

Phenotypic Variation in California Populations of Valley Oak (*Quercus lobata* Née) Sampled Along Elevational Gradients¹

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Abstract

California oaks exhibit tremendous phenotypic variation throughout their range. This variation reflects phenotypic plasticity in tree response to local environmental conditions as well as genetic differences underlying those phenotypes. In this study, we analyze phenotypic variation in leaf traits for valley oak adults sampled along three elevational transects and in young seedlings grown from their acorns in greenhouse conditions. This project represents the early phase of a long-term provenance study of valley oak (see Delfino Mix and others, Establishing a range-wide provenance test in valley oak (*Quercus lobata* Née) at two California sites, these proceedings) where we subsample the maternal families sampled along elevation gradients to assess the extent to which selection may be shaping geographical variation in leaf phenotypes of valley oak. We find that leaf thickness is the trait that shows correlations in adult populations in the field and significant variation in the greenhouse. For this trait, we also found significant differentiation across maternal families, providing preliminary evidence that this trait experiences differential selection across natural populations with different environments. This study, part of a large-scale provenance/genomic study of the genetic basis of phenotypic variation, provides an early analysis of leaf traits that may play an important role in local adaptation to climate environments and provide background for management and restoration of California oak forests.

Key words: elevational transect, phenotypic variation, *Quercus lobata*, valley oak

Introduction

Phenotypic variation, a fundamental attribute of all organisms, is the product of genetic variation and environmental variation, as well as their interactions. This variation reflects the outcome of long-term selection and the response to local environmental conditions. The study of phenotypic variation is one way to investigate the role of natural selection on phenotype due to the effect of local climate on tree populations (Endler 1986, Hedrick 2006). Phenotypic variation can be influenced by both genetic and environmental factors in natural populations, creating patterns of geographic variation in adaptive plant traits (for example, Rehfeldt and others 1999). Elevational gradients offer an opportunity to evaluate the extent of local adaptation in response to climate over small spatial scales. In a classic example, Clausen and others (1948) showed that *Achillea* phenotypes, such as growth form, size and leaf length, were associated with elevation and populations and that plants

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grew better at their home elevation than in other parts of the range, suggesting local adaptation. Evidence for local adaptation, particularly in response to climate, will be important for understanding species response to climate change. Populations in different parts of a species' range and in different microhabitats will experience and respond to climate change differently (Davis and others 2005, Rehfeldt and others 2002, Rehfeldt and others 2006). This differential response is due to both the genetic composition of local populations and the magnitude of climate change, which will vary geographically as well (Gugger and others 2013, Sork and others 2010).

In this study, we document gradients in leaf traits in adults sampled along elevational transects in order to observe how phenotypic variation changes along steep climate gradients. Because these phenotypes represent the impacts of genes, environment, and their interaction, we then plant offspring of the adults in a common greenhouse environment to assess the genetic contribution. We present the findings from the first year of a newly established long-term study to gain an initial analysis of what leaf traits may have a strong genetic component and which ones may reflect environmental conditions. Specifically, we have three objectives: (1) identify phenotypic variation in adult leaf morphology that shows significant association along three elevational transects and their corresponding climate gradients; (2) measure phenotypic variation in seedling traits to test whether they differ significantly among transects and among maternal families; and (3) test whether family differences in seedling traits correlate with spatial or climate gradients of maternal tree locations. Recognizing that maternal effects of the seed source may influence the phenotypes young seedlings, findings from the second and third objectives provide initial evidence of whether selection is shaping phenotypic variation in leaf traits and identifies which leaf phenotypic traits may be most affected by long-term selection pressures. We will briefly discuss the implications of this approach for oak management and restoration ecology.

