

Variation in leaf shape in a *Quercus lobata* common garden: tests for adaptation to climate and physiological consequences

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Introduction

Variation in leaf morphology is common in plants. Morphological variants with functional effects (e.g., via photosynthesis) should increase in frequency via natural selection, depending on environmental conditions, assuming genetic control (Givnish, 1979; Wright *et al.*, 2005). This is especially likely in widely distributed species that experience substantial variation in environmental factors (Royer, 2012; Valladeres *et al.*, 2000). Previous research has shown that leaf shape, particularly leaf dissection, can be an adaptive trait to drought tolerance (Farris, 1984).

Oaks (genus *Quercus*) are known for harboring substantial intraspecific variation in leaf morphology (Fig. 3), which may reflect different ecological strategies (Jensen, 1990; Valladeres *et al.*, 2000). Sisó *et al.* (2001), for example, found that increased leaf dissection in various *Quercus* species negatively correlated with hydraulic resistance, which suggests that leaf dissection may be an adaptive response to water stress.

We tested the hypotheses that (a) leaf dissection (perimeter:area ratio) in *Quercus lobata* was genetically based, reflecting adaptation to climatic regime and (b) variation in leaf dissection is associated with photosynthetic rate. If variation in leaf dissection is due to adaptation to local climatic conditions, we predicted that trees originating from contrasting climates would contrast in leaf dissection when grown in a common garden. If leaf dissection ameliorates water stress via photosynthesis, we predicted that more dissected leaves would have higher carbon assimilation rates.

Materials and methods

Experimental design:

Large common garden experiment: 672 maternal source trees from 97 locations throughout California, two replicates, CSO (Chico, CA) and IFG (Placerville, CA), with ~6700 trees in total (Fig. 5). To test for climate adaptation we subsampled 5 leaves from each of 54 trees grown from 27 maternal families collected from 9 locations. Both common garden sites were included. Locations representing extremes and median of multivariate climate phenospace based on principal components analysis of a 30-year georeferenced baseline dataset of 23 annual variables (Wang *et al.* 2016). To test for effects on photosynthesis, 3 leaves subsampled from each of 20 trees grown from 16 maternal families representing extremes and median of leaf dissection (perimeter:area in mm:mm²) based on a 2016 study of all common garden trees (MacDonald 2017). Trees from only one common garden (CSO) were used to measure photosynthesis (Fig. 4).

Traits measured:

Hypothesis (a): leaf dissection (perimeter:area ratio in mm:mm²) was calculated using ImageJ (Schneider *et al.*, 2012) for all leaves ($n = 270$). Hypothesis (b), carbon assimilation ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) measured with Li-Cor 6400 infrared gas analyzer for each leaf ($n=61$) during summer 2018. In addition, leaf dissection measured for each leaf as above.

Analyses:

Leaf dissection compared across climate categories (high, median, low) (Fig. 5) using mixed-model ANOVA. Variation in carbon assimilation regressed against leaf dissection using mixed-model approach. Sampling date included as fixed effect.

Results

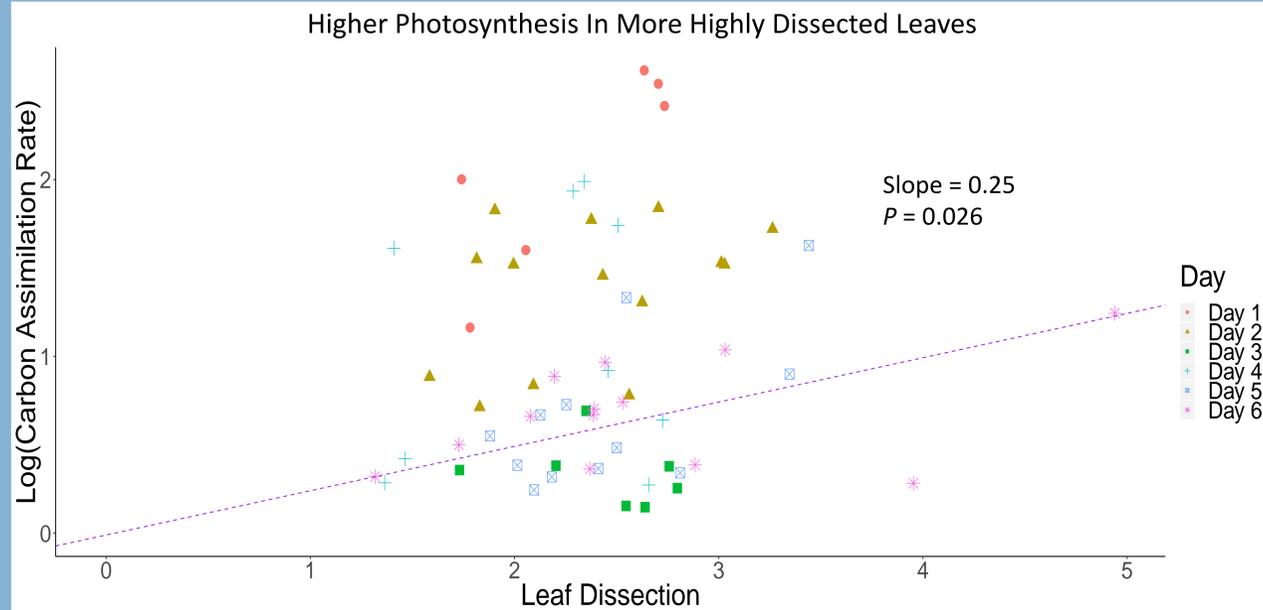


Fig. 1. Logarithm of carbon assimilation rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) as a function of leaf dissection (perimeter:area ratio in mm:mm²) in valley oaks sampled at the CSO common garden site.

