

Genetic Variation in Shoot Growth, Phenology, and Mineral Accumulation of Northern and Central Sierra Nevada Foothill Populations of Blue Oak¹

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Abstract: Genetic variation of three traits of blue oak (*Quercus douglasii*) was studied in a common garden experiment at the Sierra Nevada Foothills Field Station, Browns Valley, California. Preliminary observations over a period of 3 years suggest that some genetic variation in blue oak populations studied is expressed as differences in shoot growth, phenology, and mineral accumulation. This variation appears to be typical of neither ecotypic nor ecoclimatic variation. Interpretation of results for the development of seed transfer rules is premature at this phase of the study. A longer period of observation encompassing the adult growth phase will be required before definitive recommendations can be made.

In California, extensive clearance of blue oak (*Quercus douglasii*) from rangelands and the urbanization of blue oak-dominated vegetation types have led to a concern for the loss of biological diversity in this species and its vegetation types. Jones and Stokes Associates (1987) estimated that 591,000 acres, or 7 percent of the area supporting the Valley and Foothill Woodland type, had been completely lost between 1945 and 1980; blue oak is a major component of this Valley and Foothill Woodland type. Since 1980 urbanization has caused additional losses of blue oak woodlands and savannas, while rangeland clearance and agricultural conversion of blue oak-dominated landscapes have had less impact.

The potential impact on biodiversity caused by the loss of blue oak-dominated vegetation is threefold: first, the loss of ecosystems and ecosystem processes; second, the loss of species; and third, the loss of genetic variability (McNeely and others 1990). Losses of all three of these types of biodiversity are significant and warrant the development and application of appropriate management and planning strategies to conserve biodiversity. The research reported here addresses the genetic architecture of blue oak. Understanding the degree and geographical pattern of genetic variation is essential in evaluating the significance of the loss of local populations of a species and can serve as the basis for the development of seed source acquisition rules and gene conservation strategies.

Seed source acquisition rules are guidelines that consider the complex and irregular ways in which individual species vary genetically over the landscape. These rules are used to define geographic seed collection zones for restoration projects. Long-term seed storage, the most common *ex situ* gene conservation strategy, is not applicable to blue oak because acorns of blue oak cannot be maintained for a long time in storage facilities. Therefore, the maintenance of genetic diversity in blue oak depends on *in situ* programs combined with the *ex situ* maintenance of trees in botanical gardens.

Research on the genetic variation in blue oak has been limited to allozyme analysis of a few populations and some linkage of ecological characteristics to population variation. Both approaches indicated high within-population variation (Millar and others 1990, Rice and others 1991, Riggs and others 1991). These studies have not provided sufficient information to define the geographical pattern of genetic variation in blue oak. Research reported here was designed to

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examine genetic variability in blue oak by investigating a larger number of populations than had previously been studied.

Our study used the common garden method to characterize the geographic pattern of genetic variation in blue oak. The common garden method grew from the early reciprocal transplant experiments of Bonnier (1895) in the Pyrenees and the transplant garden experiment of Kerner (1895) in the Tirol Mountains. Turreson (1922) working in Denmark, Clausen and others (1939) working in California, and Gregor (1946) working in Scotland used the common garden method to investigate genetic variability in wild species. The method was adopted by forest geneticists for provenance testing of forest trees and by agronomists for evaluating genetic strains of crop species (Zobel and Talbert 1991). The common garden method is based on the premise that genetic variability within a species will be expressed as differences in growth, morphology, physiological characteristics, and phenology when plants, cuttings, or seeds collected over the range of a species are raised in a uniform environment.