Methods

Valley oak, *Quercus lobata*, is a dominant or co-dominant species in oak savannas, oak woodlands and riparian forests in the foothills of the Sierra Nevada, Coastal Ranges, and Transverse Ranges that surround the Central Valley of California (Griffin and Critchfield 1972). The species inhabits diverse climatic and geographical zones from sea level to 1750 m and local populations support exceptionally diverse animal and plant communities (Pavlik and others 1995). Valley oak is a species of conservation concern because it has lost significant amounts of cover in the last 300 years (Kelly and others 2005) and it currently has recruitment limitations in many areas (Tyler and others 2006). Valley oak is an important resource for acorn woodpeckers (Koenig and Haydock 1999, Scofield and others 2011, Scofield and others 2012, Thompson and others 2014), western scrub jays (Koenig and others 2009), and many rodent species (Jameson and Peeters 2004). Contemporary gene flow through pollen and seed dispersal can be sufficiently restricted to create local genetic structure that would allow local adaptation (Grivet and others 2005, Pluess and others 2009, Smouse and others 2001, Sork and others 2002), while at the same time, pollen movement has a long tail that maintains genetic connectivity among populations (Austerlitz and others 2004, Pluess and others 2009). Historical colonization has created local genetic structure that would have allowed local populations to adapt to local environments (Gugger and others 2013, Sork and others 2010). Analysis of future suitable habitats based on predicted levels of climate change indicate that some populations would have to migrate long distances to track

favorable climate (Sork and others, 2010), which is unlikely in an increasingly fragmented landscape.

During October 2012, we sampled leaves and acorns from trees along three different elevational transects (fig. 1). Leaves were typically collected from lower, sun-exposed branches, but this varied depending on the location and structure of the trees. Sample sizes and elevations, latitude, and longitude of each adult are listed in table 1. We used latitude and longitude to obtain five current climate variables (table 2), based on previous work. The data were obtained by using the custom climate data requests of U.S. Department of Agriculture, Forest Service (USDA FS) (Rehfeldt 2006): <http://forest.moscowfsl.wsu.edu/climate/customData/>.

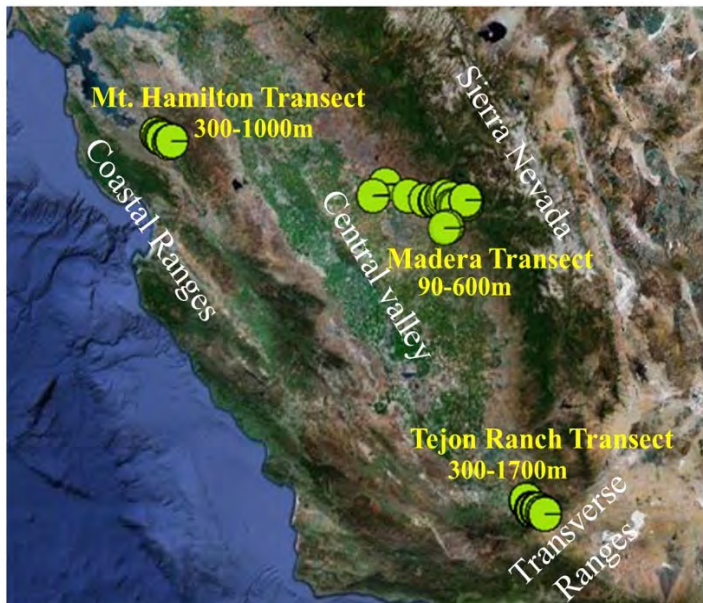


Figure 1—Locations of three elevational transects where leaf samples and acorns were collected from 127 valley oak trees.

Greenhouse methods

In the fall of 2012, we selected 59 families derived from the three elevational transects for our study. Acorns from these families were included in large provenance study initiated at the same time (see Delfino Mix and others, Establishing a range-wide provenance test in valley oak (*Quercus lobata* Née) at two California sites, these proceedings). Acorns were planted at the USDA FS, Pacific Southwest Research Station's Institute of Forest Genetics greenhouse in Placerville, California. The greenhouse is located at 38° 44' 23" N latitude and 120° 44' 32" W longitude and 849 m above sea level, at the edge of the species range of valley oak but within its climate range (see fig. 4, Delfino Mix and others, Establishing a range-wide provenance test in valley oak (*Quercus lobata* Née) at two California sites, these proceedings). In total, we planted acorns from 14 trees from Mt. Hamilton, 9 trees from Madera, and 36 trees from Tejon Ranch Transect Transects (table 1).

