

GLOBAL PATTERNS OF LEAF DEFENSES IN OAK SPECIES.

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Abstract

Plant defensive traits drive patterns of herbivory and herbivore diversity among plant species. Over the past 30 years, several prominent hypotheses have predicted the association of plant defenses with particular abiotic environments or geographic regions. We used a strongly supported phylogeny of oaks to test whether defensive traits of 56 oak species are associated with particular components of their climatic niche. Climate predicted both the chemical leaf defenses and the physical leaf defenses of oaks, whether analyzed separately or in combination. Oak leaf defenses were higher at lower latitudes, and this latitudinal gradient could be explained entirely by climate. Using phylogenetic regression methods, we found that plant defenses tended to be greater in oak species that occur in regions with low temperature seasonality, mild winters, and low minimum precipitation, and that plant defenses may track the abiotic environment slowly over macroevolutionary time. The pattern of association we observed between oak leaf traits and abiotic environments was consistent with a combination of a seasonality gradient,

which may relate to different herbivore pressures, and the resource availability hypothesis, which posits that herbivores exert greater selection on plants in resource-limited abiotic environments.

Keywords: plant-insect interaction, latitude, tannins, Ornstein-Uhlenbeck model, biogeography, macroevolution

Introduction

Plant defenses drive plants' associations with herbivores. Plants with certain life-history traits (such as trees) or habitat affiliations (such as desert plants) often invest more in defenses than other plants, and it has been the goal of several major theories of plant defense to understand these correlations (described in Stamp 2003). The defensive traits that a plant possesses today are a result of both its evolutionary heritage—the traits and constraints it inherited—and more recent adaptation of that plant species to its biotic and abiotic environment (Gould and Lewontin 1979; Agrawal 2007). Some comparative studies between plant species have stressed the importance of deep evolutionary history in driving plant-herbivore interactions (e.g. Mitter et al. 1991; Weiblen et al. 2006). Others have stressed local adaptation (which implies rapid evolution of defenses in environments where they convey a fitness benefit to the plant) (e.g. Fine et al. 2004; Kursar et al. 2009). Of course, the deep and recent impacts of history on any adaptive trait are not mutually exclusive (Futuyma and Agrawal 2009 and papers therein). Modern phylogenetic comparative approaches, such as phylogenetic least squares methods and Ornstein-Uhlenbeck (O-U) modeling, allow us to begin to tease apart the effects of evolutionary history and natural selection in the evolution of plant defenses.

Selection pressures imposed by different habitat types or climatic associations are thought to be a major driver of a plant's defensive investment (Stamp 2003). For example studies have found substantial variation in plant defenses between nutrient poor and nutrient rich habitats as well as between temperate and tropical regions (Coley and Barone 1996; Fine et al. 2006). One long-standing hypothesis suggests that plant defensive investment should be greater in regions closer to the equator (Coley and

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Barone 1996; Schemske et al. 2009), as biotic interactions such as herbivory are thought to be stronger in tropical regions near the equator. This hypothesis has recently been challenged, as some individual studies find a latitudinal gradient in plant defenses (e.g. Rasmann and Agrawal 2011), but larger meta-analyses find no clear latitudinal gradient (Moles et al. 2011; Johnson and Rasmann 2011). In order to more carefully approach the geographic variation in plant defenses, it is necessary to understand the selection pressures that drive plant defenses.

Optimal defense theory provides expectations of how natural selection should shape plant defenses (Rhoades and Cates 1976; Stamp 2003). Most simply, plants should invest more in defenses when the fitness costs of defense are outweighed by the benefits of reduced herbivory. The benefits of reduced herbivory, however, are complex: the benefit of defense will be greater to the plant, a) the more damage is decreased by the defense, such as when herbivore pressure is high, and b) the greater the fitness loss to the plant is per unit tissue lost, i.e. for plants with less tolerance of herbivory. Theories that predict a plant's investment in defense resulting from its habitat affiliation focus on variation in both of these areas: herbivore pressure and plant tolerance to herbivory.

Coley's (1985) Resource Availability Hypothesis (RAH) is the most prominent explanation of abiotic selection pressures shaping plant defenses, and it uses plant tolerance to herbivory to explain defensive investment. The RAH states that plant species that evolve under resource limitation will tend to limit herbivory through a greater investment in general (and energetically expensive) defenses against herbivores, as resource limitation makes it costly for the plant to replace tissues lost to herbivory. The RAH thus uses the plant's resource budget to predict the evolutionary origins of plant

defenses and has received substantial support from studies that compare defensive investment across plants in resource rich and resource poor environments (Coley 1983; Fine et al. 2006).

Plants in different regions almost certainly experience different herbivore pressures, and this side of optimal defense theory has received less attention recently. For example, herbivore pressure may be a function of the persistence of plant resources, as plants with year-round leaves or other resources accrue higher herbivore loads than plants with a deciduous habit (Karban 2007). At a broader scale, tropical areas have greater herbivore diversity than temperate zones, and herbivore species richness has increased dramatically during periods of global warming throughout Earth's history (Coley and Barone 1996; Schemske et al. 2009; Currano et al. 2010). There is currently debate over whether the greater diversity of herbivores found in the tropics translates into greater herbivore pressure for plants (Moles et al. 2011). Herbivore pressure likely varies between habitats, and plants in regions that experience high herbivore pressure should invest in defenses to maximize their fitness.

Almost all plant traits that affect herbivory also likely have other physiological functions (Seigler and Price 1976), so an alternative hypothesis might be that traits linked to herbivore-resistance also ameliorate the abiotic environment. Some authors have suggested that structural plant defenses or phenological avoidance of herbivores have a greater pleiotropic effect than more herbivore-specific chemical defenses (Carmona et al. 2011). For example, specific leaf area (a physical leaf trait) relates directly to water-use in plants (Knight et al. 2006), but the silencing of drought-stress pathways in tomato

plants had little effect on a suite of chemical markers of plant defense (Thaler and Bostock 2004).

In this study, we examine the evolutionary history of oaks (*Quercus*) and use that phylogeny to investigate how the evolution of a suite of 9 defensive traits correlates with various dimensions of the climatic environment and latitude. An index of these same traits has independently been shown to reduce the fitness of a generalist herbivore on oaks (Pearse 2011), and many other studies support the importance of these traits as leaf defenses against other herbivores (detailed in methods). Our hypothesis is that leaf defensive traits will evolve more readily in more stressful environments, as predicted by the resource-availability hypothesis, and in areas with less climatic seasonality, which may correspond with greater herbivore pressure. By comparing information on the defensive traits, abiotic (climatic) environment, and evolutionary history of oak species, we can begin to assess the relative importance of optimal defense theories (such as the RAH and theories that predict higher defense in regions of higher herbivore persistence) as well as non-optimal explanations for defensive investment (such as pleiotropy and evolutionary conservatism) at a global scale in oaks.

Methods

Oak taxa and assessment of plant traits Oaks provide an ideal group of plants to assess global patterns of defensive traits for several reasons. In oaks, the relationship between putative defensive traits and actual vulnerability to insect herbivory is well understood. Pearse (2011) found that indices of leaf defenses similar to those assayed in the current study predicted tussock moth caterpillar survival rates on 27 oak species. Moreover, the

effectiveness of traits such as tannins (Feeny 1970; Forkner et al. 2004), leaf phenology (Karban 2007), and trichomes (Kitamura et al. 2007) as defenses against herbivores has been substantiated to varying degrees in oaks. Also, oaks are commonly planted in arboreta, which represent quasi-common gardens in which the genetic component of plant traits can be separated from plasticity.

