

Patterns and Processes of Adaptation in Blue Oak Seedlings¹

Kevin J. Rice²

James H. Richards³

Steven L. Matzner²

Abstract: Reciprocal transplant studies examined the contribution of genetic differentiation and phenotypic plasticity to intraspecific variation in blue oak (*Quercus douglasii*) seedling survival and growth. A nested, mixed model design partitioned seedling survival and growth responses into between-population effects, within-population (among family) effects, and environmental effects. Significant between-population differences in seedling survivorship and growth were observed at both sites. Interactions between population source and planting block suggest local scale adaptation. Differences among maternal families in survival and growth were significant. Phenotypic variation in seedling performance may be related to indices of acorn quality such as embryo dry weight.

Although blue oak (*Quercus douglasii* Hook. & Arn.) is found only in California, it has a wide geographic distribution within the state. In wide-ranging plant species, it is often assumed that natural selection has resulted in the formation of genetically differentiated populations adapted to local environmental conditions. For example, provenance testing in many conifer species has indicated that there is often adaptive differentiation in response to environmental clines existing along elevational gradients (Campbell 1979, Conkle 1973, Libby and others 1969). Although it is true that natural selection can be a very powerful force in shaping the genetic structure of a species, it has also been demonstrated that gene flow, if of sufficient magnitude, can overwhelm the force of natural selection. Thus, even fairly strong natural selection may not create locally adapted populations if gene flow is very high. Gene flow is typically higher in wind pollinated, outcrossing species like blue oak. The genetic structure of a wind pollinated, outcrossing plant species is usually characterized by relatively large amounts of genetic variation within populations but relatively little genetic differentiation among populations (Hamrick 1979, 1983). Thus, all else being equal, one would expect that local adaptation would be less likely to occur in an outcrossing species.

Previous electrophoretic surveys of blue oak populations at various locations in the State have confirmed expectations that blue oak is highly outcrossing (Riggs and others 1991). In general, the allozyme markers indicated that there was little electrophoretic differentiation among populations and no evidence for the formation of geographically distinct subpopulations. If the allozyme variation described by Riggs and others (1991) also reflects patterns of genetic variation in traits with potential adaptive significance (i.e., quantitative traits), then one might argue that there is little indication of local adaptation in blue oak. This conclusion would have obvious implications on strategies for restoring blue oak populations through planting efforts. If local adaptation is not occurring in blue oak, then one would not need to be concerned about the source used in planting projects. In a very real sense one might conclude that "When you have seen one blue oak population, you have seen them all!"

However, a problem arises in trying to use patterns of variation in allozyme markers to predict patterns of variation in traits of ecological and adaptive significance. Allozyme markers are excellent markers for measuring evolutionary processes such as drift and gene flow because these markers are generally thought to be neutral to the effects of selection. Thus, patterns of allozyme

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²Associate professor and graduate research assistant, respectively, Department of Agronomy and Range Science, University of California, Davis, CA 95616

³Associate professor, Department of Land, Air, and Water Resources, University of California, Davis, CA 95616

variation can be used as indices of patterns of gene dispersal and migration unaffected by selective forces. This neutrality to selection is precisely the reason why these types of markers may not be good indicators of local adaptation. An increasing number of studies have shown that patterns of genetic structure within a species can often depend on the type of genetic marker used and that electrophoretic patterns and patterns of variation in potentially adaptive traits can differ markedly. This discrepancy may result if localized, strong selection on a quantitative trait is able to override the potential swamping effects of the gene flow indicated by allozyme markers. The potential for selection to produce locally adapted populations in spite of significant gene flow has been documented for several plant species (Endler 1977, Jain and Bradshaw 1966, McNeilly and Bradshaw 1968, Mopper and others 1991).

Reciprocal transplanting, which involves collecting germplasm from sites across an environmental gradient and then planting the material together in replicated common gardens at those sites, is the most common experimental approach used to evaluate local adaptation. Introduced by Turesson (1922) and used in classic studies by Clausen and others (1940), this technique remains the most effective method to detect the occurrence of local adaptive response in plant populations. In oaks, one of the few demonstrations of localized adaptation is provided by a reciprocal transplant study conducted on northern red oak (*Quercus rubra*). Within a 4-ha plot, Sork and others (1993) reciprocally transplanted acorns from sub-populations occupying slopes with different aspects. Using leaf damage by insect herbivores as a measure of fitness, they found that seedlings exhibited the lowest leaf damage in the sites of their maternal parent. They argued that local adaptation in this species occurred as the result of strong selection by leaf herbivory of sufficient magnitude to override effects of gene flow. The authors noted that these results, indicating very localized adaptation, were rather unexpected because northern red oak is a widely outcrossing species.

