

Phylogenetic and trait similarity to a native species predict herbivory on non-native oaks

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Introduced plants tend to experience less herbivory than natives, although herbivore loads vary widely. Herbivores may switch hosts onto an introduced plant for at least two reasons. They may recognize the novel plant as a potential host based on similarity of the plant's traits to the traits of one of its native hosts, a similarity that may or may not exhibit phylogenetic signal. Alternatively, herbivores may feed optimally, assessing which introduced plants provide the best nutrition irrespective of similarity to native species. Here, we created a phylogeny of 57 oak (*Quercus*) taxa, which were grown outside of their ranges in a common botanical garden that contained one abundant native oak (*Quercus lobata*). We used the phylogeny to estimate the phylogenetic conservatism of herbivory by two feeding guilds of insects (leaf chewers and leaf miners) and 11 plant traits expected to affect herbivore performance. We found high phylogenetic signal in chewing damage but not mining damage and all traits except for leaf maturation time. Introduced oaks that are more closely related to the native oak received more chewing and mining damage than distantly related oaks, and introduced oaks that had greater overall similarity in leaf traits also received higher chewing damage but not mining damage. These results demonstrate that interactions between introduced plants and their herbivores are driven independently by traits that track plant phylogeny and leaf traits that likely affect herbivore performance.

community phylogeny | enemy release | introduced | plant defense | *Quercus*

Plants that are introduced to regions outside of their natural ranges interact with the organisms that are already present in those regions, usually in the absence of an evolutionary history of interaction. Non-native plants may compete for resources, alter physical properties of their new ranges, and act as hosts for native herbivores (1). Understanding the interactions between an introduced plant and the local pool of herbivores has the potential to illuminate both the dynamics of economically significant non-natives, such as invasive species (2) and agricultural crops (3), and fundamental questions about the evolution of interactions between organisms that lack a history of coexistence (4). Indeed, although shared herbivores are not specifically invoked in the discussion that became known as Darwin's naturalization hypothesis (5), recent authors have suggested that herbivore sharing may complement competitive exclusion in geographically partitioning closely related species (6). Using non-native plants as a model, we tested long-standing evolutionary predictions about the relationships among phylogenetic affinities, plant traits, and host association by herbivores (7).

Over the past 50 years, ecologists have used the hypothesis of "enemy release" to understand and manipulate interactions between introduced plants and herbivores (8). The enemy release hypothesis posits that fewer herbivores use a plant in its introduced range than in its native range and that this release from herbivory may lead to the plant having higher performance or becoming invasive. In this model, the herbivores in the introduced range of the plant are less able to use it as a resource than the insects in the plant's native range because they are not adapted to the plant. Recent studies, however, have shown that some introduced plants

can receive at least as much herbivory as closely related native plants and that the relationship between introduced status and degree of herbivory may change from one system to the next (9, 10).

Why do herbivores interact with some non-native plant species but not others? For herbivores to use a novel host they must be able to both recognize the introduced plant as a potential host and profitably digest the plant (11). In one scenario, often associated with specialist herbivores, an herbivore may not distinguish the introduced host from its native host. In this case non-native plants that are more similar to a native host will receive more herbivory than unique non-natives, and the predictor of herbivore damage will be trait similarity to a recognized native species. In another scenario, typical of generalist herbivory, an herbivore may simply assess the utility of a novel host without reference to its native hosts. In this case, non-native plants with traits that reduce herbivore performance will receive less herbivory than those plants that are more readily exploited by the herbivore, irrespective of their similarity to the native host, and the predictor of herbivore damage is likelier to be a combination of traits, potentially irrespective of trait similarity to the native species. In both of these cases the evolutionary history of the plant's traits will pattern herbivory across the phylogeny of non-natives (12); however, in the case of specialist insects, the similarity of traits (13), or potentially the proxy of shared evolutionary history (7), between the native and non-native should predict the amount of herbivory on non-native species.

We assess herbivory and leaf traits that may affect herbivory on 57 species of introduced oaks in a common botanical garden in the Central Valley of California, where there is one dominant native oak species, *Quercus lobata* Née. We create a molecular phylogeny for the genus *Quercus* and use it to ask whether non-native trees that are more closely related to *Q. lobata* receive more herbivory than more distantly related *Quercus* species. We assess whether trees that are closely related to *Q. lobata* have more similar traits that could affect herbivore performance than more distantly related *Quercus* species and to what extent phylogenetic signal in host traits accounts for observed herbivory. We also assess whether herbivores feed on introduced plants based on the digestibility (or low level of defense) of the introduced oak and estimate the evidential support for the relative effect of each of 11 plant traits on herbivory, using a phylogenetic generalized least squares (GLS) multiple regression approach in an information theoretic framework.