In the current study, we sample 56 oak taxa, representing each of the five major oak clades (Manos et al. 1999; Pearse and Hipp 2009), including representatives from eastern North America, western North America, Mexico, Europe, and Asia. Species were selected based on their presence in a 40-year old stand of oaks at University of California- Davis Arboretum. While this sample represents less than 15% of the entire genus, it represents a broad spectrum of the phylogenetic, geographic, and ecological diversity of the genus. Each taxon was represented by 3 individuals except in cases where fewer were present in the arboretum (Pearse and Hipp 2009). Nomenclature follows the Oaks Names Database (Trehane 2007). Leaf traits were taken from previous studies on these same trees (Pearse and Hipp 2009; Pearse and Baty in press).

To assess the relationship between defensive traits and environment, leaf defensive traits were assembled into three defensive indices using principal component analysis (PCA) to derive a chemical traits defensiveness index (DI_{CHEM}) composed of five traits: phenolics, condensed tannins, summer tannins, peroxidase (POX), and proteins; a physical traits defensiveness index (DI_{PHYS}) composed of four traits: leaf toughness, specific leaf area (SLA), trichome density on the upper leaf surface, and trichome density on the lower leaf surface; and an all-traits index (DI_{ALL}) composed of all nine traits. For all three defensiveness indices, we utilized the first and second principal

component (PC). PC1 extracts 33%, 54%, and 42% of the total trait variance in DI_{ALL} , DI_{PHYS} , and DI_{CHEM} respectively. In preliminary analyses, we found no association between PC2 of DI_{ALL} or DI_{PHYS} and any environmental predictors, so these are not shown. Preliminary analyses using PC2 of the DI_{CHEM} suggested that this axis (extracting 29% of the variation in DI_{CHEM}) showed covariation with environmental predictors. These four defensiveness indices are our primary response variables in the models evaluated in this study (details under “Comparative methods” below).

Estimation of climatic variables Oaks occur on every major landmass in the Northern Hemisphere, so transitions between different habitat types have likely occurred independently from one another (Manos et al. 1999). Moreover, niche convergence is well known within oaks, such that each of the major oak clades has undergone many of the same habitat transitions (Cavender-Bares et al. 2004). The ranges of most oak species have been well documented in forestry and herbarium records, enabling quantitative assessment of climatic niches for each species in this study by associating fine-scale climatic data (Hijmans et al. 2005) with numerous herbarium and observational records for each species.

Herbarium and forestry records of oaks were taken from three different sources: the Global Biodiversity Information Foundation (GBIF; <http://data.gbif.org>), the Consortium of California Herbaria (ucjeps.berkeley.edu/consortium/), and herbarium sheets housed in the Field Museum Herbarium (F). Erroneous records were culled from the dataset based on the following criteria. Taxonomically uncertain records were removed, using nomenclatural stability according to the Oak Names Database as the criterion (Trehane 2007). Records that lacked georeferenced coordinates were

georeferenced manually when descriptions with locality information could be confirmed within 5 km. Urban plantings and arboretum records were removed from the dataset, and records that fell more than 100 km outside of the established geographic range based on taxonomic literature (Flora of North America Editorial Committee 1993+; le Hardy de Beaulieu and Lamant 2007) were removed from the dataset. Climate information from the WorldClim database (Hijmans et al. 2005) was associated with each locality at a resolution of 2.5 arc minutes (ca. 4.8 km) using the R packages *dismo* and *raster* (Hijmans and Phillips 2010; Hijmans and van Etten 2010). For each oak species, the mean of each climatic estimate was calculated across the entire species range as represented by specimen data above and used for subsequent analyses. Because this approach does not correct for spatial autocorrelation, the resulting climatic means are weighted by collection intensity (Fig. 1A). While intraspecific variation in both climate and traits bears further investigation in oaks, we find that species identity explains an average of 44–85% of total variance in climatic variables, which supports an earlier study in which leaf physiological traits show strong among-species variance relative to within-species variance, even across a strong latitudinal gradient (Cavender-Bares et al. 2011).

We utilized eight of the 19 WorldClim climatic measures in our study, as follows: BIO1, *mean annual temperature*; BIO4, *temperature seasonality*, estimated as the standard deviation of temperature among months; BIO5, *maximum temperature* (mean temperature of the warmest month); BIO6, *minimum temperature* (mean temperature of the coldest month); BIO12, *mean annual precipitation*; BIO13, *maximum precipitation*, estimated as the mean precipitation of the wettest month; BIO14, *minimum precipitation* of the driest month; and BIO15, *precipitation seasonality*, estimated as the coefficient of

variation in precipitation across months. We use the italicized abbreviations throughout this paper for sake of readability. The eight climatic predictors were selected *a priori* from the 19 provided in WorldClim: the excluded predictors comprise 8 that are quantified by quarter rather than month and 3 that are based on climatic ranges rather than extremes or means, and thus are derived from data already reflected in the 8 we included. We also include mean latitude as a predictor. Among the nine predictors, six pairs exhibited covariance of $|r| > 0.70$.

We dealt with covariation among predictor variables in three ways: first, we condensed all of the 8 climatic variables using PCA. PC1 of these the environmental predictors accounted for 40% of the variance in all 8 predictors. Second, in order to compare the importance of different aspects of the abiotic environment, we utilized an information theoretic modeling approach to evaluate the importance of predictors across a wide range of plausible predictor combinations, as described below. Third, we used AIC to reduce models in order to avoid problems of collinearity.

Phylogeny We reconstructed oak phylogenetic relationships based on the same oak individuals from which we had gathered leaf trait data, using minimum evolution on an AFLP dataset (Pearse and Hipp 2009). This phylogeny was topologically identical in most particulars to a phylogeny estimated using Markov chain Monte Carlo under an asymmetric binary model. To integrate over phylogenetic uncertainty, a set of 200 minimum evolution bootstrap trees was utilized for most analyses presented in this paper. Because of the tendency for AFLP phylogenies to exhibit long terminal branches, we pruned each bootstrap tree back to the most recent common ancestor for each species that possessed at least two individuals per tip. For species with only a single exemplar per tip,

tips were pruned to 50% of their original length. Each resulting tree was ultrametricized using penalized likelihood with the smoothing parameter set at 0.1 (Sanderson 2002), and the resulting trees pruned to include only the taxa for which we could assess leaf traits and geographic ranges.

Phylogenetic comparative methods: overview We present two types of phylogenetic comparative analyses in this study: Generalized Least Squares phylogenetic regression models that assess the relationship between plant traits and environmental associations and Ornstein-Uhlenbeck models that specifically model the contribution of phylogenetic inertia and trait adaptation. All variables throughout the paper were rescaled by the standard deviation of the sample, so regression coefficients reported here are in estimated-standard deviation units.

Phylogenetic comparative methods 1.1: multivariate measures of environment and latitude predicting leaf defensive investment. As an estimate of the combined effects of latitude and climate on defensive leaf traits in oaks, we used a GLS framework to assess the influence of a multivariate estimate of environment (PC1 of WorldClim variables) and latitude on a multivariate estimate of plant defenses (DI_{ALL}). We ran these GLS regressions both in a simple regression and multiple regression framework.