Table 1—Location and sample size of adult trees of valley oak collected along the three elevational transects and the number of families from those adults grown in greenhouse conditions

Transect	Number of Adults	Number of Families	Number of Seedlings	Latitude	Longitude	Elevation (m)
Madera	2			36.9950	-120.0010	78
	5			37.0269	-119.8115	97
	6			36.8848	-119.4907	165
	4			36.9605	-119.4963	245
	5			36.9657	-119.4846	260
	3			36.9641	-119.4450	314
	2			36.9247	-119.3306	377
	3			36.9570	-119.3004	394
	3			36.9379	-119.3135	408
	1			36.9553	-119.2327	439
	1			36.9927	-119.4132	472
	3	3	36	36.9737	-119.3679	581
	3	2	23	36.9717	-119.3679	587
	1	1	12	36.9693	-119.2470	597
4	3	34	36.9689	-119.2474	605	
Subtotal	46	9	105			
Mt. Hamilton	5			-121.7753	37.3775	388
	2	2	24	-121.7267	37.3579	467
	5	2	24	-121.7409	37.3587	496
	3	2	22	-121.7351	37.3557	510
	1	1	12	-121.7313	37.3510	517
	1	1	12	-121.7314	37.3505	521
	2	1	11	-121.6688	37.3241	649
	2	2	24	-121.6701	37.3261	665
	1	1	9	-121.6690	37.3264	688
	3			-121.6567	37.3366	993
	2			-121.6561	37.3374	1002
	2	1	7	-121.6534	37.3383	1041
	1	1	10	-121.6499	37.3423	1112
Subtotal	30	14	155			
Tejon Ranch	8			-118.7475	35.0605	360
	8	5	52	-118.7071	35.0075	600
	6	5	60	-118.6931	35.0050	900
	8	7	71	-118.6675	35.0053	1230
	8	7	83	-118.6498	35.0018	1519
	8	7	77	-118.5937	34.9755	1639
	5	5	59	-118.6199	34.9879	1750
Subtotal	51	36	402			

Acorns were bulk weighed by family before planting. Acorn collecting and planting are described in Delfino Mix and others (Establishing a range-wide provenance test in valley oak (*Quercus lobata* Née) at two California sites, these proceedings) using acorns collected in October and measured after 1 year of growth (fig. 2).

Adult and seedling measurements

From pressed vouchers of adult leaves sampled, we measured the following traits for three leaves per tree: leaf area, leaf thickness, leaf thickness per unit leaf area, leaf dry mass, leaf mass per unit area (LMA), leaf length, perimeter, and leaf perimeter per square root of leaf area as an index of leaf lobedness for each tree. Thickness was measured with digital calipers. Area, length and perimeter were measured in ImageJ (Rasband 2010) using binarized images of leaves from a standard flat bed scanner.

After acorns were planted in November, we recorded days until germination. In fall of 2013, at the end of the first growing season, we recorded the following seedling traits (sample sizes in table 1): diameter of stem base (mm), and height (cm) (see figure 2, bottom). To standardize the developmental states of leaves across seedlings, we selected the three youngest fully emerged leaves from each seedling and then pressed and dried them in paper envelopes. From the pressed vouchers, we measured the same leaf traits measured for adults and listed above. All analyses are based on average across three leaves per seedling.



Figure 2—Overview of methods: Acorns were sampled in October 2012 from adults (top photo), planted in November, and measured in September 2013 (bottom).

Statistical analyses

For Objective 1, examining adult phenotypic variation, we first describe the environmental differences among the three sites by conducting a canonical discriminant function (CDF) analysis with the five climate variables and then describe morphological differences among the three sites using a discriminant function analysis with six leaf variables using IBM SPSS 20. For each transect, we

used the six leaf measurements as dependent variables and elevation and the five climate variables as independent variables.