Results

Description of Traits and Evolutionary Relationships. Using a combination of minimum evolution (ME) (distance-based) and

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Data deposition: The data matrix and AFLP phylogeny have been deposited in TreeBase, www.treebase.org (study accession no. S2405; matrix accession no. M4552).

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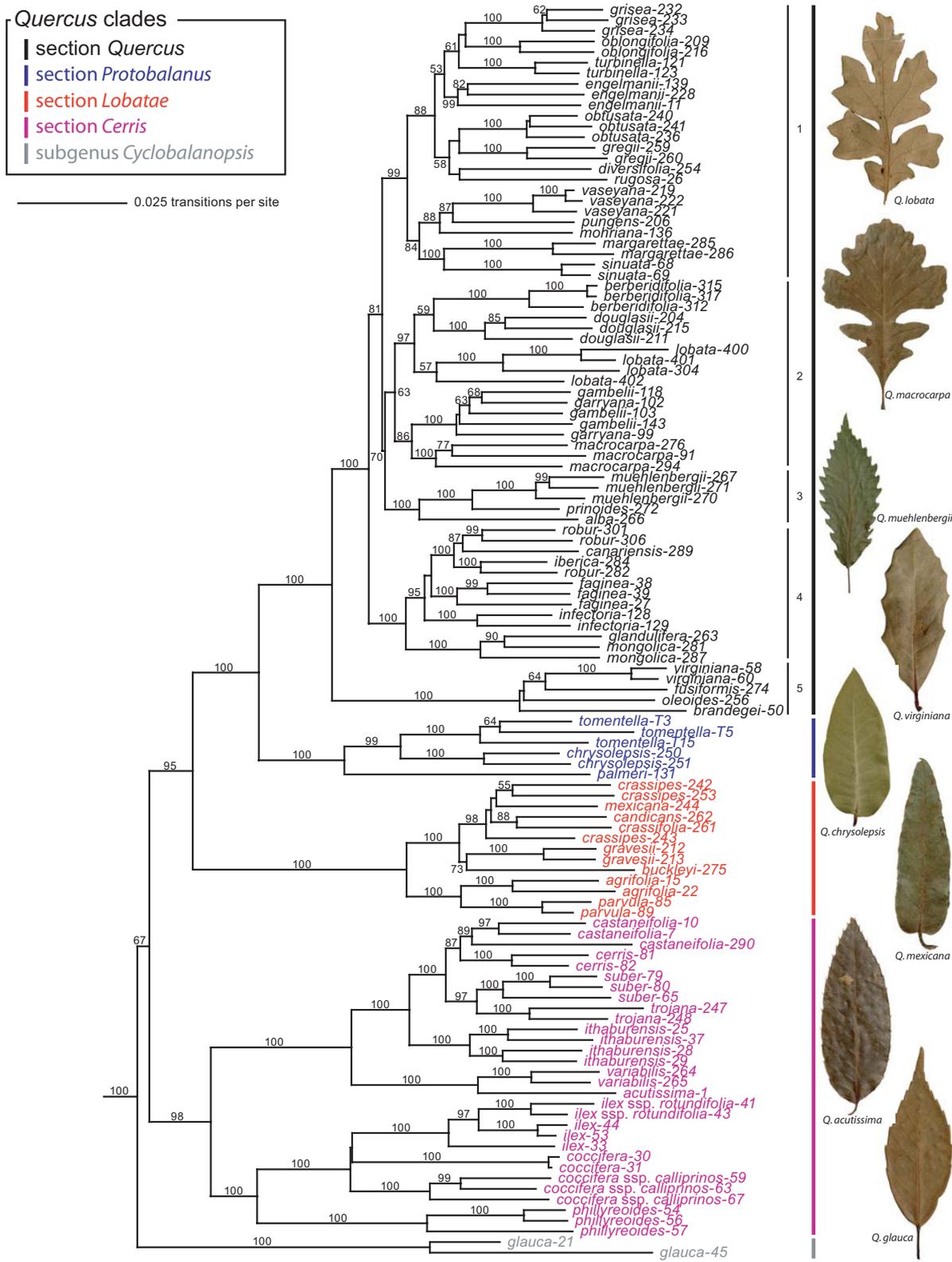


Fig. 1. A ME tree of *Quercus* based on AFLP data. Support values based on nonparametric bootstrap analysis are shown. All recognized major clades of oaks (48) are well-supported.