Phylogenetic comparative methods 1.2: individual components of the environment predicting particular leaf traits. We used an information theoretic approach to estimate the relative importance of each of the nine potential predictors in explaining the variance in our three defensive traits indices. First, we analyzed all possible regression models of 1–4 predictors for each of the three indices, a total of 255 models for each index. We corrected for phylogeny using generalized least squares (GLS) on the minimum evolution

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estimate of the optimal phylogenetic tree and calculated the small-sample Akaike information criterion (AIC_c) weight for each GLS regression. Relative importance of each climatic variable in predicting trait variance was estimated as the sum of AIC_c weights for models possessing that climatic variable. Model weights sum to 1.0 for each analysis, and all parameters occur in the same number of models, according each parameter equal prior weight and integrating our estimates of parameter importance over model uncertainty (Burnham and Anderson 2002; cf. discussions in Hipp et al. 2007). From this analysis (Table A2) we identified those predictors that have ≥ 0.40 evidential support.

In this analysis, we report the R^2 for the best-fit model (assessed using AIC_c weight). Note that while these GLS R^2 values may be interpreted as the total variance explained in the response variable by the system of predictors, partial r^2 values from a GLS analysis are not readily interpreted and consequently not presented in this study (Lavin et al. 2008). The R^2 are provided from a single model for purposes of comparison, but we make no attempt in this paper to select a single best model or set of models, as the 95% confidence set of models (estimated using cumulative reverse-sorted AIC_c weights on the single minimum evolution tree (Burnham and Anderson 2002) included 87 to 208 models for each response variable (Table A2), with no model receiving more than 0.105 AIC_c weight, and all potential predictors appear in at least one of the models in the confidence set. Rather, our analyses emphasize the relative importance of the potential predictors in predicting leaf traits and model-averaged estimates of the effect strength and direction based on model averaging.

Phylogenetic comparative methods 1.3: individual components of the environment

predicting leaf defensive investment We estimated regression coefficients model-averaged over the 255 models for each leaf defensiveness index as well as for each of the individual leaf traits that contribute to these indices. However, these regressions condition on a single phylogeny and a Brownian motion model of character evolution, and they include models that are highly parameter-rich in comparison to the size of our dataset (the largest models include 11 free parameters). Incorrect phylogeny point-estimates, incorrect modeling of the relationship between branch lengths and trait covariance, and model over-parameterization all have the potential to increase the variance of parameter estimates (Revell 2010). Consequently, we performed a second set of analyses aimed at estimating regression coefficients more precisely. To reduce model complexity, we included in this analysis only the environmental predictors that were supported at AICc weights ≥ 0.40 (see Comparative methods 1 above), for a maximum of 3–4 predictors for each defensiveness index. To integrate over phylogenetic uncertainty, each set of regression models was conducted in a GLS framework over 200 phylogenetic trees subsampled from our bootstrap phylogenetic analysis, with the covariance matrix separately for each tree. Finally, to relax the assumption that traits evolve according to a Brownian motion process, the covariance matrix for each model on each tree was rescaled using Pagel's λ , a scalar by which all off-diagonal elements of the covariance matrix are multiplied (Pagel 1999). All model parameters, including λ and the regression coefficients, were optimized simultaneously using maximum likelihood in the *gls* function of the nlme package in R (Pinheiro et al. 2009). In this context, λ estimates the phylogenetic signal in the regression residuals, and rescaling by λ has been found to

minimize the variance of GLS regression coefficients (Revell 2010). Parameter estimates are presented as means over the bootstrap sample, with a 95% phylogenetic uncertainty interval on the bootstrap sample calculated by percentile (empirical).

Phylogenetic comparative methods 2: Modeling the evolution of oak traits as a product of both phylogenetic inertia and adaptation

It has been noted that the Brownian motion model of character evolution underlying phylogenetic GLS and phylogenetic independent contrasts is a poor model for studying the effects of natural selection on character evolution, and that an Ornstein-Uhlenbeck (O-U) process more accurately describes the history of traits evolving by natural selection (Felsenstein 1985; Hansen 1997; Butler and King 2004). Unlike a Brownian motion model, in which trait evolution is treated as a purely stochastic process, O-U models treat trait evolution as a result of effectively stochastic evolutionary processes (e.g., genetic drift or adaptation to a randomly shifting optimum) as well as a deterministic process (e.g., adaptation to a modeled optimum). In this paper, we utilize Hansen et al.'s (2008) extension of the O-U model as implemented in SLOUCH (Hansen et al. 2008), in which the evolution of the predictors (in our case, variables describing the abiotic environment) is modeled according to a Brownian process, while the evolution of the traits (in our case, the all-traits leaf defensiveness index) is modeled according to an O-U process that tracks an optimum determined by the predictors. The model is represented by coupled stochastic differential equations:

$$dy = -\alpha(y - \theta(\mathbf{X}))dt + \sigma_y dW_y, \quad (\text{Eq. 1})$$

$$d\mathbf{X} = \sigma_x dW_x \quad (\text{Eq. 2})$$

(Hansen et al. 2008), where y is the response (in our case, leaf trait or index), \mathbf{X} is the system of predictors, θ is the evolutionary optimum of y , and α is the rate of adaptation of

y toward θ . Equation 2 models the evolution of the environmental predictors according to a multivariate Brownian motion process. Equation 1 models the evolution of the dependent trait, which is taken as evolving toward an optimum θ that is a function of the environmental predictors, with a rate that is determined by the inherent rate of adaptation (α) and the distance between the trait value and its optimum. The “phylogenetic half-life” (Hansen 1997; Hansen et al. 2008)—the expected time (expressed in the same units that the tree is scaled to) for a trait to evolve halfway from its ancestral state to its optimum—is a direct function of the rate of adaptation: $t_{1/2} = \ln(2) / \alpha$. At equilibrium, when the dependent trait is fully adapted to the optimum determined by the predictors in the model, the residual trait variance is estimated as $v_y = \sigma_y^2 / 2\alpha$. The O-U process is sometimes referred to a “rubber band” process (Hansen and Martins 1996; Martins et al. 2002) because the rate of evolution toward a trait optimum is a function of the distance of the trait from that optimum; in other words, natural selection “pulls” harder on traits that are far from their optimum. Phylogenetic regression parameters, in this case, are equivalent to phylogenetic GLS regression parameters, and are obtained by multiplying the coefficients of the optimal regression by a phylogenetic correction factor $\rho(\alpha t) = (1 - (1 - e^{-\alpha t}) / \alpha t)$, which approaches a limit of 0 if there is no phylogenetic inertia (i.e., as α goes to ∞) and 1 if there is no effect of adaptation (i.e., as α goes to 0; Hansen et al. 2008). It is worth noting here that phylogenetic inertia here denotes “a resistance to or slowness in the adaptation to a specific optimum,” as contrasted with phylogenetic signal as “any statistical influence of phylogeny on the trait” (Hansen et al. 2008, p. 1966). The all-traits defensiveness index was modeled as evolving in five multivariate environments, chosen for the importance of their parameters in GLS models: BIO4, BIO4 + BIO5,

BIO4 + BIO6, BIO4 + BIO5 + BIO6, and BIO4 + BIO5 + BIO6 + BIO14 (Table 2). For computational reasons, all analyses were performed on the single best minimum evolution estimate of the phylogeny. Model coefficients are reported \pm standard errors as estimated using GLS. Confidence intervals around $t_{1/2}$ and v_y were estimated by plotting the joint likelihood surface from the maximum likelihood value to 2 log-likelihood units below the maximum likelihood value. All parameter estimates are reported for the separate models evaluated as well as model averaged over all five models.