Given that the electrophoretic studies by Riggs and others (1991) have indicated substantial gene flow in blue oak, we initiated a series of reciprocal transplant experiments in order to determine whether selection during the early stages of seedling establishment might be strong enough to result in local adaptation. The study used a hierarchical design to examine adaptive differentiation at both the between- and within-population scale. Acorns from trees at two different geographic locations were planted in common gardens at each of the sites in order to detect local adaptation on a regional scale (i.e. between planting sites). By incorporating the maternal family origin of each acorn into the nested design, we were also able to estimate the amount of within-population genetic variation for adaptive traits. This within-population variation represents the evolutionary potential of a population to respond to new selective pressures.

Methods and Materials

In fall 1991, acorns were collected from blue oak populations at the University of California Hopland Research and Extension Center in Mendocino County and the University of California Sierra Foothill Research and Extension Center in Yuba County. Average annual rainfall at the Hopland site is about 95 cm while the Sierra site is somewhat drier with about 70 cm of rainfall per year. To assure good sampling of the genetic variation at each of the geographic locations, acorns were collected from at least 10 different trees located at various locations within each site. Trees were sampled without regard to tree size or health. Acorns were considered to be ready for harvest from a tree when (1) they detached very easily from their cups, (2) there was no tissue damage when the

acorns were removed from their cups, (3) the attachment tissue at the base of the acorn had dried, and (4) the acorns were turning yellow to orange-brown. Acorns with weevil damage were not collected. Although the experimental design required at least 80 acorns from each tree, because of high acorn production in 1991 there was ample seed to complete the experimental design. After collection, acorns were placed within plastic bags and stored for approximately 2 weeks at 3 °C. Acorns were then placed outdoors into a germination bin filled with sand that was shaded and periodically irrigated. At both Hopland and Sierra, a series of 10 planting blocks (10 m by 20 m) were established across a range of microhabitats differing in elevation, slope, and aspect. The goal was to use the widely distributed blocks to sample the regional environment of each field station so that results could be properly extrapolated to the regional scale. Within each of the planting blocks, four germinating acorns from each of the 10 maternal families from each site were planted in fall 1991, yielding a total of 80 acorns planted per block. Before planting, the fresh weight of each acorn was recorded and used as a covariate in the analysis. Within each block, acorns were planted at random with respect to family and population. Acorns were planted at a depth of 5 cm and were covered by protective netting ("Hopland tents"). Although only germinating acorns were used in the field planting, acorn germinability and viability were checked for each maternal family and used in calculating overall survival. Demographic monitoring of the reciprocal transplants began in spring 1992 and took place in April, June, and September. Within each planting block, seedlings were scored for survival and growth (i.e., height). Using the GLM procedure in the SAS statistical package, survival and growth data were analyzed as a hierarchical ANOVA with population (Hopland vs. Sierra) as a fixed effect and block and maternal family as random effects. To estimate the relative importance of the random effects (block and family) on seedling performance, variance components were estimated using the VARCOMP procedure in SAS.

A random subsample of 25-50 acorns from each of the maternal family collections from the field planting were used to examine variation in acorn size parameters at the between- and within-population (i.e., family) level. After taking fresh weights, acorns were placed in 65 °C drying ovens until no change in weight was detected. Acorns were then re-weighed to obtain dry weights and then "hulled" in order to obtain an estimate of embryo dry weight. The effects of both population source and maternal family on acorn size parameters were analyzed using the GLM and VARCOMP procedures in SAS.

Results and Discussion

Evidence for Local Adaptation

Overall, the survival of seedlings was higher at the Hopland planting site ($P < 0.01$) throughout the course of the study. Gopher and squirrel predation at Sierra were important factors contributing to lower seedling survival at this site. Using first-year seedling survival as an index of fitness, local adaptation is indicated by the results at the Sierra site (fig. 1a). At the Sierra site, the local Sierra germplasm had significantly higher survival than the non-local Hopland seedlings. This significant ($P < 0.05$) "home" advantage for the Sierra seedlings also occurred during the second and third years of the study (data not shown) but did not persist into the fourth year (fig. 1b). Low overall survival to the fourth year at the Sierra site (< 5 percent) regardless of acorn source was probably the major factor eliminating the home advantage for the Sierra source. In contrast to the results for the Sierra populations, the survival results for the Hopland population indicate relative maladaptation. This somewhat unexpected result is indicated by the poorer survival of the Hopland seedlings at their home site throughout

Figure 1—Interactive effects of population source and planting site on percent seedling survival after one and four growing seasons. Within a planting site, survival percentages (± 1 standard error) with different superscripts are significantly different ($P < 0.05$).