Bayesian (character-based) phylogenetic analysis of 2,932 amplified fragment-length polymorphism (AFLP) markers based on 14 primer pairs, we estimated the phylogeny of 57 *Quercus* species growing in the University of California-Davis Shields

Oak Grove (Fig. 1 and see *SI Methods* in *SI Appendix* for analysis details). Bootstrap support values and Bayesian posterior probabilities are high at a wide range of phylogenetic depths, indicating high phylogenetic certainty for the topology reported,

Table 1. Description of herbivore damage types and oak leaf traits

Damage/trait	r^2	λ
Damage		
Chewing	0.869	1.0000 \pm 0.0000 ($P = 1.0000$ [1.000])
Mining	0.659	0.1541 \pm 0.0010 ($P = 0.0096$ [0.134])
Traits		
Maturation time	0.653	0.3984 \pm 0.0021 ($P = 0.0067$ [0.094])
Bud break	0.886	0.7160 \pm 0.0032 ($P = 0.0991$ [1.000])
Evergreenness	0.944	0.9950 \pm 0.0010 ($P = 0.9646$ [1.000])
Toughness	0.842	1.0000 \pm 0.0000 ($P = 0.9998$ [1.000])
Percent H ₂ O	0.815	1.0000 \pm 0.0000 ($P = 1.0000$ [1.000])
Specific leaf area	0.846	1.0000 \pm 0.0000 ($P = 1.0000$ [1.000])
Phenolics	0.701	0.9886 \pm 0.0017 ($P = 0.9586$ [1.000])
Condensed tannins	0.871	0.2932 \pm 0.0292 ($P = 0.3352$ [1.000])
Tannins	0.757	0.1784 \pm 0.0258 ($P = 0.5685$ [1.000])
Peroxidase	0.714	0.9623 \pm 0.0026 ($P = 0.8124$ [1.000])
Protein	0.793	0.9171 \pm 0.0042 ($P = 0.6796$ [1.000])
All 11 traits	n/a	0.8040 ($P = 0.0084$ [0.067])

The proportion of variance in damage type or trait explained by among-species differences is estimated by r^2 . The degree of phylogenetic signal in each damage type or trait is estimated by Pagel's λ (ranging from 0 = character variance is not predicted by the phylogeny, 1 = all variance in the character can be explained by phylogenetic signal under a Brownian motion model of character evolution). P values are from a likelihood ratio test comparing an unconstrained model in which λ is estimated from the data to the Brownian motion model, in which $\lambda = 1$; P values in brackets are Bonferroni-corrected ($N = 13$ tests). Both λ and P were estimated on 200 nonparametric bootstrap replicate trees, and λ is reported as mean \pm phylogenetic SE as a way of assessing the effect of phylogenetic uncertainty on parameter estimates. n/a, not applicable.

and the two analyses are broadly congruent (Fig. 1 and Fig. S1 in *SI Appendix*). Our analyses are also congruent with the large-scale topologies supported by recent oak phylogenetic work based on a combination of nuclear DNA regions (14). Moreover, multiple accessions of most species clustered together with high support, suggesting that our AFLP data are recovering the oak species identified by traditional oak taxonomy (Fig. 1). The results of comparative analyses were robust to different methods for estimating branch lengths on an AFLP phylogeny (Table S3 in *SI Appendix*), and standard errors on comparative analysis results conducted over a subset of 200 bootstrap trees indicate that phylogenetic uncertainty has negligible effect on our conclusions. The identity of oak species explained a significant amount of the variation (65–87%) in the amount of herbivory received from each type of herbivore and oak leaf traits (Table 1; ordinary least-squares ANOVA). Leaf traits and herbivory predominantly showed high phylogenetic signal as estimated by using Pagel's λ , indicating that these traits exhibit a degree of phylogenetic signal consistent with evolution according to a constant-variance [in the sense of Pagel (15)], Brownian motion process (Table 1 and Table S4 in *SI Appendix*). Under a Brownian motion process, character states of sampled taxa are nonindependent, with expected covariance between taxa a linear function of the recency of their most recent common ancestor. Only leaf maturation time ($\lambda = 0.398 \pm 0.002$; the SEM reported here and hereafter reflects phylogenetic uncertainty in the parameter point estimate) and leaf mining ($\lambda = 0.154 \pm 0.001$) exhibited a substantially reduced phylogenetic signal, but Bonferroni-corrected P values are nonsignificant even for these traits ($P > 0.05$ in a standard likelihood ratio test, where the constrained mode ($\lambda = 1$, Brownian motion model of phylogenetic signal) is nested within the unconstrained model). For all 11 traits in combination, multivariate $\lambda = 0.804$ (Table 1).