All statistical analyses presented in this study were conducted in R packages APE, NLME, OUCH, VEGAN, and SLOUCH (Pinheiro et al. 2009; Oksanen et al. 2009; Paradis et al. 2004; Hansen et al. 2008; R Core Development Team 2008). Trait data are archived in Dryad (Accession # ____), and phylogenetic data in TreeBase (S10065).

Results

Description of oak biogeography, habitat association, and leaf traits

Oak taxa are separated into the taxonomically-defined oak sections of white oaks (nearctic and palearctic), red oaks (nearctic), intermediate oaks (nearctic), Cerris "black" oaks (palearctic), and the Cyclobalanopsis group (palearctic) (Figs. 1A, 1B, Manos et al. 1999). Climatic variables except for temperature of the hottest month and seasonality of precipitation exhibited high phylogenetic signal (Table A1). Oak defensive traits varied in their degree of phylogenetic signal, with evergreenness, summer water content, specific leaf area, and protein content of oak leaves exhibiting strong phylogenetic signal, while all other leaf traits showed weaker phylogenetic signal (Table A1).

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Because of covariation within sets of climatic variables and leaf traits, we used PCA to assess the general effects of differences in "multivariate environment" on leaf defenses. The first principle component of climatic variables ("multivariate environment") was comprised predominantly of precipitation seasonality, temperature seasonality, minimum temperature, and minimum precipitation. The first principle component of all leaf traits (DI_{ALL}) was comprised of summer SLA, summer toughness, and phenolic content. Trichomes, and leaf toughness loaded most heavily onto PC1 of physical leaf traits (DI_{PHYS}). Phenolic content and protein content (negative) loaded most heavily onto PC1 of chemical leaf traits (DI_{CHEM-1}). In preliminary analyses, PC2 of chemical leaf traits (DI_{CHEM-2}), and corresponded with various aspects of the oak environment, so we report this in further analyses. Variance in DI_{CHEM-2} was comprised mostly of condensed tannins.

Latitude as a predictor of oak leaf defense

Because of the interest in latitude as a predictor of leaf defenses and herbivore intensity (e.g. Pennings et al. 2009), we first investigated the simple relationship between latitude and defensiveness indices using univariate regression. Species mean latitude has a significant effect on all indices we investigated (DI_{ALL} : $\beta = -0.291$, $\lambda = 1.0$, $R^2 = 0.089$, $P = 0.029$, Fig. 2; all other indices (DI_{CHEM-1} , DI_{CHEM-2} , and DI_{PHYS}) are comparable in effect and significance). However, these simple regressions become insignificant when environmental covariance is taken into account using multiple regression ($\beta = -0.046$, $P = 0.778$, Fig. 2). The low cumulative model support for mean latitude (cumulative AICc weight < 0.4 for all herbivory indices; Table A2) also reflects the fact that little variance in defensiveness traits is explained by latitude once climatic predictors are factored out.

Multivariate environment as a predictor of oak leaf defense

The PC1 of oak climatic environment ("multivariate environment") predicted the total defensive investment (DI_{CHEM}) of 56 oak species, where oak species from regions with less seasonality in temperature and precipitation as well as higher minimum temperatures invested more in defenses (Figs. 1b, 2; GLS regression: $\beta = 0.10$, $P = 0.001$). An index of physical defenses (DI_{PHYS}) also increased with higher scores for "multivariate environment" (GLS regression: $\beta = 0.29$, $P = 0.003$), as did PC1, but not PC2 of chemical defenses (DI_{CHEM-1} , DI_{CHEM-2}) in oaks (DI_{CHEM-1} : $\beta = 0.24$, $P = 0.03$; DI_{CHEM-2} : $\beta = 0.13$, $P = 0.17$). The multivariate environment remained strongly predictive of total defensive investment of leaves even when analyzed in conjunction with latitude (multiple regression: ($\beta = 0.35$, $P = 0.013$, Fig. 2).

Components of the abiotic environment that explain variance in leaf defensive

investment. Cumulative AICc weights on generalized linear regression models estimated on a single phylogenetic tree were used to estimate the importance of environmental predictors (Table 1, A2). In order to obtain a better estimate of regression coefficients for each environmental variable, we also reduced the model of climatic variables to include only those with a cumulative AICc weight > 0.4 . Model-averaged standardized regression coefficients from this reduced model-set were estimated using phylogenetic generalized linear regression models over 200 bootstrap trees and are our best estimate the relative strength and direction of environmental variables in predicting leaf defensive indices (Table 1). Each index of defense (DI_{ALL} , DI_{CHEM-1} , and DI_{PHYS}) decreased as *temperature seasonality* increased (Table 1). Cumulative AICc weights indicate that DI_{ALL} , DI_{PHYS} , and to a lesser degree DI_{CHEM-1} were strongly influenced by temperature

seasonality (cum. AICc = 0.807, 0.892, and 0.413 respectively). DI_{ALL} and DI_{CHEM-1} increased as the *minimum precipitation* decreased ($\beta_{DI_{all}} = -0.180$, $\beta_{DI_{chem-1}} = -0.162$), and influence of *minimum precipitation* was stronger for DI_{CHEM-1} than for other indices (cum. AICc = 0.565). DI_{ALL} and DI_{CHEM} also increased as the *maximum temperature* increased ($\beta_{DI_{all}} = 0.311$, $\beta_{DI_{chem-1}} = 0.461$), and the influence of *maximum temperature* was stronger for DI_{ALL} than for other indices (cum. AICc=0.659). DI_{ALL} and DI_{PHYS} increased as the *minimum temperature* decreased ($\beta_{DI_{all}} = -0.491$, $\beta_{DI_{phys}} = -0.137$). *Maximum precipitation* had an effect on the second component of chemical-traits defenses (DI_{CHEM-2}), which was positively correlated with increasing precipitation ($\beta_{DI_{chem-2}} = 0.434$, AICc=0.757). This response is governed chiefly by condensed tannins, which respond almost as strongly and with equal support ($\beta = 0.352$, cumulative AICc support = 0.762; Table A2).

Components of the abiotic environment that explain variance in individual leaf traits.

For the most part, climatic niche is a poorer predictor of individual leaf traits than combined leaf traits (see R^2 values in Table A2). Striking exceptions among physical traits are summer SLA and trichome density on the leaf underside, which correlated negatively with *temperature seasonality* ($\beta = -0.360$ and -0.350 respectively; Table A2); summer toughness, which correlated negatively with *annual precipitation* ($\beta = -0.264$); and trichome density on the lower and upper leaf surfaces, which correlated negatively with mean latitude ($\beta = -0.299$ and -0.302 respectively). Among chemical traits, condensed tannins and protein content were both greater in habitats higher *maximum precipitation* ($\beta_{condensedTannins} = 0.352$) and *minimum preceipitation* ($\beta_{springProtein} = 0.295$;

Table A2). All of these individual trait responses are congruent with the responses in trait indices (Tables 1, A2).

Modeling the evolution of oak traits as a product of both phylogenetic inertia and adaptation. If traits evolved without phylogenetic inertia —i.e., if ancestral trait values had no effect on trait values of current-day samples except through correlation in selective regimes between ancestors and descendents—then the phylogenetic regressions we have already discussed would be the best estimates of the optimal fit between traits and their environment. The positive side of this is that phylogenetic inertia provides us with the data needed to estimate the rate of adaptation, as divergence from Brownian motion expectations for trait evolution serve as evidence of causal correlations between traits and environment (Hansen and Martins 1996; Butler and King 2004). While the model we utilize in this paper (Hansen et al. 2008) does not partition trait variance into a phylogenetic component and an adaptive component, it does provide a direct estimate of the effect of phylogeny on our estimates of regression coefficients by translating the phylogenetic regression into an estimate of the optimal regression, taking into account the rate of adaptation (α) and phylogenetic autocorrelation in regression residuals.