For Objective 2, we tested whether days to germination, seedling diameter, and seedling height in the first year were significantly different among transects and across maternal plants within transect, using mixed model nested ANOVAs where transect is a main effect and maternal family nested within transect is a random effect. We used this same model for all morphological measurements of seedlings leaves using the mean values per seedling as the response variable. Resulting *P*-values were adjusted for multiple testing using the false discovery rate (FDR) method of Benjamini and Hochberg (1995).

For Objective 3, we examined the association between seedling types and environmental variables in two ways. First, we estimated the individual correlation coefficients for seedling traits of maternal families and environmental variables associated with the locality of maternal tree. *P*-values were FDR-adjusted as above. Second, we examined the association between phenotype and environment with a redundancy analysis (RDA) multivariate model to identify the most important seedling traits and climate variables taking into account the covariance among variables within each type.

Results

Objective 1

The CDF analysis of climate environments at the sampling localities of the adult populations yielded two functions (F1 and F2) that explain 85.9 percent and 14.1 percent of total variation, respectively. Highly significant climatic discrimination was found among the three elevational gradients (F1, Wilks' $\lambda = 0.002$; $\chi^2 = 765.3$; $df = 10$; $P = 0.0001$; F2, Wilks' $\lambda = 0.102$; $\chi^2 = 277.9$; $df = 4$; $P = 0.0001$).

The three transects have very different climate environments, with much less variation within each transect than between (see fig. 3). The standardized canonical discriminant function coefficients indicated that two climate variables, GSDD5 and T_{\min} , explain most of the differences among the three transects (table 2). The trends

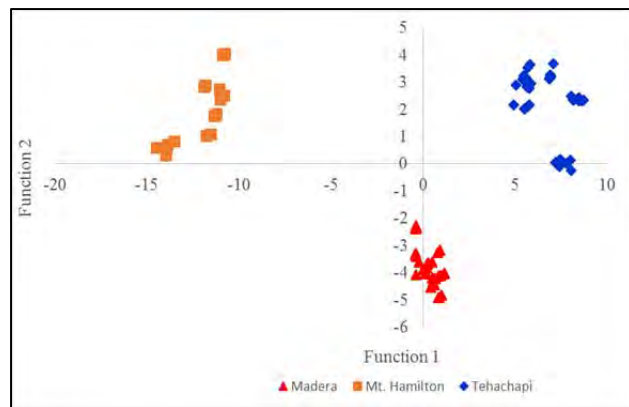


Figure 3—Plot of canonical function scores of climate variables for three transects.

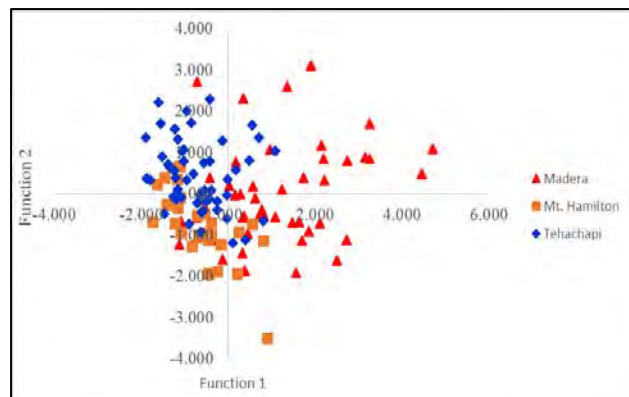


Figure 4—Plot of canonical function scores of morphological variation along the three transects.

indicate that the Tejon Ranch transect was the coldest and Madera was the warmest. Within each transect, it is interesting to note that the slope of the points for the Tejon Ranch and Madera transects was similar, indicating similar climate variables varied along those transect. The Mt. Hamilton transect points have a very different slope, suggesting a different pattern of climate variation along the transect.

