them were added with the 'text' and 'abline' functions. View at 20 x 20.

#Creates object 'Ranks', a named vector of Metric-Based Rankings.
Ranks<-setNames(traits$RankAICc,rownames(traits))
#Plots a tree with bars, where the bars are scaled by Metric-Based Ranking
#and color coded by native region.
plotTree.wBars(tr.pruned, Ranks, col= Colors, tip.labels=TRUE)
```

Ancestral Character State Reconstruction (R Script)

```
#Set Working Directory to Location of Data Files
setwd("C:/Users/Alhex/Desktop/R")

###LIBRARIES###
library(ape)
library(magrittr)
library(nlme)
library(phytools)
library(tidyverse)


###READING IN DATA###

#Read in Metric Data from Excel (.csv)
traits <- read.csv("MainMetrics3821.csv",  as.is=TRUE)
#Read in List of Species with Data Deficiencies
NADropList <- read.csv("NADropList.csv", as.is=TRUE)
#Read in the primary phylogenetic tree
tr <- read.tree("tr.singletons.GlobalOaks2019.tre")
#strip genera from each species binomial
tr$tip.label <- gsub('Quercus_', '', tr$tip.label, fixed = T)
#make a list of species in .tre
tr$tip.label <- sapply(strsplit(tr$tip.label,  "|", fixed = T),
'[', 1) %>% make.unique
#Creates tr.nonafinal; tree with only species that have no data deficiencies
tr.nonafinal <- drop.tip(tr, which(!tr$tip.label %in% NADropList$Species))
#Changes row numbers of Metric Data table to species names
```

```
traits <- read.csv("MainMetrics3821.csv",  as.is=TRUE, row.names = 1)

###ESTIMATING AND PLOTTING ANCESTRAL STATES

#Creates vector of species names on the tree
nonafinaltips <- tr.nonafinal[["tip.label"]]

#Create object of Metric-Based Rankings of Specialization, 'SpecRanks'
SpecRanks <- traits[nonafinaltips, 27]
#Assign species names to SpecRanks
names(SpecRanks) <- nonafinaltips

#Changes Data to Correct Input Format
SpecRanksDF <- as.data.frame(SpecRanks, row.names = nonafinaltips)
SpecRanksMTX <-as.matrix(SpecRanksDF)
SpecRanksFinal<-as.matrix(SpecRanksMTX)[,1]

#Perform the Ancestral State Estimations
AncestralStates <- fastAnc(tr.nonafinal, SpecRanksFinal, vars=FALSE,CI=FALSE)

#This Plots Ancestral States as a colored gradient across branches
#View at 17x17 Window size
cont<-contMap(tr.nonafinal,SpecRanksFinal,plot=FALSE)
plot(cont,legend=0.7*max(nodeHeights(tr.nonafinal)), mar=c(5,5,5,5))