Regression coefficients estimated in the two best-supported O-U models (three- and four-predictor models) closely track the GLS estimates of regression coefficients (cf. Table 1). These models included *temperature seasonality*, *maximum temperature*, *minimum temperature*, and to a lesser degree *minimum precipitation*. The variance across O-U models in the estimate of the optimal regression coefficient is on average 40.0% lower than the variance across the same models in the phylogenetic regression coefficient estimates (Table 2), due to the fact that in the current study, the estimated rate

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of adaptation (α) increases as the absolute value of the coefficient estimate increases. The model-averaged estimates of the optimal regression coefficients are on average 33.6% greater in magnitude than the model-averaged estimates of the phylogenetic regression coefficients. Under the model-averaged estimates of optimal relationships, the magnitude of the *temperature seasonality* effect ($\beta = -1.611$) is 2.2–2.5 \times greater than the magnitude of the effect of *maximum temperature* ($\beta = 0.637$) or minimum temperature in the coldest month ($\beta = -0.730$).

The phylogenetic half-life ($t_{1/2}$, the amount of time required for leaf defenses to evolve halfway toward their optimum) ranges from 0.14 in the three-predictor model to 0.72 in the *temperature seasonality* model, where $t_{1/2}$ is scaled in tree-length units (Table 2). In the one- and two-predictor models, the two-log-likelihood-unit confidence interval extends to a phylogenetic half-life greater than 20, which suggests that the no-adaptation hypothesis cannot be rejected for those models. However, the best-supported models are the three-predictor model (AICc weights = 0.404, $R^2 = 0.453$) and four-predictor model (AICc weight = 0.396, $R^2 = 0.468$), both of which have a narrow confidence interval around $t_{1/2}$. Both of these well supported models and the model-averaged estimate of $t_{1/2}$ support an important role for both phylogenetic signal ($t_{1/2} > 0$) and adaptation ($t_{1/2} < \infty$, though any $t_{1/2}$ that is substantially greater than the total tree depth (tree depth is 1.0 in our study) implies that ancestry dominates trait values).

Discussion

The magnitude of leaf anti-herbivore defenses in oaks tracks the abiotic environment of oak species' habitats (Figs. 1b, 2b). Part of this variation corresponds to a latitudinal

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gradient in defense (Fig 2b), but an additional portion of the variation in leaf defenses is explained by environment alone. This is not the case for the portion of leaf defenses predicted by latitude, as all of the variation in leaf defenses explained by latitude can also be explained by environment (Fig 2a). This suggests that there is a latitudinal gradient in oak defenses and that this gradient driven predominantly by climatic differences between latitudes. Additionally climatic differences irrespective of latitude also influence oak defenses.

In general, oaks from less seasonal and, to a lesser extent, drier locations invest more in defenses than oaks from more temperate, wetter environments (Fig. 2; Tables 1, 2). In addition, after correcting for correlation among climatic predictors, *temperature seasonality* is the climatic variable most predictive of oak leaf traits. (Tables 1, 2). Other aspects of temperature as well as the precipitation of the driest month also play a role in the most predictive models of oak leaf defenses (Tables 1,2). These comparative patterns might result from any combination of several evolutionary processes (Endler 1982). We assess the relative contribution of two adaptive hypotheses, the resource-availability hypothesis and gradients in herbivore pressure, and two non-adaptive hypotheses (evolutionary conservatism and pleiotropy) in determining the current association of oak defenses with climatic environments.

Evolutionary conservatism of leaf defenses and climatic niche

Leaf defenses might correlate with climatic niche without being adapted to climate if there were only infrequent evolutionary transitions between different leaf defenses and habitat affiliations. Modern phylogenetic comparative methods were developed precisely because non-independence among species can cause such spurious

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correlations to appear significant if phylogeny is not accounted for (Felsenstein 1985). In oaks, however, the parallel evolution of leaf defenses in different lineages (Fig. 1b) and the strong support for correlations even assuming strong phylogenetic autocorrelation (Tables 1, 2, A2; Fig. 2) suggests that evolutionary conservatism in leaf traits cannot solely account for the association of leaf defenses with particular habitats. Nonetheless, groups of extant species tend to have similar leaf defenses and abiotic habitats (Fig. 1b), and the strong phylogenetic signal in the residuals of traits regressed on their environmental predictors suggests that evolutionary conservatism explains a significant component of the variance in trait values. The phylogenetic lag time suggested by the stochastic linear Ornstein-Uhlenbeck analyses (Table 2) along with the significant contribution of rate of adaptation as a model parameter suggests that both adaptation and phylogenetic inertia contribute to the observed interspecific trait variance.

Similarly, rare, geographically isolated events may drive current patterns in plant defense. For example, kelps that are found along the coast of North America consistently have lower concentrations of phlorotannins and experience less herbivory than kelps from the coast of Australia, a trend that has been attributed to top-down limitation of kelp herbivores by sea otters in North America but not in Australia (Steinberg et al. 1995). We might, then, see a spurious correlation between climatic niche and leaf defenses if oaks had made few transitions among habitats and levels of herbivore defenses. Oaks, however, exhibit parallel responses to climatic transitions in multiple places over multiple continents (Fig. 1A, B) and parallel adaptations to various aspects of their abiotic environment (Cavender-Bares et al. 2004). It is consequently unlikely that a geographically isolated event determined the multiple occurrences of plant defenses.

Pleiotropy of plant defensive traits as traits that ameliorate harsh environments

A second hypothesis is that leaf traits that confer defense against herbivores may also ameliorate harsh environments; the correlation between leaf traits and climatic niche, then, might have nothing to do with herbivore pressure. In any comparative or correlative study it is impossible to eliminate the possibility of pleiotropy driving the observed patterns, and that is true here. Many leaf traits that have been implicated in antiherbivory defense have also been implicated in conveying drought-, UV-, or heat-tolerance in plants. Flavonoid compounds (a class of phenolics) protect corn from UV radiation (Stapleton and Walbot 1994). Similarly, trichomes protect photosynthetically active portions of leaves of *Quercus ilex* from UV radiation (Skaltsa et al. 1994). In a common garden of cork oaks (*Q. suber*) collected from across Spain, low SLA was associated with drought-tolerant populations, and trees showed plasticity in SLA such that SLA was higher in wetter years (Ramirez-Valiente et al. 2010). Trichomes may also reduce water loss and contribute to drought tolerance (Espigares and Peco 1995).

In this study, temperature seasonality has approximately twice as much effect (estimated by beta coefficients) on the combined defensiveness index (DI_{ALL}) as maximum temperature and minimum temperature in the most extreme months and 5–18 times the effect of drought intensity (Tables 1, 2). This suggests that heat-, freezing-, or drought-tolerance alone is not driving the evolution of traits we studied. Rather, the effects of seasonality on insect intensity or resource limitation have stronger effects on the evolution of herbivore-resistance traits (Karban 2007; Coley and Barone 1996; Coley et al. 1985).