When we examined the CDF of adult leaf traits, we found more variation among individuals within each transect (fig. 4) than we observed for the climate variables (fig. 3). The CDF of adult morphological leaf traits revealed 77.6 percent is significant associated with F1 (Wilks' $\lambda = 0.46$; $\chi^2 = 95.28$; $df = 12$; $P = 0.0001$) and 22.4 percent with F2 (Wilks' $\lambda = 0.82$; $\chi^2 = 24.8$; $df = 5$; $P = 0.0001$). Leaf thickness and leaf dry mass per unit area had high coefficients on the first F1 and lobedness index and leaf dry mass per unit area on F2 (table 3). The trees sampled along the Madera Transect showed the most variation along the first axis providing an indication of the balance between leaf size and thickness at this drier site.

Table 2—Standardized canonical discriminant function coefficients (F1 and F2) of the five climate variables analyzed to determine climatic differentiation among the three elevational transects of valley oak

Climate variables	F1	F2
Growing season precipitation from April through September (GSP)	0.176	-0.334
Mean maximum temperature in the warmest month (T_{max})	-1.895	-3.897
Mean minimum temperature in the coldest month (T_{min})	-4.948	0.012
Growing season growing degree-days greater than 5 °C (GSDD5)	6.883	4.082
Summer/spring precipitation balance: precipitation of July + August divided by precipitation of April + May (SmSprPB)	0.200	1.114

Table 3—Standardized canonical discriminant function coefficients of six morphological traits of adult leaves analyzed to determine morphological differentiation among the three elevational transects of valley oak

Leaf traits (Means per three leaves per adult)	F1	F2
Thickness (millimeter)	0.997	0.400
Thickness per area (millimeter per centimeter ²)	0.308	-0.480
Leaf dry mass per unit area (LMA; grams per centimeter ²)	-0.621	0.612
Mean leaf length (cm)	-0.136	0.577
Lobedness Index = perimeter (centimeter) per square root of leaf area (centimeter)	-0.367	0.642
Leaf dry mass (gram)	0.053	-0.533

Objective 2

Using separate nested ANOVA for each seedling trait, we find that all families within transect were significantly different using the overall error term (ANOVA, $df = 56/603$, $P < 0.0001$). Our nested ANOVA model also reveals that many seedling traits were significantly different among transects using family within transect as the error term (P -values shown in table 4). We found that leaf thickness showed the strongest differences across transects, with the Madera transect having the thickest and smallest leaves on average (see table 4).

Tables 4—Summary of transect means for first year seedling traits measured in greenhouse at Institute of Forest Genetics, Placerville, California. We tested the significance using separate nested ANOVAs where the main effect of transect was tested over family nested within transect as error term, with false discovery rate (FDR)-adjusted *P*-values associated with degrees of freedom= *df* = 2, 46 of numerator and denominator.

Seedling trait	FDR-adjusted <i>P</i> -value	Transect means		
		Mt. Hamilton	Madera	Tejon Ranch
Days until germination	NS	87.8	86.2	94.0
Mean family seed weight (gram)	NS	7.3	8.9	8.3
Seedling stem diameter (millimeter)	0.017	2.8	2.5	3.1
Seedling height (centimeter)	NS	121.8	161.0	149.7
Mean leaf thickness (millimeter)	0.001	0.131	0.160	0.155
Mean leaf thickness per area (millimeter per centimeter ²)	0.007	0.011	0.016	0.012
Mean leaf length (centimeter)	0.031	6.26	5.84	6.60
Leaf mass per unit area (g/cm ²)	NS	0.009	0.009	0.009
Lobedness (centimeter per centimeter)	NS	5.8	5.6	5.7
Mean leaf dry mass (gram)	0.010	0.112	0.092	0.126
Mean leaf area (centimeter ²)	0.015	12.7	10.9	14.3

Objective 3

Many seedling leaf traits were correlated with geographical and climate gradients of the maternal plant locations suggesting that these traits may reflect selection on maternal trees. Leaf thickness was most highly correlated with T_{\max} and longitude, but also with latitude and GSDD5 (table 5). Latitude and SmSprPB were most frequently correlated with seedling leaf traits (table 5). Elevation was correlated with only two traits—days to germination and seedling leaf area, and longitude was correlated with leaf thickness only.