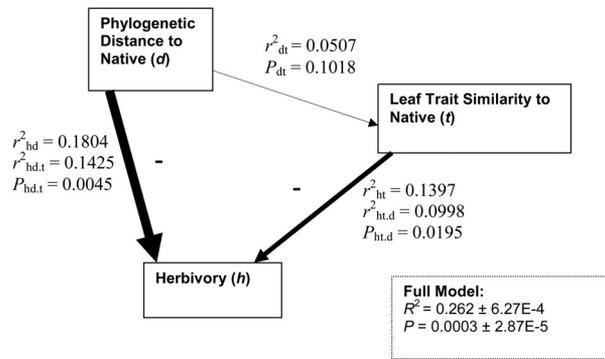
Effect of Oak Relatedness and Trait Similarity to the Native on Herbivory. Both the phylogenetic relationship and the similarity of defensive traits between non-native oak species and the native oak (*Q. lobata*) were predictive of the degree of chewing-guild herbivory that non-native oaks received in the common garden (Fig. 2). Phylogenetic proximity to the native predicted 18.04% (± 0.00 , $P = 0.001$) of the total variance in chewing damage; this relationship

remained after controlling for trait similarity by using the matrix of 11 traits we measured (partial $r^2 = 0.143 \pm 0.001$, $P = 0.004$; Fig. 2). Overall trait similarity to the native also predicted a significant, although slightly lower, component of the total variance in chewing damage (simple $r^2 = 0.140 \pm 0.000$, $P = 0.004$; partial $r^2 = 0.100$, $P = 0.020$). Phylogenetic similarity to the native species predicted a low and nonsignificant component of the total variation in trait similarity to the native (simple $r^2 = 0.051 \pm 5.3E-5$, $P = 0.102$). However, given the high phylogenetic signal of each of the predictors separately and in combination (multivariate $\lambda = 0.8040$), the fact that the correlation is not significant is likely because the comparison of trait similarity to a particular (native) species deals with a relatively small set of the total pairwise distance matrix, whereas global phylogenetic signal uses all pairwise distant estimates. This explanation is consistent with the fact that a Mantel permutation on the same regression applied to the entire pairwise trait and phylogenetic distance matrices for the tree likewise shows small, but moderately significant, correlation (simple $r^2 = 0.021 \pm 3.3E-05$, $P = 0.042$).

Mining damage was also predicted by phylogenetic proximity to the native oak, even after correcting for trait similarity (partial $r^2 = 0.126 \pm 0.001$, $P = 0.005$), but not by trait similarity after correcting for phylogenetic proximity (simple $r^2 = 0.072 \pm 0.000$, $P = 0.049$; partial $r^2 = 0.040 \pm 0.000$, $P = 0.144$; Fig. 2). These trends were robust to topological or branch length differences that arose between phylogenies inferred by using ME or Bayesian techniques (Table S5 in *SI Appendix*).

Effect of Oak Traits on Herbivory Without Reference to the Native. We estimated the relative importance of 11 oak leaf traits in predicting chewing and mining damage by using a series of 561 GLS regression models. The models evaluated include all permutations of one to four of the oak leaf traits and incorporated nonindependence of phylogenetic traits by assuming a Brownian motion model of character evolution ($\lambda = 1$). We report two estimates of parameter importance: the cumulative small-sample Akaike information criterion (AICc) weight for each parameter over all GLS models in which that parameter appears; and the standardized (or β) GLS regression coefficients based on a multiple regression using all 11 predictors. The standardized regression coefficients represent the amount of change in leaf herbivory expressed in SD caused by a