Resource limitation selects for greater investment in defensive traits

The third hypothesis is the resource availability hypothesis (RAH; Coley et al. 1985), which argues that selection for defensive leaf traits should be stronger in resource-poor environments, as the cost of herbivory is greater for plants in environments where leaf construction costs are higher. Our most strongly supported predictor of leaf defensive traits, temperature seasonality, does not represent an obvious gradient in resource availability for plants. Along gradients of temperature and precipitation, however, leaf defensive traits respond in the direction expected under the RAH. Along the precipitation gradient, physical and chemical leaf defenses increase as *minimum precipitation* decreases (i.e., in environments that pose the greatest drought stress; Tables 1, 2, A2). Likewise, the one chemical trait that is expected to favor herbivory (leaf protein content) increased marginally with increasing precipitation in the driest month ($\beta = 0.2950$, cumulative AICc weight = 0.6325; table A2). This is compatible with the RAH, as water is often a limiting resource for plants (Shields 1950). Similarly, along temperature gradients, allocation to leaf defenses increased marginally as minimum temperature decreases and as maximum temperature increases (Tables 1, 2, A2). This suggests that temperature extremes select for increased allocation to leaf defenses, which is also compatible with the RAH. These dimensions of oaks' abiotic niche are likely to affect insect density (discussed below) as well as tree growth, suggesting that it may be difficult to separate the effects of climate on insect density from selective gradients on herbivore defenses that accord with the RAH.

Seasonality drives plant defenses

In this study, we found that oak defenses were higher in regions with low seasonality (Tables 1, 2; Fig. 2). Indeed, temperature seasonality was the most consistent predictor of oak traits and defense indices in our dataset (Tables 1, 2, A2; Fig. 2). One potential explanation for a greater defensive investment in less seasonal areas would be if there were greater herbivore pressure in less seasonal areas, as optimal defense theory predicts that plants that are exposed to high herbivore pressure should invest more in defenses (Stamp 2003; Rhoades and Cates 1976). Indeed, seasonality of leaf retention has been shown to strongly affect herbivore pressure in some systems including oaks (Karban 2007, 2008). Nevertheless, differences in herbivore pressure are difficult to assess at a global scale, as different plant taxa tend to have unique herbivore associates (Weiblen et al. 2006; Ehrlich and Raven 1964). Some studies have found a robust difference in herbivore pressure between tropical and temperate zones, which differ in their temperature seasonality, but others have not (Schemske et al. 2009; Rasmann and Agrawal 2011; but see Moles et al. 2011). This study shows that temperature seasonality is the strongest climatic predictor of oak defenses.

Conclusion

In this study, we found a strong latitudinal gradient in oak defenses, where oak species from lower absolute latitudes invested more in defenses. The latitudinal gradient seems to be driven by differences in climate, and abiotic climate also explains more variance in oak defenses than latitude alone (Fig 1, Table 1). Specifically, temperature seasonality and to a lesser degree precipitation are strong climatic determinants of oak defenses (Tables 1). We cannot entirely identify the selection pressures that drove the

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correlation between environment and leaf defense (whether differences in leaf defenses are driven by pleiotropy, lack of tolerance to herbivory in resource-limited habitats, or differences in herbivore pressure). Explicit models of trait adaptation and evolutionary conservatism suggest that both recent selection as well as evolutionary history shape the current leaf defenses in oaks. Our results suggest that differences in herbivore pressure along a seasonality gradient and differences in resource availability along a drought gradient are likely factors that have shaped investment in leaf defenses. Both resource limitation and seasonality may be involved in driving the global pattern of leaf defenses in oaks.

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Figure 1: Panel A - a world map, whose background color denotes temperature seasonality (standard deviation of temperature within a year), and dots represent localities of oak collections used in this study. Dot colors denote the 5 major subgroups of *Quercus*. In oaks of different lineages exit in both seasonal and aseasonal environments multiple times. Panel B - a phylogeny of the 56 oak species used in this study. Colors of the phylogeny on the left represent an evolutionary reconstruction of a multivariate measure of leaf defenses in oaks. Colors of the phylogeny on the right represent an evolutionary reconstruction of a PCA ordination of abiotic environment experienced by oaks, which roughly corresponds to a temperate - tropical/mediterranean gradient. Estimation of either defense or environment at deep nodes is likely inaccurate due to multiple transitions in traits within the phylogeny.

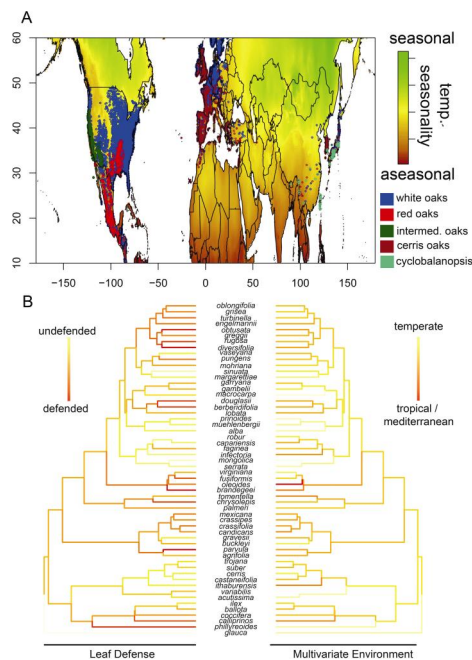


Figure 2: Scatterplots showing an index of leaf defenses (DI_{ALL}) plotted against latitude and a multivariate estimation of oak environment: Solid trend lines indicate a significant ($P < 0.05$) relationship using phylogenetically controlled GLS in a simple regression framework; bold dotted lines indicate a significant ($P < 0.05$) relationship using GLS in a multiple regression framework; small dotted lines indicate the phylogenetic error around simple regression estimates.