We also examined the association between leaf traits and environmental variables using a multivariate model, Redundancy Analysis (RDA), which is essentially a multivariate correlation test. We found that the overall models showed a significant association between seedling leaf traits for families sampled at Mt. Hamilton and Tejon Ranch transects, but not Madera Transect, possibly because of a low sample size and only a small section of the gradient represented in the common garden. Based on the scores showing the association between individual traits and the two main axes (RDA1 and RDA2), we find that stem diameter, leaf thickness per area, leaf length, and leaf dry mass were the traits most important for Mt. Hamilton families (table 6.A), while GSDD5, elevation, and T_{\max} were the most important environmental variables. For Tejon Ranch, leaf dry mass, height, leaf length and stem diameter were the traits most important and T_{\max} , GSDD5, longitude, and elevation were the important environmental variables. In the Madera transect, most traits had similar associations and longitude, elevation, and T_{\min} were the most important environmental variables. Leaf thickness per area and leaf dry mass were important across the three transects and were correlated with several climate variables in the single correlation tests across all samples. These multivariate tests indicate that leaf morphology is correlated with climate and spatial gradients.

Table 5—Correlations of mean leaf traits per family with spatial and climate variables of localities of maternal plants (top number) with false discovery rate adjusted *P*-values (bottom number). Significant correlations are in bold. Sample sizes are 59 families, except seed weight with 58 families.

	Days to germination	Diameter	Leaf thickness	Leaf thickness per area	Leaf length	Leaf dry mass	Leaf area
Latitude	-0.302 0.056	-0.331 0.044	-0.3450 0.039	0.087 0.522	-0.286 0.060	-0.327 0.044	-0.328 0.044
Longitude	0.238 0.115	0.184 0.224	0.4760 <0.0001	0.123 0.378	0.146 0.314	0.153 0.295	0.173 0.241
Elevation	0.406 0.019	0.254 0.097	-0.1330 0.347	-0.244 0.107	0.196 0.195	0.236 0.115	0.262 0.087
GSP	-0.143 0.319	-0.316 0.049	-0.2470 0.105	0.178 0.236	-0.304 0.056	-0.382 0.028	-0.323 0.046
T _{min}	-0.354 0.037	-0.276 0.071	-0.2470 0.105	0.078 0.556	-0.232 0.120	-0.269 0.078	-0.291 0.060
T _{max}	-0.29 0.060	-0.134 0.347	0.5700 0.003	0.403 0.022	-0.108 0.437	-0.159 0.286	-0.175 0.240
GSDD5	-0.396 0.022	-0.193 0.199	0.3210 0.046	0.308 0.056	-0.157 0.286	-0.213 0.156	-0.231 0.120
SmSprPB	0.358 0.037	0.288 0.060	0.2880 0.060	-0.106 0.438	0.286 0.060	0.355 0.037	0.336 0.044

Discussion

Natural populations of valley oak contained significant phenotypic variation among three elevational transects that were geographically separated. The trait that differed most strongly in adult trees was leaf thickness. To understand the extent to which phenotypic variation is due to underlying genetic variation, we planted acorns from a subsample of those adult trees in a greenhouse and found that every leaf trait we examined showed significant differentiation in seedlings among maternal families. In particular, when we compared genetic differentiation among transects, the most significant traits were leaf thickness per area and leaf dry mass. On average, the Madera site had the thickest leaves and it also had the warmest temperature. When we looked to see whether leaf thickness showed an association with climate, which would be initial evidence that this trait is under selection, we found a positive correlation with maximum temperature. Given that leaf thickness can be a structural mechanism to reduce evaporative water loss, we suggest that future work looking for locally adaptive traits should pay attention to this character. For example, *Q. ilex* seedlings from the driest provenance, when grown in a common garden from seeds of different provenances, showed higher leaf mass area and total leaf thickness (Gratani and others 2003). These morphological traits were associated with higher water use efficiency, whereas, smaller leaf area was associated with a higher photosynthetic capacity in low air temperature conditions, suggesting potentially adaptive features to provenance climate.