A Chewing Damage



B Mining Damage

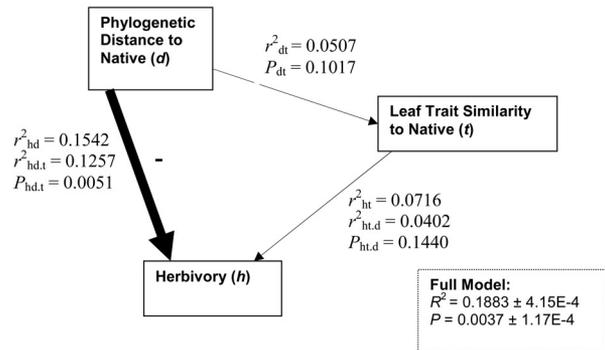


Fig. 2. Damage to introduced oaks caused by herbivory from chewing (A) or mining (B) insect feeding guilds as predicted by the phylogenetic distance [d : pairwise patristic (branch-length) distance] between the non-native and native oak (*Q. lobata*) and trait dissimilarity (t : pairwise Euclidean distance based on 11 leaf traits) of the introduced oak to the native. The simple correlation (r^{2yx} , where y is the response and x is the predictor) and partial correlation ($r^{2yx,w}$, where y is the response, x is the predictor, and w is a potential covariate whose effect on both y and x is accounted for before assessing the correlation) of each path was estimated. Significance of r^2 (one-tailed P values) was assessed by using a phylogenetic permutation test, where the null hypothesis simulated is no correlation between the damage measurement for each species and the trait or phylogenetic distance. Phylogenetic uncertainty was incorporated by running all analyses >200 bootstrap replicates of the oak phylogeny, and the correlation and P value shown are averaged over bootstrap replicates. Total model correlation and P value are reported as mean \pm SEM, where the error is caused by topology and branch length differences among bootstrap replicates. The width of each path arrow is proportional to the strength of the relationship. A plus or minus sign along a path indicates the direction of any significant correlations.

change of 1 SD in each predictor variable. Cumulative AICc weights estimate the relative evidential support for the importance of each predictor in explaining the observed damage, taking into account the expected covariance among traits and degree of damage assuming a Brownian motion model of trait evolution.

Leaf maturation time and evergreenness most strongly explained mining damage in the GLS models (cumulative AICc weights = 0.463 and 0.356, respectively; β coefficients = -0.228 and -0.229 , respectively; Table 2). Phenolics also play an important role (AICc weight = 0.225, β coefficient = 0.146; Table 2). Chewing damage was best explained and most affected by condensed tannins (cumulative AICc weight = 0.718, β coefficient = -0.271); all other predictors had relatively low explanatory power, although total tannins, specific leaf area, and day of bud break all had strong effects on chewing damage (β coefficients = 0.145, -0.152 , and -0.205 , respectively). These effects were robust to

topological or branch length differences that arose between phylogenies inferred by using ME or Bayesian techniques (Table S6 in *SI Appendix*).

Discussion

This study demonstrates that herbivory on introduced plant species is a function of their phylogenetic similarity to a local native. Leaf traits that are typically viewed as affecting insect performance, e.g., tannin content and specific leaf area, also affected the degree and type of herbivory on introduced plant species. However, phylogenetic similarity still predicted a significant portion of the variance in herbivory even when the effects of trait similarity to a native species were accounted for; for leaf mining, it predicted almost all of the explained variance.

Phylogenetic proximity to a native species and trait similarity to that species were poorly correlated with one another in this study, despite the fact that they both predicted some variance in herbivory. These facts in combination suggest either that the leaf traits that we measured (i.e., traits traditionally thought to influence herbivory) are poor predictors of a plant's utility to herbivores, or herbivores cue in on a complex set of traits in recognizing plants species, including traits unmeasured in this study that may not directly influence edibility but that accurately predict the relatedness of non-native oak species to the native oak. Moreover, our study may help explain observed trends of phylogenetic overdispersion of plant communities (16, 17), because shared herbivores may prohibit closely related plants from co-occurring geographically.

Although broad patterns of taxonomic conservatism in host preference have long been recognized (18, 19), our study shows fine-scale conservatism among plants within the same genus. By observing herbivory on oaks that are relatively recently displaced from their native range, our study minimizes the erosion of initial recognition signals between insects and edible plants during the coevolution between insect and host. By observing host switches of insects onto non-native taxa, it is possible to determine what plant traits convey the identity of the native host to specialist insects. Our study finds that non-native oaks that are more related to the native oak suffer more herbivory, and that the leaf traits typically studied with reference to insect performance (e.g., tannins, protein content, defensive enzymes) do not capture the majority of variance in herbivory.