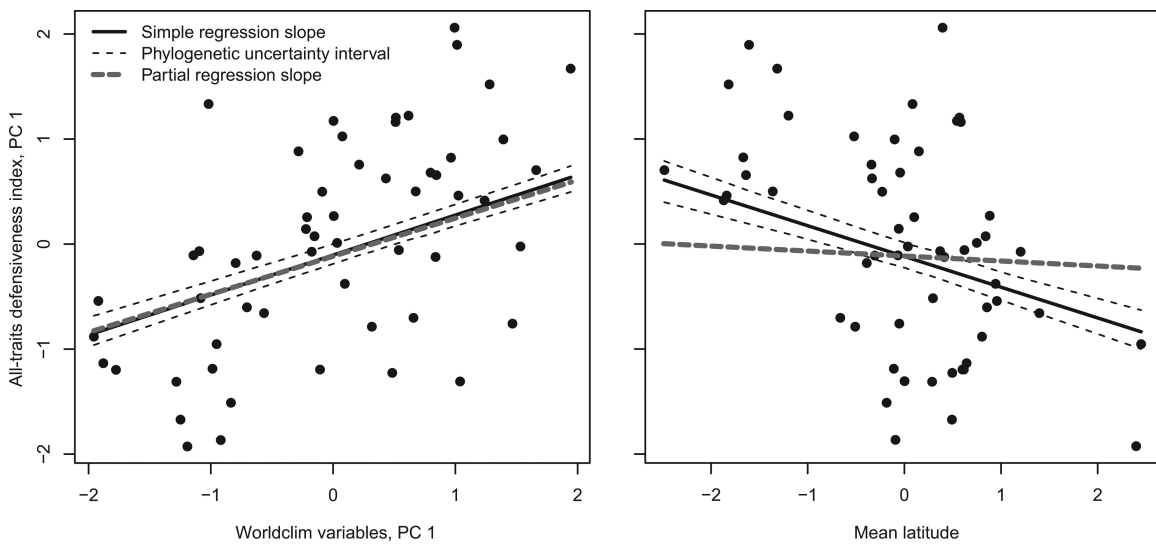


Table 1: Individual components of environment predict oak leaf defenses. GLS models that use environmental variables and latitude to predict leaf defensive traits in oaks. Model predicted either the first principal component of all oak leaf traits (DI-ALL), physical leaf traits (DI-PHYS), or chemical leaf traits (DI-CHEM1) or the second principal component of chemical leaf traits (DI-CHEM2). Regression coefficients are model-averaged over trees for which ML estimates of Pagel's lambda converged, using AICc weights for weighted averages of coefficients; the number of trees successfully analyzed for each model varies due to lack of convergence in estimating Pagel's λ on some trees. Cells without any information represent parameters for which cumulative AICc weight < 0.40, suggesting that models including that parameter are more poorly supported than models that exclude the parameter. Abbreviations: β = standardized regression coefficient over the reduced model, including only predictors supported at > 0.40 AICc support; W = cumulative AICc weight over all models allowing anywhere from 1 to 4 predictors on a fixed tree

Environmental Variable	DI _{ALL}	DI _{CHEM-1}	DI _{CHEM-2}	DI _{PHYS}
Annual Mean Temperature	–	$\beta = -0.378$ (–0.678, –0.052), W = 0.404	–	–
Temperature Seasonality	$\beta = -0.874$ (–1.294, –0.098), W = 0.807	$\beta = -0.445$ (–0.872, 0.000), W = 0.413	–	$\beta = -0.620$ (–0.763, –0.004), W = 0.892
Max Temperature of Warmest Month	$\beta = 0.311$ (–0.013, 0.499), W = 0.659	$\beta = 0.461$ (0.062, 1.101), W = 0.458	–	–
Min Temperature of Coldest Month	$\beta = -0.491$ (–0.703, 0.049), W = 0.481	–	–	$\beta = -0.137$ (–0.233, 0.345), W = 0.536
Annual Precipitation	–	–	–	$\beta = -0.140$ (–0.282, –0.012), W = 0.413
Precipitation of Wettest Month	–	–	$\beta = 0.434$ (0.419, 0.448), W = 0.757	–
Precipitation of Driest Month	$\beta = -0.180$ (–0.367, –0.077), W = 0.413	$\beta = -0.162$ (–0.375, 0.000), W = 0.565	–	–
Precipitation Seasonality	–	–	–	–
Mean Latitude	–	–	–	–
Number of trees analyzed	175 / 200	63 / 200	200/200	195 / 200

Table 2. Random covariates Ornstein-Uhlenbeck model results. The random covariates Ornstein-Uhlenbeck (O-U) model is a regression model as implemented in SLOUCH. The response variable for all models is the total defensive index (DI_{all}), and the predictors for each model are indicated in the column headers. For each predictor, the evolutionary regression coefficient (+/- standard error) is presented, followed by a the optimal regression coefficient +/- standard error in parentheses. The evolutionary regression coefficient is the regression coefficient taking phylogeny into account, while the optimal regression coefficient estimates the relationship between the predictor and response variables if there were not phylogenetic inertia. The adaptive 1/2-life estimates the amount of time (relative to a tree length of 1) required for the response variable to evolve halfway toward its adaptive optimum, and is presented as the best estimate followed by the 2-log-likelihood-unit confidence interval. The stationary variance is the estimated variance in trait value after the trait evolved fully to its optimum; stationary variance is also presented as the maximum likelihood estimated followed by the 2-log-likelihood-unit confidence interval. Abbreviations: AIC_c = small-sample Akaike Information Criterion value; t_{1/2} = adaptive 1/2-life; v_y = stationary variance.

O-U model	model support				model parameters		predictors				
	ln(L)	AICc	AICc w _i	R ²	t _{1/2}	v _y	Temperature Seasonality (BIO4)	Max Temp. Warm. Month (BIO5)	Min Temp Coldest Month (BIO6)	Precipitation Driest Month (BIO14)	
DI _{ALL} ~ BIO4	-65.1217	139.0278	0.0789	0.1642	0.72 (0.14, >20)	1.00 (0.46, >20)	-0.395 ± 0.119 (-1.105 ± 0.333)	-	-	-	
DI _{ALL} ~ BIO4 + BIO5	-63.7663	138.7326	0.0915	0.2243	0.54 (0.06, >20)	0.72 (0.36, >20)	-0.542 ± 0.136 (-1.241 ± 0.311)	0.218 ± 0.120 (0.498 ± 0.274)	-	-	
DI _{ALL} ~ BIO4 + BIO6	-64.8696	140.9392	0.0304	0.1776	0.83 (0.15, >20)	1.00 (0.43, >20)	-0.531 ± 0.191 (-1.649 ± 0.594)	-	-0.162 ± 0.181 (-0.504 ± 0.561)	-	
DI _{ALL} ~ BIO4 + BIO5 + BIO6	-61.0250	135.7644	0.4035	0.4531	0.14 (0.10, 0.29)	0.05 (0.01, 0.69)	-1.418 ± 0.247 (-1.774 ± 0.309)	0.621 ± 0.147 (0.777 ± 0.184)	-0.731 ± 0.213 (-0.915 ± 0.266)	-	
DI _{ALL} ~ BIO4 + BIO5 + BIO6 + BIO14	-59.7352	135.8037	0.3957	0.4683	0.17 (0.13, 0.49)	0.09 (0.01, 0.65)	-1.236 ± 0.285 (-1.628 ± 0.376)	0.533 ± 0.159 (0.702 ± 0.210)	-0.663 ± 0.219 (-0.874 ± 0.289)	-0.163 ± 0.119 (-0.215 ± 0.157)	
model averaged	-	-	-	-	0.255	0.231	-1.158 (-1.611)	0.481 (0.637)	-0.562 (-0.730)	-0.064 (-0.085)	

Table A1: Estimates of phylogenetic signal (lambda) for each of the environmental associations, leaf traits, and combined trait measures (principal component axes). AIC support for a model where lambda is less than 1 (i.e. a weaker phylogenetic signal) is shown. All estimates were made across 200 bootstrap trees in order to estimate the effect of phylogenetic uncertainty in lambda (95% uncertainty interval shown in parentheses)

trait	lambda	AICc weight
mean annual temperature (BIO1)	0.8363 (0.7655, 0.9083)	0.5592
temperature seasonality (BIO4)	0.8841 (0.6545, 1)	0.3660
maximum temperature (BIO5)	0.2211 (0.1686, 0.2916)	0.9663
minimum temperature (BIO6)	0.7329 (0.6406, 0.8277)	0.7051
mean annual precipitation (BIO12)	0.7689 (0.6666, 0.875)	0.6335
maximum precipitation (BIO13)	0.8034 (0.687, 0.9356)	0.5674
minimum precipitation (BIO14)	0.6891 (0, 0.8613)	0.5465
precipitation seasonality (BIO15)	0 (0, 0.0001)	0.9082
Mean Latitude	0.879 (0.7885, 0.9799)	0.4482
phenolics	0.849 (0.7235, 1)	0.3501
condensed tannins	0.1849 (0, 1)	0.6934
total tannins	0.4155 (0.0548, 1)	0.3608
peroxidase activity	0.9819 (0.9198, 1)	0.2590
Protein content	0.8754 (0.7775, 1)	0.3369
Leaf toughness	0.9037 (0.8246, 0.9704)	0.3769
Specific leaf area	0.9149 (0.8625, 0.9682)	0.3565
Bottom Trichomes	0.5034 (0, 1)	0.4566
Top Trichomes	0.1372 (0, 0.4916)	0.7881
PC1 of all traits (DIALL)	0.9984 (0.9834, 1)	0.2466
PC1 of physical traits (DIPHYS)	0.9967 (0.9566, 1)	0.2484
PC1 of physical traits (DICHEM)	0.9827 (0.9037, 1)	0.2518