#Plots the Tree by Current and Estimated States as a phenogram across time
#View at 15x15 window size
par(mar=c(5,4,4,5))
```

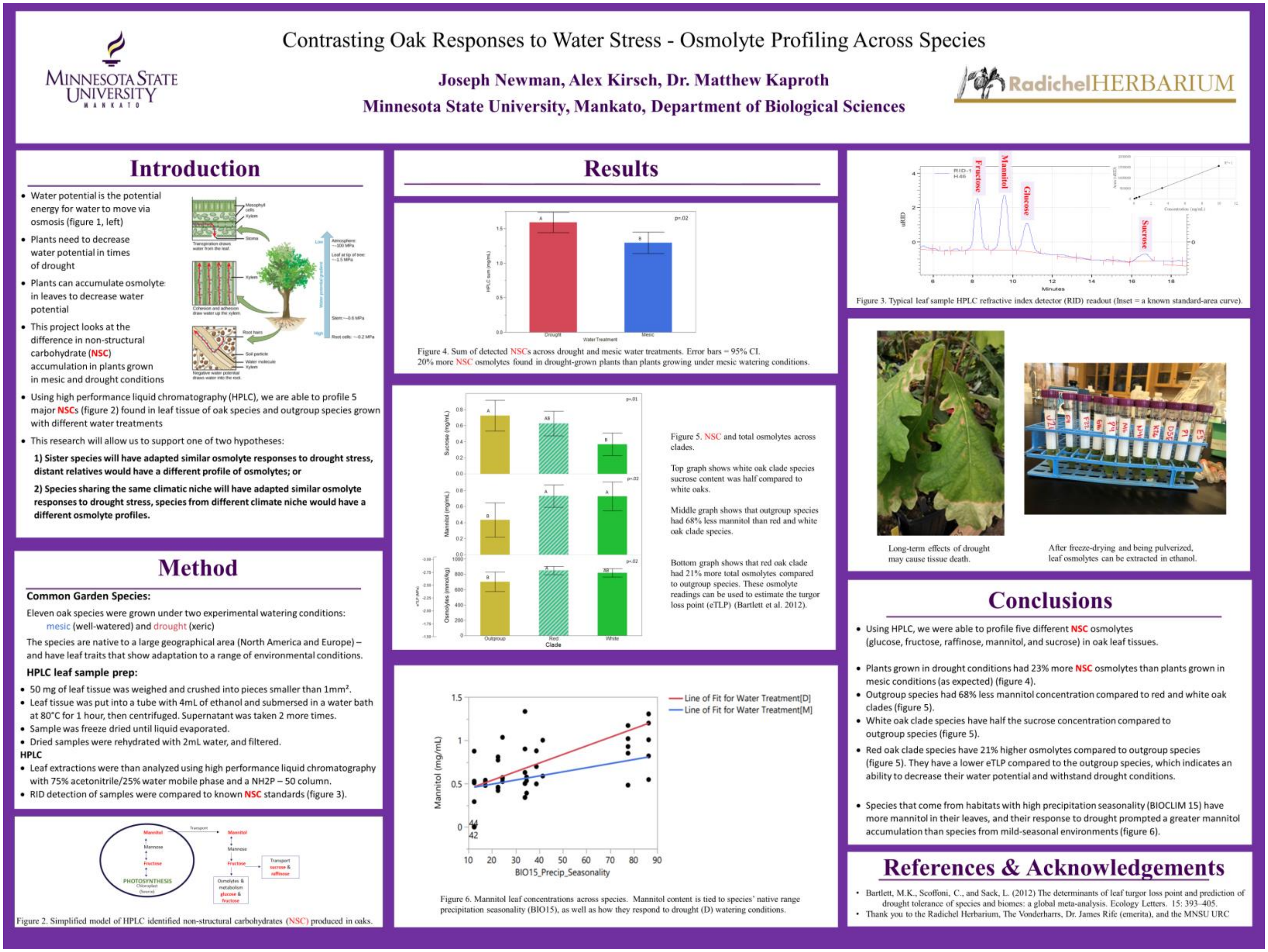
```
phenogram(tr.nonafinal,SpecRanksFinal,
        spread.labels=TRUE,spread.cost=c(1,0),
        link=2.5 , offset=0)

#This Plots the Plain Tree with Nodes Labeled by Circles,
#Scaled by Estimated State
#View at 15x15 window size
plot(tr.nonafinal)
nodelabels(pch = 21, cex=(AncestralStates/20),
         bg=ifelse(AncestralStates>50,"Black","White"))
```

Phylogenetic Generalized Least Squares (R Script)

```
#Set Working Directory to Location of Data Files
setwd("C:/Users/Alhex/Desktop/R")

###LIBRARIES###
library(ape)
library(magrittr)
library(nlme)

###READING IN DATA###

#Read in Data from Excel (.csv)
traits <- read.csv("WaterPGLS.csv", as.is=TRUE)
#Read in the primary phylogenetic tree
tr <- read.tree("tr.singletons.GlobalOaks2019.tre")
#Strip genera from each species binomial
tr$tip.label <- gsub('Quercus_', '', tr$tip.label, fixed = T)
#Make a list of species in .tre
tr$tip.label <- sapply(strsplit(tr$tip.label, "|", fixed = T),
                                '[', 1) %>% make.unique
#Create tr.pruned; tree with only species in Specialization Study
tr.pruned <- drop.tip(tr, which(!tr$tip.label %in% traits$Species))
#Changes row numbers to species names
traits <- read.csv("WaterPGLS.csv", as.is=TRUE, row.names = 1)
#creates vector of species name
tips <- tr.pruned[["tip.label"]]
```

```
###PREPPING VARIABLES###

#Save metrics to be tested as vectors, then name them with the respective
#species names.
Rank <- traits[tips, 3]
IMGS <- traits[tips, 1]
Bio15 <- traits[tips, 2]
names(Rank) <- (tips)
names(IMGS) <- (tips)
names(Bio15) <- (tips)