Plant traits that affect herbivore success are important mechanistic determinants of herbivory across different species of plants (20). In our study, a suite of 11 plant traits was predictive of chewing damage when using a phylogenetically corrected multiple regression approach (Table 2) and when reduced to a single trait distance between each non-native oak and the native *Q. lobata* (Fig. 2). This trend was significant even after correcting for phylogenetic distance between the native and introduced tree, suggesting that multivariate trait similarity in oaks is an important predictor of herbivory that does not track phylogeny as closely as the underlying individual traits [compare Becerra's observations of leaf beetles on different species of trees in the genus *Bursera* (13)]. Leaf traits in our study were less predictive of mining damage than they were of chewing damage, probably because mining feeding styles bypass the defenses measured in this study. Our finding that measures of similarity to a native and measures of quantitative leaf defenses are predictive of damage by native herbivores to non-native oak species may reflect a general pattern of response by native insect communities to novel plants, because local insect communities contain a mixture of both generalist and specialist herbivores, which likely differ in how they assess potential hosts.

How is phylogenetic conservatism of herbivores related to plant traits, and what does this mean for macroevolutionary trends in plant-insect interactions? As the plant defensive traits assayed in this study explain a surprisingly small component of the total variance in herbivory (and less than phylogenetic similarity), there must be traits or other factors not investigated in this study that

Table 2. Standardized regression coefficients based on the multiple (all-predictors) model and cumulative small-sample AICc weights for including each of 11 herbivory predictors summed over all models including one through four parameters, based on a GLS model of the effect of oak leaf traits on herbivory by chewing and mining feeding guilds

Trait	Chewing		Mining	
	Standardized coefficient	AICc weight	Standardized coefficient	AICc weight
Condensed tannins	-0.271±0.001	0.718±0.004	0.003±0.001	0.209±0.001
Total tannins	0.145±0.001	0.275±0.002	-0.048±0.001	0.195±0.001
Specific leaf area	-0.152±0.002	0.273±0.002	0.090±0.002	0.202±0.001
Day of bud break	-0.205±0.001	0.270±0.001	0.016±0.001	0.227±0.001
Evergreenness	0.030±0.001	0.206±0.001	-0.229±0.002	0.356±0.004
Phenolics	-0.038±0.001	0.201±0.001	0.146±0.002	0.225±0.001
Peroxidase	-0.109±0.001	0.198±0.001	-0.070±0.001	0.224±0.001
Protein content	0.036±0.001	0.198±0.001	0.094±0.001	0.199±0.001
Leaf toughness	0.021±0.001	0.193±0.001	-0.135±0.001	0.260±0.001
Maturation time	-0.070±0.001	0.189±0.001	-0.228±0.001	0.463±0.003
Percent H ₂ O	0.028±0.001	0.185±0.001	-0.033±0.002	0.207±0.001

Standardized regression coefficients estimate the relative effect of each predictor on the response, scaled in units of SD; cumulative AICc weights estimate the importance of each parameter in explaining the predictor over a wide range of possible models. Parameter estimates are presented as the mean over 200 bootstrap replicate trees ± phylogenetic SE.

accurately convey phylogenetic proximity to a native species or edibility of introduced oaks to their potential herbivores. Oaks are thought to have classic quantitative defenses (21), which have been shown to be convergent across broad taxonomic groups (20). As a consequence, plant traits involved in host recognition, such as leaf volatile chemicals, may be more phylogenetically informative to insects than the classic defensive traits. If this is the case, however, phylogenetic niche conservatism by herbivores on non-native oaks will often result in suboptimal foraging, where recognition cues do not accurately predict the quality of the leaf material as food.

The disconnect between well-established plant defensive traits and less obvious plant traits that accurately convey plant phylogeny to insects may help to explain why phylogenetic similarity of natives to introduced species has been a robust predictor of invasiveness (6) that may be more tractable than metrics of plant traits (22). This study finds that phylogenetic similarity to a native is a robust proxy for an unknown suite of traits that promote herbivory by local insects. We conclude that classical leaf defensive traits do not on their own drive the phylogenetic niche conservatism that we observe in the oak herbivore community, but that they predict an independent component of herbivory on non-native oaks.