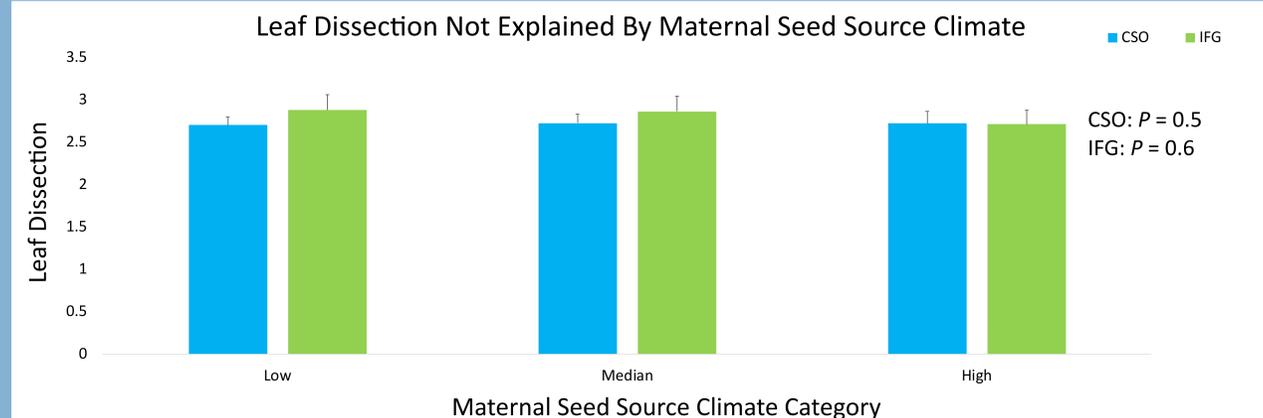


Fig. 2. Least squares mean (± 1 S.E.) leaf dissection (perimeter:area ratio in mm:mm²) for *Q. lobata* in common gardens at Chico Seed Orchard (blue) and Institute of Forest Genetics (green). CSO: $F_{2,65} = 0.61$, $P = 0.5$; IFG: $F_{2,65} = 0.54$, $P = 0.6$



Fig. 3. Intraspecific variation in *Quercus lobata* leaves collected from trees growing in a common garden, Spring 2017 (CSO).

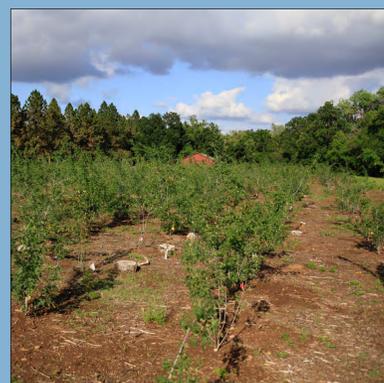


Fig. 4. Chico Seed Orchard (CSO) common garden of valley oak trees, Spring 2016.

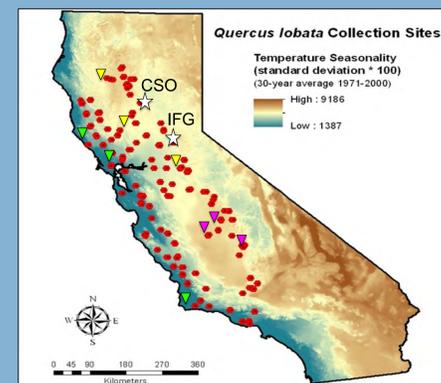


Fig. 5. Locations (red circles) from which valley oak acorns were collected for experiment, which was replicated in two common gardens (white stars). Locations of maternal trees sampled and associated climate category (triangles): high = pink; median = yellow; low = green.

Discussion

Oak leaves are famously variable in shape. We explored two ideas to explain that variation. **First**, we tested the hypothesis that variation in leaf shape is genetically based and is influenced by local adaptation to prevailing climatic conditions. Previous studies have reported that leaf morphological traits including dissection vary with climate and other site characteristics (Ogaya and Peñuelas 2007, Peppe *et al.* 2011, Royer 2012, Albarán-Lara *et al.* 2015). If this hypothesis were true, we expected to see climate-associated variation maintained in progeny growing in a common garden, because of inherited variations from the maternal source trees. We found no evidence to support this idea (Fig. 4), which suggests that leaf dissection is principally influenced by the environment.

Phenotypic plasticity can itself be adaptive in variable environments if shifts in phenotype lead to improved performance. We also tested the **second** hypothesis that variation in leaf shape has a functional (physiological) consequence. Prior studies have found leaf shape in oaks to be associated with physiological performance (Sisó *et al.* 2001). We found that more dissected leaves had higher photosynthetic rates (Fig. 5), which is consistent with this hypothesis.

Carbon assimilation measurements were collected during the summer (June and July). Prior studies have shown that leaf dissection may be a way that oak trees cope with water stress (Sisó *et al.* 2001). Thus, the positive relationship between photosynthesis and leaf dissection that we observed may be more likely to occur under the hot, dry conditions in which we conducted the study.

Why variation in leaf shape persists in this common garden remains elusive (Fig. 3). The expression of plasticity in common gardens may be constrained if it varies across maternal lineages. Alternatively, leaf shape may be influenced by other, unmeasured environmental variables.

Acknowledgments

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