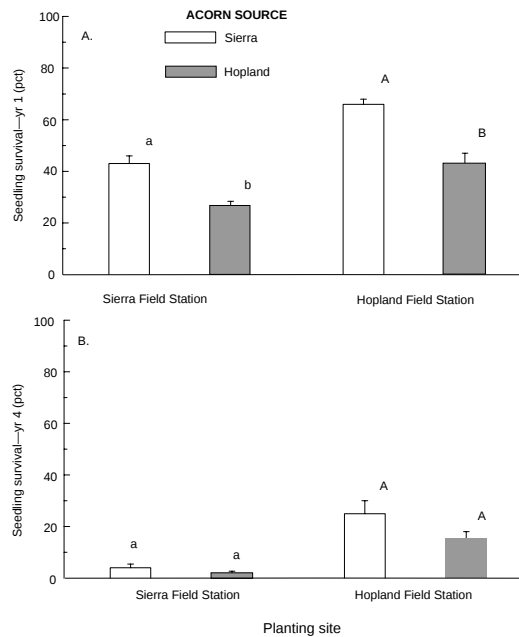
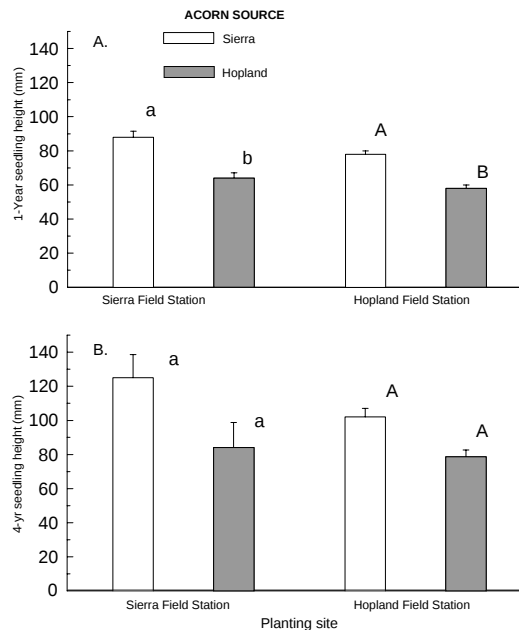


Figure 2—Interactive effects of population source and planting site on average height in 1- and 4-year-old seedlings. Within a planting site, average heights (± 1 standard error) with different superscripts are significantly different ($P < 0.05$).



the course of the study (figs. 1a and 1b). Similar results are indicated in an analysis of seedling “size” as measured by seedling height (fig. 2). Local adaptation is indicated for the Sierra population at its “home” site while local maladaptation is indicated for the Hopland source at its “home” site. It is interesting to note that one might argue that the data on both seedling survival and size indicate that the Sierra germplasm is “better” overall. We would caution against such an interpretation because (1) these data address only differences among seedlings at a relatively early stage, (2) differences among sources in seedling performance appear to diminish as time goes by (e.g., no differences in seedling survival or growth at both sites by the end of the study), and (3) a much larger array of germplasm sources would need to be tested before robust

conclusions could be made regarding the overall superiority of the Sierra germplasm. Long-term monitoring at both sites is planned in order to determine whether these patterns of adaptation (and maladaptation) persist.

In addition to these population comparisons, localized adaptation was also indicated by rank correlation analysis that indicated that family performance was highly site-specific. In other words, seedlings from one tree might exhibit high survival or growth at one site but typically performed relatively poorly at another site. Further evidence for very localized adaptation in blue oak is provided by a comparison of population performance among different planting blocks located within a regional site. In practice, one examines whether there is a statistically significant population source by planting block interaction. At both regional sites, this interaction component was highly significant ($P < 0.01$), suggesting a potential adaptive response to the variation in light, moisture, and soil nutrients that we have observed to occur among planting blocks within sites (unpublished data)⁴.

Analysis of acorn size parameters may offer some explanation for variation in seedling survival and growth. At both the population and family level, acorn dry weight and embryo allocation were found to be positively correlated ($P < 0.05$) with seedling survival and growth. Variance component analysis indicated that much of the variation in acorn dry weight and embryo allocation was caused by differences among maternal trees in the initial collection ($P < 0.01$). This suggests at least some genetic control of acorn size. Similar patterns of strong differences in seed weight among trees and weaker but still significant differences among populations have been found for ponderosa pine (Ager and Stettler 1983). Ager and Stettler (1983) proposed that the large differences in seed size among ponderosa pine trees reflect a local selective regime that is highly heterogeneous in space and time. Before we can contemplate similar explanations for the observed between-tree variation in blue oak acorn size, we need to obtain better estimates of the magnitude of genetic control of acorn size parameters. We are currently trying to understand the relative contribution of environmental and genetic factors to variation in these size parameters by analyzing differences among maternal trees across multiple years. The rationale for this analysis is that if acorn size parameters for a given tree remain relatively constant across years varying widely in acorn production, it is more likely that acorn size has a significant genetic component.