Methods

Field Site. Tree traits, herbivory measures, and genetic information were collected from oak trees at the Shields Oaks Grove of the University of California-Davis Arboretum. Three mature (acorn-bearing) individuals of each of 60 species of oaks were chosen for this study, except in cases where <3 trees per species were present at the site. Trees used in this study were planted from 1963 to 1996. Collection notes for each oak individual in this study are found in Table S1 in *SI Appendix*. *Q. agrifolia* and *Q. suber* have existed in the Central Valley for over a century as ornamental trees; these trees were treated as non-native despite being present for a longer time in this region. Morphologically aberrant taxa were removed from all analyses (*SI Methods* in *SI Appendix*).

Herbivory Measures, Herbivore Natural History, and Plant Traits. Cumulative herbivory over the 2008 growing season was assessed in August 2008 on 20 leaves per oak individual between a canopy height of 2 and 6 m (except on trees that did not reach the height of 2 m) and was recorded as mean percentage of leaf damage by using a transparent grid. Herbivory types were recognizable as “chewing” or “mining.” Tree phenology (evergreenness, bud break, and leaf maturation time) were estimated by observing trees every 2 days between February 15, 2008 and May 12, 2008. Evergreenness class was estimated as [percentage of green leaves remaining on February 15 (1: 0%; 2: 1–50%; 3: 51–99%; 4: 100%)]. Leaf maturation time was estimated as days between bud break and full leaf expansion.

Herbivores were collected at the University of California-Davis Arboretum during the summers of 2007, 2008, and 2009. When possible they were reared from larvae feeding on the oaks, and additional specimens were collected by blacklighting. Herbivore feeding type was either observed directly or inferred

from the historical collections and literature (23, 24) and host breadth was inferred from the same literature. Common insects responsible for chewing damage on the oaks included lepidopterans: *Archips argyrospila* Walker and *Epinotia emarginata* Walsm., native tortricids known predominantly from oaks, but also from other tree species; *Chionodes trichostola* Meyrick, a native gelechiid that feeds on multiple sections of *Quercus*; *Ypsolopha cervella* Walsm., a native pluteid that feeds on multiple species of oaks within the red and white groups. Insects responsible for mining damage include native Leptidoptera within the family Gracillariidae (probably *Cameraria* sp. and *Caloptilia* sp.). Historical insect collections indicate the presence of known oak-feeding lepidopterans, *Stigmella* sp. [Nepticulidae]; *Lithocollitis* sp. [Gracillariidae]; *Chionoides restio*, *C. powelli* [Gelechiidae], and *Henricus umbrabasana* [Tortricidae] within 2 km of the study site; although their presence at the study site could not be confirmed, they are likely part of the herbivore fauna at the study site.

Fresh leaf material was bulk collected from at least five leaves per oak individual and ground in liquid nitrogen. Ground leaf material (100 μL) was suspended in 1 mL of 70% acetone. After 1 h, the insoluble material was removed by centrifugation. Relative total phenolic concentration was estimated by using a colorimetric Prussian Blue assay (25) with the modifications of Graham (26). Leaf extract (100 μL), 3 mL of dH₂O, 1 mL of 0.02 M K₃Fe(CN)₆, and 1 mL of FeCl₃ were mixed. After exactly 10 min, 5 mL of 0.2% Gum Arabic in 17% phosphoric acid was added and the absorbance was determined at 700 nm. Total tannins were estimated by using a radial diffusion assay of tannin-mediated protein precipitation (27). Leaf extract (16 μL) was placed in a 4-mm hole in a ≈2-mm-thick, 1% (wt/vol) agarose plate with 0.1% (wt/vol) BSA. Plates were incubated at room temperature and the area of precipitated proteins was measured with calipers after 96 h. Tannin concentrations were standardized to tannic acid and reported as mg tannins/g fresh weight leaf. Relative condensed tannin concentration was estimated colorimetrically after reaction with acidified butanol in the presence of Fe³⁺ salts (28). Leaf extract (150 μL) was incubated with 900 μL of acidified butanol and 30 μL of 2% FeNH₄(SO₄)₂ in 2 M HCl at 100 °C for 50 min., cooled to room temperature. Absorbance of the mixture was read at 550 nm. Ground fresh leaf tissue (100 mg) was suspended in 1.5 mL of 0.1 M potassium phosphate buffer (pH 7) with 7% polyvinylpyrrolidone. Insoluble tissue was removed and the supernatant (10 μL) was assayed for soluble proteins with Bradford reagent. Ground fresh leaf tissue (100 mg) was suspended in 1.5 mL of 0.1 M potassium phosphate buffer (pH 7) with 7% PVPP and 3% Triton-X (Fisher). Insoluble material was removed and 30 μL of supernatant was added to 270 μL of 0.1 M potassium phosphate (pH 8) with 27 mM guaiacol and 12 mM H₂O₂. Peroxidase activity was recorded as Δ(Absorbance_{470nm})/min. Leaf toughness was recorded on three leaves per tree with a penetrometer (Chatillon & Sons) as (g/3 mm ø needle). Specific leaf area was measured as g/cm² dry weight. Water content was measured as (fresh weight – dry weight)/fresh weight.