Table A2: A summary of models that use environmental variables and latitude to predict each oak leaf trait (or PC combination of traits). GLS models were constructed that incorporated all possible combinations of up to 4 environmental predictors (n=255). Parameter estimates and cumulative AICc weights were averaged over all models. Additionally, the best fit model (as estimated by AICc) was identified, and its AICc weight and R² are shown. Environmental variables with AICcThe high number of models that fall within the 95% confidence interval of model-fit strongly suggests that model averaging is appropriate.

leaf trait	Best-fit model	Models in 95% confidence set	Best-fit AICc weight	Best-fit R ²	mean annual temperature (BIO1)	temperature seasonality (BIO4)	maximum temperature (BIO5)	minimum temperature (BIO6)	mean annual precipitation (BIO12)	maximum precipitation (BIO13)	minimum precipitation (BIO14)	precipitation seasonality (BIO15)	Mean latitude
PC1 all traits (DIALL)	~ BIO1 + BIO4 + BIO5 + BIO15	117 / 255	0.1048	0.3687	β = −0.1971, W = 0.3897	β = −0.7506, W = 0.807	β = 0.3600, W = 0.6593	β = −0.3008, W = 0.4809	β = −0.0076, W = 0.1465	β = 0.0020, W = 0.1163	β = −0.1297, W = 0.4132	β = 0.0761, W = 0.3453	β = −0.0254, W = 0.1646
PC1 phys. traits (DIPHYS)	~ BIO4 + BIO6 + BIO13 + latMean	87 / 255	0.0482	0.3867	β = −0.0206, W = 0.2991	β = −0.6278, W = 0.8919	β = 0.0020, W = 0.2736	β = −0.2232, W = 0.5359	β = −0.0928, W = 0.4126	β = −0.0865, W = 0.3873	β = −0.0267, W = 0.1951	β = 0.0138, W = 0.1558	β = −0.1175, W = 0.3788
PC1 chem. traits (DICHEM1)	~ BIO1 + BIO4 + BIO5	151 / 255	0.0570	0.2197	β = −0.2227, W = 0.4036	β = −0.2778, W = 0.4126	β = 0.2689, W = 0.4581	β = −0.0443, W = 0.2544	β = 0.0614, W = 0.2838	β = 0.0243, W = 0.2377	β = −0.2324, W = 0.5649	β = 0.0449, W = 0.2759	β = −0.0438, W = 0.2284
PC2 chem. traits (DICHEM2)	~ BIO13	156 / 255	0.0632	0.1765	β = 0.009, W = 0.2092	β = −0.0251, W = 0.2251	β = −0.0111, W = 0.212	β = 0.0041, W = 0.2044	β = 4e−04, W = 0.3526	β = 0.3461, W = 0.7569	β = 0.0036, W = 0.2322	β = 0.0340, W = 0.2627	β = −0.0908, W = 0.351
phenolics	~ BIO1 + BIO4 + BIO5	191 / 255	0.0365	0.1305	β = −0.0853, W = 0.3051	β = −0.1188, W = 0.3423	β = 0.1893, W = 0.5045	β = −0.0482, W = 0.2710	β = 0.0143, W = 0.2336	β = 7e−04, W = 0.2121	β = −0.1155, W = 0.4219	β = −0.0012, W = 0.2385	β = −0.0341, W = 0.2424
condensed tannins	~ BIO4 + BIO13	146 / 255	0.0495	0.2833	β = 0.0119, W = 0.2148	β = −0.0936, W = 0.3752	β = −0.0492, W = 0.3119	β = 0.0220, W = 0.2311	β = 0.0350, W = 0.3323	β = 0.3522, W = 0.7618	β = 0.0165, W = 0.2135	β = 0.0232, W = 0.2214	β = −0.1284, W = 0.4127
total tannins	~ BIO14	208 / 255	0.0332	0.0420	β = −0.0849, , W = 0.32	β = −0.0729, W = 0.3155	β = 0.0137, W = 0.2655	β = 0.0273, W = 0.2601	β = −0.0275, W = 0.2997	β = −0.0448, W = 0.2795	β = −0.0883, W = 0.3863	β = 0.0255, W = 0.2677	β = 0.0126, W = 0.2264
peroxidase activity	~ BIO15	188 / 255	0.0436	0.1045	β = 0.0385, W = 0.2392	β = 0.0635, W = 0.2709	β = −0.0454, W = 0.2580	β = −0.0046, W = 0.2202	β = −0.1099, W = 0.3518	β = 0.0213, W = 0.2805	β = 0.1612, W = 0.4986	β = −0.1155, W = 0.4489	β = 0.0195, W = 0.2199
Protein content	~ BIO6 + BIO13 + BIO14	146 / 255	0.0394	0.2544	β = 0.117, W = 0.3186	β = 0.1038, W = 0.2855	β = −0.1593, W = 0.4089	β = 0.1254, W = 0.3979	β = −0.0116, W = 0.3751	β = −0.1900, W = 0.4697	β = 0.2950, W = 0.6325	β = −0.0418, W = 0.2553	β = 0.0730, W = 0.2619
Leaf toughness	~ BIO12	157 / 255	0.0493	0.1746	β = −0.0234, W = 0.2481	β = −0.1322, W = 0.3969	β = 0.0098, W = 0.2507	β = −0.0701, W = 0.2883	β = −0.2638, W = 0.6985	β = −0.0316, W = 0.3330	β = −0.0658, W = 0.3191	β = 0.0229, W = 0.2408	β = 0.0160, W = 0.2060
Specific leaf area	~ BIO4 + BIO13 + BIO15	124 / 255	0.0607	0.3203	β = −0.0455, W = 0.243	β = −0.3603, W = 0.7962	β = −0.0238, W = 0.2539	β = −0.0187, W = 0.2577	β = −0.0788, W = 0.4803	β = −0.1696, W = 0.5017	β = −0.0336, W = 0.2301	β = 0.1578, W = 0.5076	β = −2e−04, W = 0.1507
Bottom Trichomes	~ BIO5 + latMean	124 / 255	0.0672	0.2217	β = −0.011, W = 0.3197	β = −0.3498, W = 0.6091	β = −0.1057, W = 0.4348	β = −0.1513, W = 0.3829	β = 0.0077, W = 0.1858	β = 0.0160, W = 0.1935	β = 0.0097, W = 0.1837	β = −5e−04, W = 0.1714	β = −0.2985, W = 0.6583
Top Trichomes	~ BIO5 + BIO13 + latMean	163 / 255	0.0656	0.2089	β = −0.0616, W = 0.2647	β = −0.1813, W = 0.4572	β = −0.1476, W = 0.4950	β = 0.0353, W = 0.2555	β = −0.0054, W = 0.2270	β = −0.1207, W = 0.4126	β = −0.0153, W = 0.1877	β = −0.0271, W = 0.2065	β = −0.3022, W = 0.6337