Evolutionary Potential Within Populations

Within-population differences among trees (maternal families) in both survival and growth are significant for both populations examined (*table 1*). These inter-family measurements allow us to examine the genetic variation within a population and thus its evolutionary potential for further adaptive change. Variance component analyses indicated that in spite of the large environmental effects indicated by the between-block and within-family (error) terms, variation among maternal families within a population was a significant contributor to phenotypic variation in seedling growth and survival. This inter-family variation was relatively strong for seedling growth parameters (e.g., height) and slightly less pronounced for survival. The lower inter-family variation in survival might be expected because factors reducing survival (e.g., small mammals) are often spatially stochastic and thus less likely than factors affecting individual seedling growth to involve genetic differences among families. Overall, the significant variation among families indicates that there is genetic variation within both populations for seedling growth and survival; genetic variation that can allow these populations to adapt to new evolutionary challenges.

Within-family (error) variation reflects a combination of genetic variation, microenvironmental variation, and an interaction of both genetic and

⁴Unpublished data on file at 161 Hunt Hall, Department of Agronomy and Range Science, University of California, Davis, CA 95616

Table 1—Variance component analysis of the percent contribution of among-family, among-block, block by population, and within-family (error) variance to total phenotypic variance in seedling survival and height at both planting sites

Variance component	Seedling survival (by year)				Seedling height (by year)			
	Hopland □□□□□		Sierra □□□□□□□		Hopland □□□□□□□		Sierra	
	1992	1995	1992	1995	1992	1995	1992	1995
	----- Percent of total variance -----				----- Percent of total variance -----			
Family	23.4	10.3	16.6	6.1	25.4	10.3	19.8	4.5
Block	14.9	19.5	14.8	6.2	15.5	9.3	8.6	13.4
Block x population	2.2	8.2	0.7	0.9	3.2	9.6	15.7	16.8
Within-family (error)	59.5	62.0	67.9	86.9	55.8	70.9	55.9	65.3

environmental factors. Although we cannot estimate the effects of microenvironmental variation directly, we suspect that a large amount of the within-family variation was caused by substantial microenvironmental variation within a block. Highly localized microenvironmental variation within a block would not be surprising because we have found that physical parameters including soil depth, soil nutrients, soil moisture, relative humidity, and light vary widely within a block (unpublished data)⁵. The relative importance of inter-family variation in explaining phenotypic variation in seedling growth and survival appeared to decline over the course of the study. For example, in 1992, inter-family variation accounted for 25 percent of the variation in seedling height at the Hopland planting site. In 1995, the proportion of the variance explained by differences among families has dropped to about 10 percent. This result suggests that potential importance of genetic effects on seedling performance may be most pronounced during the first years of establishment. In addition, there was a significant site effect in that the importance of inter-family variation was less at the Sierra planting site for all 4 years of the study. This site difference may reflect the stochasticity of small mammal predation that was so predominant at the Sierra site.

Conclusions

Despite strong evidence for significant gene flow among populations of blue oak (Riggs and others 1991), our reciprocal transplant studies indicate that regional populations represent genetically distinct entities. Further, results suggest that local adaptation has occurred within at least one of the regional populations examined. These results for blue oak challenge the assumption that wind-pollinated, outcrossing trees with nearly continuous geographic distributions are always characterized by high genetic variation within populations and by low genetic diversity between populations (Hamrick 1979, 1983; Hiebert and Hamrick 1983). Our study is not unique in this conclusion; other studies that have tried to examine the potential for strong selection to form locally adapted populations in trees have also detected significant differences between populations. In addition to the work on red oak discussed above (Sork and others 1993), studies on regional differentiation in lodgepole pine (Yeh and others 1986), elevational variation in ponderosa pine (Conkle 1973) and Douglas-fir (Campbell 1979), and local adaptation in pinyon pine populations (Mopper and others 1991) have also found differentiation between populations despite potentially high gene flow. This is not to say that within-population genetic

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diversity of outcrossing tree species is low. Results from our analyses of inter-family differences indicate that there is significant genetic variation within both blue oak populations for traits such as acorn size and seedling survival and growth. This diversity within each population may indicate that high gene flow within each population may limit scales of adaptation in blue oak to “coarse grained” regional populations. On the other hand, our study was not designed to examine microhabitat scale adaptation as found in red oak (Sork and others 1993). It is possible that the inter-family differences that we detected result from our sampling strategy to use widely distributed maternal trees from a variety of microhabitats. Instead of just representing within-region genetic diversity, differences among these trees may indicate that a large number of sub-populations are locally adapted to a variety of microhabitats. Taken together, these results suggest a complex genetic architecture for blue oak and thus argue for a serious consideration of genetic issues when restoration of blue oak populations involves a significant planting effort.

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