Phylogeny Estimation. Phylogeny of 58 oak species was estimated by using Bayesian Markov chain Monte Carlo (29) and ME (30) methods on an AFLP dataset (Fig. 1). DNA was extracted from lyophilized leaf tissue by using DNeasy (Qiagen) or cetyltrimethylammonium bromide (30) techniques. AFLP protocols were performed by using standard procedures optimized for *Quercus* (31, 32). Fifteen primer pairs (Table S2 in *SI Appendix*) were selected based on prior screening of 128 primer pairs in *Quercus* (32). Fragment analysis was performed on an Applied

Biosystems 3730 capillary sequencer with a 6-carboxyl-x-rhodamine (ROX) -labeled internal lane standard (GeneFlo 625; CHIMERx). Alignment, binning, and scoring of fragments between 70 and 550 bp were conducted with GeneMapper version 3.7 (Applied Biosystems). Fragments were scored as present or absent, and the resulting binary data matrix was used to calculate a pairwise genetic distance matrix by using Nei and Li's (49) restriction site distance, which is derived from the probability of two individuals inheriting the same copy of the recognition sites flanking an AFLP fragment, based on the Jukes-Cantor (33) model of nucleotide substitution (see discussion in refs. 34 and 35). Trees were constructed by using ME (36) and tree bisection and reconnection branch swapping on a neighbor-joining start tree (Fig. 1). Clade support was estimated by using 2,000 nonparametric bootstrap replicates under the same search parameters. Trees were also estimated by using the two-state Bayesian model implemented in MrBayes (37) allowing for unequal stationary distributions of the two-state AFLP characters (Fig. S1 in *SI Appendix*).

Statistical Analysis. To account for phylogenetic uncertainty, all phylogenetic comparative analyses were conducted on a subsample of 200 trees from the ME bootstrap sample. Each bootstrap tree was pruned at random to one representative per species. Phylogenetic signal was estimated for each of 11 traits by using Pagel's phylogenetic dependence estimator (λ) (38, 39). Multivariate phylogenetic signal for all 11 traits in combination was estimated on the single ME tree for computational reasons using the method of Freckleton et al. (39) as implemented in the program PCCA (40). The importance of each trait for herbivore damage was assessed by using GLS multiple regression, with trait importance estimated by summing information criterion weights over all fitted models (41) and covariance in trait values among taxa estimated from the ME bootstrap trees assuming a Brownian motion character model. To ensure that parameters were equally weighted, all 561 possible models that allow at least one and at most four predictors were fitted, with each parameter occurring in 176 of these. We report small-sample AICc weights, which are not susceptible to the dimension constancy

assumption of the Bayes information criterion and converge on the standard AIC weight at large sample sizes (41). All variables were standardized to mean = 0 and unit variance. Additional analyses were conducted to assess the sensitivity of our conclusions to branch-length estimates and model complexity (SI S5). Collinearity of plant traits as predictors of herbivory was assessed by using the variance inflation factor statistic and was determined to be unproblematic. Finally, the effects of phylogenetic and trait distance to the native species (*Q. lobata*) on variance in the amount of herbivore damage sustained among non-native species were estimated by using partial and multiple regression. Significance of the multiple and partial coefficients of determination (R^2 and r^2 , respectively) was assessed by using a phylogenetic permutation test. The distribution of the test statistic was simulated under the null hypothesis of no correlation between the damage measurement for each species and the trait or phylogenetic distance by permuting the damage vector while leaving the phylogenetic and trait distance vectors in observed order and calculating the test statistics for each permutation. Significance (one-tailed P value) was estimated as the percentage of permutations in which the test statistic was equal to or larger than the observed value. *Q. lobata*, the native oak, was excluded from all comparative analyses. Partial and multiple regression were performed by using standard methods (42), and all analyses were conducted in R version 2.8.1 (43), using the ape, geiger, nmlme, and vegan packages (44–47).

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