Use of common garden experiments declined after the 1960's with the advent of biochemical techniques to characterize genetic variability. The high cost of maintaining common gardens also played a role in the decline of common garden experiments. Various nucleic acid and allozyme methods developed in the past 30 years have yielded rapid and precise information at the gene and gene product level. However, as Millar and Libby (1991) pointed out, common garden experiments over several years of study provide more information on traits directly related to adaptation than biochemical studies. Hamrick and others (1991) further contend that dependence on biochemical techniques has not usefully demonstrated positive association among different adaptive traits and genetic variation in outcrossing species with wide geographic ranges. One must be cognizant that genetic variation in trees that affects morphological and physiological expression can be expected to involve multiple gene interaction (Namkoong and others 1988). These interactions are expressed through the physiological and developmental processes that are impossible to observe out of the environmental context in which the organism operates. The common garden can serve as an environment to observe the various outcomes of these multiple gene interactions. It can provide, in combination with various nucleic acid and allozyme methods, a successful approach to understanding genetic variability within a species.

Establishment of the Common Garden

A common garden for blue oak plantings was established at the Sierra Foothill Field Station in spring 1992 on a site within the natural range of the species. Seed collections for this common garden were made at 15 locations in the foothills of the Sierra Nevada. Collection sites were established along four west-to-east transects located approximately 100 km apart in the northern and central Sierra Nevada foothills. Collections were made in 1990 at elevations of approximately 150, 300, 600, and 900 m along these transects, with the exception of transect number 2 where collections were made at 150, 450, and 750 m (*table 1*).

At each site approximately 150 to 200 acorns were collected from each of 10 trees. Trees used in the collection occurred within 30 m of the chosen elevation (e.g., 150, 300, 600, 900 m) but were separated from each other by at least 100 m. Acorns first were subjected to a float test. Those that floated were held for 24 hours in a mist bed and float-tested a second time because a majority of acorns that did not sink in the initial test appeared to be sound. One hundred acorns representing the size distribution of the acorns that sank in the float test were selected for each tree. The sets of 100 acorns, from each of the 10 trees used for collection at the various elevations along the transects, were then bulked. These bulked samples

were dried in open trays to near 25 percent on a dry weight basis. During the drying process they were sequentially exposed to temperatures of 20, 15, 10, and 5 °C over a period of 2 weeks. After drying, the acorns were placed in air-tight polyethylene bags for storage at 1 °C until they were planted. Before planting they were soaked for 24 hours in aerated tap water.

Acorns from the 15 seed sources collected in 1990 were sown at the Magalia Nursery, California Department of Forestry and Fire Protection, Magalia, Calif., in January 1991. Seedlings were lifted from the nursery beds and planted in the common garden at the Sierra Nevada Foothill Station in March 1992.

The plantation planting design used in the common garden consisted of 10 major blocks each with 15 minor plots per seed source. Each of the minor plots had nine planting spots on 1.2-m centers. Seedlings were planted in holes dug to 45 cm with a power auger. Two bare-root seedlings were planted in each augered planting spot. Weeds were controlled before planting with a glyco-phosphate herbicide. After planting weeds were killed by a combination of hand weeding and herbicide application. Approximately 10 percent of the seedlings needed replacement during the first growing season because of predation by voles and pocket gophers. Replacement seedlings were grown outside in Berkeley, California, in 8-cm-diameter by 30-cm-deep containers. These were planted in January 1992 at Berkeley and used for replacement of seedlings in the Sierra Nevada Foothills common garden during the first growing season. Seedlings were thinned to one per spot in January 1993.

Analysis of soil conditions in the common garden indicated a uniformity of soil characteristics. Soil samples were collected from six randomly located sample spots in the common garden and were analyzed in spring 1995. Soil was collected at depths of 10, 30, and 60 cm at each sample location. Soil samples were air dried and sifted to remove particles of >2 mm. The pH of each sample was determined on a saturation paste, total carbon by combustion, total nitrogen by macro-kjeldahl, cation exchange capacity by ammonium acetate extraction, phosphorus by sodium bicarbonate extraction, and exchangeable cations by both acetate extraction at pH 7 and potassium chloride (KCl) extraction at the unbuffered pH of the soil. Standard soil moisture contents at pressure plate pressures of 0.03 and 1.5 MPa were also determined.