Table 6—Summary of scores of association of eight morphological seedling leaf traits with two Redundancy Axes (A) and scores of environmental variables with two RDA axes (B)

A. Seedlings leaf traits	Madera		Mt. Hamilton		Tejon Ranch	
	RDA1	RDA2	RDA1	RDA2	RDA1	RDA2
Stem diameter	-0.733	-0.135	-0.957	-0.053	0.923	-0.196
Height	-0.697	0.327	-0.863	-0.054	1.046	0.109
Thickness	-0.771	-0.457	0.035	-0.943	0.470	1.222
Thickness per area	0.639	-0.331	1.022	-0.249	-0.644	0.838
Length	-0.497	0.327	-1.012	0.053	0.940	-0.162
LMA	-0.446	-0.589	0.200	-0.713	0.461	0.349
Lobedness	-0.419	0.274	-0.760	-0.315	0.636	0.154
Leaf dry mass	-0.741	-0.003	-0.942	-0.157	1.080	-0.068

B. Climate and geographical variables	Madera		Mt. Hamilton		Tejon Ranch	
	RDA1	RDA2	RDA1	RDA2	RDA1	RDA2
GSP	0.249	0.832	-0.138	0.407	-0.107	-0.845
T _{min}	0.263	0.839	0.101	-0.344	0.121	0.612
T _{max}	-	-	0.241	0.035	0.279	0.643
GSDD5	-0.092	0.864	0.212	-0.165	0.285	0.558
SmrSprPB	-	-	0.152	-0.139	-0.166	0.314
Elevation	0.370	0.672	-0.178	0.252	-0.246	-0.657
Longitude	0.262	0.840	-0.066	0.586	-0.304	-0.818
Latitude	-0.122	-0.752	-0.120	-0.879	0.149	0.887

This study suggests that leaf traits are highly differentiated across maternal families and geographical regions, but these findings must be considered cautiously because maternal effects may enhance differentiation among families and possibly overestimate the degree of genetic differentiation. We also found that many leaf traits other than leaf thickness that were related to mass and size, such as leaf dry mass, leaf length and leaf area, were also significantly correlated with geographical and climate gradients, though interestingly seed weight, considered a measure of maternal investment, was correlated only with T_{max}. Again, we caution that these correlations may also be enhanced by maternal effects. Nonetheless, change in morphological traits consistent with a direct response to climate change was observed in *Dodonaea viscosa* subsp. *angustissima*, where leaf width and leaf area were associated with temperature along latitudinal and elevational gradients, respectively (Guerin and others 2012). Thus, other studies have shown a genetic basis for traits such as the ones measured here.

Through the greenhouse experiment, we also found that days until germination showed genetic differentiation among transects and was correlated with latitude and elevation for this trait. The correlation with latitude might suggest that either photoperiod changes or growing season associated with climate might select for more delayed germination. The correlation with elevation as well as several climate variables also indicates that growing season may shape the delay in germination. For example, the number for growing degree-days above 5 °C was negatively correlated

with the days until germination such that provenances from places with a longer growing season germinated faster.

In summary, this study identifies extensive phenotypic variation in leaf morphology in adult populations of valley oak and initial evidence that much of that variation is due to geographical differences and to genetically based differences among families that appears to be under selection. Future analysis of phenotypic traits in these individuals and the full provenance study when they are older and less likely to reflect maternal effects will provide valuable insight about the genetic basis of valley oak phenotypes associated with local climate environments.

Next steps and implications for oak management and restoration

This study is part of a large-scale, species-wide provenance study where we will be measuring many phenotypic traits in the future (see Delfino-Mix and others, Establishing a range-wide provenance test in valley oak (*Quercus lobata* Née) at two California sites, these proceedings). The findings, which were based on trees sampled from sharp climate gradients along three elevational transects, demonstrate that at least a portion of the phenotypic variation we observe in natural populations may reflect the impact of natural selection. Using next generation sequencing tools, we are also conducting a parallel genomic study to look for marker genes that are associated with geographical, climate and trait variation in these populations of trees. As plants in the provenance test become older, we will associate phenotypes important to local adaptation with DNA sequence data to find genetic markers for adaptive genetic variation.

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