###RUN TEST AND VIEW TEST RESULTS###
#PGLS model of Metric-Based Rank against Bioclimatic Variable 15
pglsModel1 <- gls( Rank ~ Bio15,
               correlation = corPagel(value = 1, phy = tr.pruned, fixed = FALSE, form = ~1),
               data = traits, method = "ML")

#Display test summary and coefficient
summary(pglsModel1)
coef(pglsModel1)
```

Appendix: Part Three – *“Contrasting Oaks Responses to Water Stress – Osmolyte Profiling Across Species”*



Contrasting Oak Responses to Water Stress - Osmolyte Profiling Across Species

Joseph Newman, Alex Kirsch, Dr. Matthew Kaproth
Minnesota State University, Mankato, Department of Biological Sciences

Introduction

- Water potential is the potential energy for water to move via osmosis (figure 1, left)
- Plants need to decrease water potential in times of drought
- Plants can accumulate osmolytes in leaves to decrease water potential
- This project looks at the difference in non-structural carbohydrate (**NSC**) accumulation in plants grown in mesic and drought conditions
- Using high performance liquid chromatography (HPLC), we are able to profile 5 major **NSC**s (figure 2) found in leaf tissue of oak species and outgroup species grown with different water treatments
- This research will allow us to support one of two hypotheses:

  1) Sister species will have adapted similar osmolyte responses to drought stress, distant relatives would have a different profile of osmolytes; or

  2) Species sharing the same climatic niche will have adapted similar osmolyte responses to drought stress, species from different climate niche would have a different osmolyte profiles.

Method

Common Garden Species:

Eleven oak species were grown under two experimental watering conditions:
mesic (well-watered) and drought (xeric)

The species are native to a large geographical area (North America and Europe) – and have leaf traits that show adaptation to a range of environmental conditions.

HPLC leaf sample prep:

- 50 mg of leaf tissue was weighed and crushed into pieces smaller than $1mm^2$.
- Leaf tissue was put into a tube with 4mL of ethanol and submersed in a water bath at 80°C for 1 hour, then centrifuged. Supernatant was taken 2 more times.
- Sample was freeze dried until liquid evaporated.
- Dried samples were rehydrated with 2mL water, and filtered.

HPLC

- Leaf extractions were than analyzed using high performance liquid chromatography with 75% acetonitrile/25% water mobile phase and a NH2P – 50 column.
- RID detection of samples were compared to known **NSC** standards (figure 3).

Figure 2. Simplified model of HPLC identified non-structural carbohydrates (NSC) produced in oaks.

Results

Figure 4. Sum of detected NSCs across drought and mesic water treatments. Error bars = 95% CI.
20% more NSC osmolytes found in drought-grown plants than plants growing under mesic watering conditions.

Figure 5. NSC and total osmolytes across clades.

Top graph shows white oak clade species sucrose content was half compared to white oaks.

Middle graph shows that outgroup species had 68% less mannitol than red and white oak clade species.

Bottom graph shows that red oak clade had 21% more total osmolytes compared to outgroup species. These osmolyte readings can be used to estimate the turgor loss point (eTLP) (Bartlett et al. 2012).

Figure 6. Mannitol leaf concentrations across species. Mannitol content is tied to species' native range precipitation seasonality (BIO15), as well as how they respond to drought (D) watering conditions.

Figure 3. Typical leaf sample HPLC refractive index detector (RID) readout (Inset = a known standard-area curve).

Long-term effects of drought may cause tissue death.

After freeze-drying and being pulverized, leaf osmolytes can be extracted in ethanol.

Conclusions

- Using HPLC, we were able to profile five different **NSC** osmolytes (glucose, fructose, raffinose, mannitol, and sucrose) in oak leaf tissues.
- Plants grown in drought conditions had 23% more **NSC** osmolytes than plants grown in mesic conditions (as expected) (figure 4).
- Outgroup species had 68% less mannitol concentration compared to red and white oak clades (figure 5).
- White oak clade species have half the sucrose concentration compared to outgroup species (figure 5).
- Red oak clade species have 21% higher osmolytes compared to outgroup species (figure 5). They have a lower eTLP compared to the outgroup species, which indicates an ability to decrease their water potential and withstand drought conditions.
- Species that come from habitats with high precipitation seasonality (BIOCLIM 15) have more mannitol in their leaves, and their response to drought prompted a greater mannitol accumulation than species from mild-seasonal environments (figure 6).

References & Acknowledgements

- Bartlett, M.K., Scoffoni, C., and Sack, L. (2012) The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. Ecology Letters. 15: 393–405.

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