Table 1—Location of blue oak (Quercus douglasii) seed sources

Transect	Geographic location	Latitude	Seed source no.	Elevation
				<i>m</i>
1	Route 36	40° 19'	1A	150
			1B	300
			1C	600
			1D	900
2	Route E21	39° 20'	2A	150
			2B	450
			2C	750
3	Route 50	38° 46'	3A	150
			3B	300
			3C	600
			3D	900
4	Route 120	38° 00'	4A	150
			4B	300
			4C	600
			4D	900

A nutrient analysis showed that the soil is not limiting for plant growth (*table 2*). The soils showed 27 and 12 percent moisture at pressure plate pressures of 0.03 and 1.5 MPa, respectively. Summer soil moisture levels in the common garden were above the permanent wilting point, during each year of the study, because of late spring precipitation and the elimination of herbaceous cover. The soil in the common garden is probably a complex of the Auburn, Los Pasos, and Argonaut series that are derived from greenstone and metamorphic parent material. These soils tend to be well weathered with a red color tending to yellowish in the C horizon at 70-80 cm. Soil bulk densities ranging from 1.20 to 1.32 were measured in the common garden.

Table 2—Soil nutrient characteristic in a common garden at the Sierra Nevada Foothill Field Station¹

Depth	pH	C	N	P	Milliequivalents / 100 g soil						
					Ca	Mg	K	Na	Mn	CEC	Ca/Mg
<i>cm</i>		<i>pct</i>	<i>pct</i>	<i>ppm</i>							
10	6.0	1.05	0.11	17.10	11.05	1.45	0.62	0.13	0.79	14.3	7.6
30	6.1	0.51	0.05	5.10	1.87	2.96	0.41	0.13	0.64	14.1	4.0
60	6.2	0.25	0.04	2.20	15.38	5.08	0.22	0.14	0.34	15.8	3.0

¹C = carbon; Ca = calcium; CEC = Cation exchange capacity; K = potassium; Mg = magnesium; Mn = manganese; N = nitrogen; Na = sodium; P = phosphorus.

Methods

Plant Growth and Phenology

Basal diameter and length of the longest stem of each plant were measured each year in late winter. Diameter measurements were made using a digital, electronic caliper at a point 3 cm above the soil level. The longest stem was measured using a flexible steel carpenter's tape. Many plants did not develop a central dominant leader, but grew in a more shrubby habit. Wilting of the second flush of growth and attacks of mildew resulted in the mortality of the terminal portion of the main stems of some plants over the 3-year period of measurements; however, this isolated mortality did not result in a disruption in any of the height growth trends shown by the populations.

Phenology of bud break, leaf expansion, and shoot elongation was followed during the spring shoot development period in 1993; the timing of bud swell, bud break (leaf emergence), and shoot elongation were recorded for the major shoot on all plants.

Plant Tissue Analyses

In spring 1995, eight of the 15 blue oak populations in the common garden were sampled for plant nutrient analysis. Populations were chosen to represent the latitudinal and elevational range of populations planted in the common garden. Three mature leaves were cut from each seedling within each of six randomly selected blocks. Leaves were pooled by block for analysis. Recently matured leaves were used as stable benchmark and to avoid leaves that may be dominated by the transient nutrient fluxes involved in (1) the cell division or osmotic adjustments of expanding leaves or (2) the cell wall thickening and re-transport

of nutrients in older leaves. Leaf tissue samples were oven dried at 65 °C, ground to pass through a 20-mesh screen, analyzed for nitrogen by micro-kjeldahl, and suitably prepared for atomic absorption and ICP spectroscopy by nitric acid digest (Bradstreet 1965, Zarcinas and others 1987).

Results and Discussion

Plant Growth

Analysis of diameter and height growth did not reveal a consistent trend in relation to elevation and latitude (*table 3*). Both variables first increase and then

Table 3—Average diameters and heights of blue oak (Quercus douglasii) in a common garden at the Sierra Nevada Foothill Field Station in February, 1994

Latitude	Elevation (m): 150	300	600	900	Avg.
----- Diameter (mm) -----					
40° 19'	13.6	12.6	12.8	13.2	13.0a ¹
39° 20'	16.2	16.8 ²	15.7 ³	-	16.2b
38° 46'	14.2	17.0	16.0	14.3	15.4b
38° 00'	13.8	16.3	14.3	14.5	14.7b
Avg.	14.4ab	15.7ab	14.7ab	14.0ab	
----- Height (cm) -----					
40° 19'	97	88	100	92	94.2c
39° 20'	104	111 ²	112 ³	-	109.0c
38° 46'	94	123	109	95	105.2c
38° 00'	100	121	89	103	103.2c
Avg.	98.8c	111.4c	102.5c	97.5c	

¹Numbers with the same letter are not significantly different at the 0.01 percent level

²450-m elevation

³750-m elevation

decrease along both gradients. The average diameters and heights were greater in those populations occurring in central latitudes and middle elevations. The term trend is used here with caution because a statistical analysis (a one-way ANOVA; Tukey test for multiple comparison of means) indicated that only the average of the diameters of the northernmost populations was significantly different (0.05 percent level) from the diameters of the other populations averaged by latitude. The trend in greater growth exhibited by the central latitude and mid-elevation populations suggests a better adaptation of the genotypes of these populations to the environment of the common garden at the Sierra Nevada Foothills Station. This location falls within the range of central latitudes and mid-elevation. It is assumed that populations from higher and lower latitudes and higher and lower elevations are not as well adapted to the environment of the common garden. Blue oaks occurring at these higher and lower elevations and latitudes may have evolved a more conservative growth strategy in response to drought-induced desiccation at lower elevations and latitudes and early frost damage at higher elevations and higher latitudes. In either case, limiting growth and entering into dormancy early would be a conservative strategy that results in decreased growth. Mid-elevation and mid-latitude populations would be less vulnerable to either extremes of drought or

frost damage. Continued growth of these populations over a longer season may have contributed to their increased diameters and height.

Plant Phenology

A large data set was produced from the observations of the phenology of the different seed sources. We have chosen the percentage of seedlings that were dormant on March 16, 1993 as an indicator of the difference in the phenological development of the various populations. On this date about 60 percent or more of the individuals in each population had experienced bud break. The data show that all individuals from the lower-elevation populations collected along the latitude of the common garden (latitude N39°20'') had initiated bud break (*table 4*). Eighty-nine percent of the individuals from the highest-elevation population along this same latitude (latitude N39° 20'') had also initiated bud break. Populations collected along latitudes N40° 19'' showed an increasing percentage of dormant individuals as elevation increased. At latitudes N38°46'' and N38°

Table 4—Percentage of blue oaks that were dormant on March 16, 1993 in a common garden at the Sierra Nevada Foothill Field Station

Latitude	Percent dormant				
	Elevation (m): 150	300	600	900	Avg.
40° 19'	10	15	38	40	25.8a ¹
39° 20'	0	0 ²	11 ³	-	2.8b
38° 46'	17	26	15	30	22.0a
38° 00'	20	12	18	22	18.0a
	Avg.	11.8a	13.2a	20.5ab	30.6b

¹Numbers with the same letter are not significantly different at the 0.01 percent level
²450-m elevation
³750-m elevation

00'' there was no trend in increasing dormancy and elevation; however, the highest-elevation populations along these two transects exhibited the greatest dormancy percents. An average of the percentages of dormant plants at each elevation across the various latitudes showed an increase in dormancy with increasing elevation. This trend of increasing dormancy with latitude and elevation suggests that release from dormancy may be related to temperature. The fact that nearly all individuals from populations along the latitude of the common garden had broken bud suggests that photoperiod may also be involved. Kramer and Kozlowski (1979) proposed a general model for the control of dormancy and bud break that involved both photoperiod and temperature.

One would expect that trees at the locations from which the acorns were collected would break bud dormancy in relation to increasing temperature and day length. Trees at the lowest elevation (150 m) along the latitude N38°00'' would be the first to break bud. However, in the common garden at latitude N39° 20'', 20 percent of the trees from this seed source were still dormant on March 16, 1993. There may be attributes of the common garden used in this experiment that are influencing the bud break of seedlings that are not understood and have resulted in what does not seem to be a logical pattern of bud break.

Plant Tissue Analyses

Leaf tissue analysis showed a greater accumulation of nitrogen, phosphorus, and sulfur in high-elevation populations than in low-elevation populations (*table 5*). This pattern was not apparent in the other nutrients studied. This increased

Table 5—Foliar nutrient content of blue oak in a common garden at the Sierra Nevada Foothill Field Station in 1995

Latitude	Elevation	
	Low (150 m)	High (900 m)
Nitrogen (pct)		
40° 19'	2.28	2.39
39° 20'	2.40	2.37
38° 46'	2.31	2.44
38° 00'	2.35	2.45
Avg.	2.33a ¹	2.41a
Phosphorus (ppm)		
40° 19'	1772	1987
39° 20'	1739	1946
38° 46'	763	1908
38° 00'	1761	1918
Avg.	1758.8b	1939.8c
Sulfur (ppm)		
40° 19'	1603	1668
39° 20'	1681	1748
38° 46'	1649	1662
38° 00'	1571	1695
Avg.	1626.0d	1693.2d
Calcium (pct)		
40° 19'	1.15	0.96
39° 20'	1.29	1.04
38° 46'	1.04	1.23
38° 00'	1.09	1.23
Avg.	1.14e	1.12e
Magnesium (pct)		
40° 19'	0.25	0.20
39° 20'	0.23	0.22
38° 46'	0.23	0.22
38° 00'	0.22	0.23
Avg.	0.23f	0.22f
Potassium (pct)		
40° 19'	0.48	0.56
39° 20'	0.51	0.55
38° 46'	0.52	0.50
38° 00'	0.54	0.54
Avg.	0.51g	0.54g
Manganese (ppm)		
40° 19'	606	556
39° 20'	605	582
38° 46'	477	628
38° 00'	488	586
Avg.	544h	588h
Iron (ppm)		
40° 19'	108	119
39° 20'	100	93
38° 46'	113	99
38° 00'	96	101
Avg.	104i	103i
Calcium/Magnesium		
40° 19'	4.61	4.85
39° 20'	5.69	4.95
38° 46'	4.39	5.87
38° 00'	5.03	5.29
Avg.	4.93j	5.24j

¹Numbers with the same letter are not significantly different at the 0.01 percent level

concentration of these macronutrients may be an indication of more effective mineral accumulation by seed sources from high elevations. We hypothesize that the shorter growing seasons and the trend toward less nutrient-rich soils, with increasing elevation, may have been selective forces for a greater capacity for absorption in higher-elevation populations.

Conclusions

The basic approach of common garden studies is to observe the phenotypic expression of survival, growth, and development over time. Phenotypic expression may indicate a stepwise change in genetic variation associated with distinct ecotypes or a gradual variation typical of ecoclines (Heslop-Harrison 1964). Our data have not demonstrated either stepwise or gradual variation. Few of the characteristics of the plants measured have been statistically definitive for delineating the geographic pattern of genetic variation in blue oak. Genetic variation in blue oak may be sufficiently large within local populations to obscure statistical delineation of either ecotypes or ecoclines. It is possible that genetic variation between populations, selected at the intervals of latitude and elevation studied in this report, is slight and that neither ecotypes nor ecoclines occur in the area studied. We, therefore, cannot at this time suggest definitive seed source transfer rules that could be used in restoration projects. We intend to follow these populations over the next decade to see if a more definitive characterization of the geographic variation within blue oak can be achieved.

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