Evidence for Local Adaptation in Closely Adjacent Subpopulations of Northern Red Oak (Quercus rubra L.) Expressed as Resistance to Leaf Herbivores

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EVIDENCE FOR LOCAL ADAPTATION IN CLOSELY ADJACENT SUBPOPULATIONS OF NORTHERN RED OAK (*QUERCUS RUBRA* L.) EXPRESSED AS RESISTANCE TO LEAF HERBIVORES

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Abstract.—Many studies of herbaceous plant populations have illustrated the potential of adjacent subpopulations to adapt to local ecological conditions. However, the extent to which local adaptation on a small geographical scale can occur in outcrossing tree populations is not well understood. In this study, we reciprocally transplanted acorns from adjacent subpopulations of northern red oak (*Quercus rubra* L.) occupying north-, southwest-, and west-facing slopes within a 4-ha plot in a Missouri oak-hickory forest. The quantitative character we measured was leaf damage by herbivores on first-year seedlings, because it reflects resistance to insect herbivores—a quantitative trait that could be under different selective pressures in dissimilar microhabitats. We found that seedlings showed the least damage when planted at the site of the maternal plant. This finding provides initial but strong evidence of local adaptation and illustrates that selection associated with leaf herbivory may have a strong impact on the genetic structure of local tree populations. Such a result is unexpected for a widely outcrossing species on such a small geographical scale but indicates that genetic structuring is possible within other plant populations occupying heterogeneous environments.

Local adaptation results when a population has evolved through natural selection in response to the specific ecological conditions of its local environment. Because plants usually have restricted seed and pollen dispersal and are immobile once they are established, selection by the local environment may cause adaptive genetic differentiation among or within plant populations (Bradshaw 1972). For example, the classic experiments of Clausen et al. (1940) using reciprocal common garden experiments showed that populations of *Potentilla glandulosa* occurring along an elevational gradient survive and grow best at the elevation from which they were derived. In general, reciprocal transplant experiments provide a valuable approach toward identifying the relationship between the genetics and ecology of plant populations.

For some herbaceous plant species, it has been shown that significant genetic differentiation via local adaptation can occur among adjacent subpopulations (Bradshaw and Jain 1966; Antonovics 1968; McNeilly and Bradshaw 1968; Schemske 1984; Parker 1985; Schmitt and Gamble 1990). This result is not surprising for plant species that have restricted gene flow through limited pollen or seed

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dispersal, because restricted gene flow prevents swamping of locally favored genes (Wright 1943, 1969; Slatkin 1973, 1985, 1987). However, restricted gene flow can also create small effective population sizes, which increase the potential of genetic drift to reduce the effects of natural selection (Wright 1943, 1969; Slatkin 1973, 1985, 1987). In contrast, for outcrossing plant species with high gene flow, such as most temperate tree species (Loveless and Hamrick 1984), the efficacy of local selection in creating genetic differentiation among adjacent subpopulations is reduced by high rates of gene flow unless selection is strong (Slatkin 1973; Endler 1977). Forest geneticists have documented genetic differences among populations occupying different latitudes and elevations with isozymes as genetic markers (see, e.g., Yeh and O'Malley 1980; El-Kassaby and Sziklai 1982; Sork et al. 1993) and have shown population differentiation in quantitative characters such as tolerance to drought and frost or resistance to pests (see Libby et al. 1969). However, few studies have looked for adaptation on a relatively small geographical scale for outcrossing tree species (Gregorius 1985; for noteworthy exceptions, see Mitton et al. 1977; Mopper et al. 1991).

In our study, we are interested in whether selection by insect herbivores can be sufficiently strong to cause local adaptation in adjacent subpopulations of northern red oak (*Quercus rubra* L.). The broad range of plant characteristics that seemingly have evolved as evolutionary responses to selection by herbivores suggests that herbivores can impose intense selection on plants (Marquis 1992). Nonetheless, only a few studies have shown a genetic basis for resistance traits in natural populations (Crawford-Sidebotham 1972; Berenbaum et al. 1986; Rausher and Simms 1989; Simms and Rausher 1989; Marquis 1990), and in some cases it has been suggested that this has resulted in local adaptation (Zangerl and Berenbaum 1990). A complication for the evolution of resistance is that most plant species experience damage by a number of different herbivore species, which could impose qualitatively different selection pressures on the host plant. Further, selection by herbivores can vary in space and time (Marquis 1990). A difficult aspect of identifying resistance to herbivores is that the actual trait conferring resistance is often unknown. However, one can measure resistance as a function of lower susceptibility to herbivores, that is, a smaller percentage of leaf area damaged (Simms and Rausher 1989; Marquis 1990).

The purpose of our study is to determine the extent to which a population of northern red oak (*Q. rubra* L.) occupying a physically heterogeneous environment might be genetically differentiated among microhabitats. The quantitative character we use to measure genetic differentiation is susceptibility to herbivores. This character can be expected to differ within a heterogeneous environment because the community of insect species as well as the abundance of insects is likely to differ across dissimilar microhabitats within a forest. For example, one study found that the insect communities in clear-cuts and partial cuts differed significantly (Linit et al. 1986). If insect herbivore communities differ qualitatively and quantitatively across microhabitats within a site, selection by such herbivore communities could contribute to local adaptation. As a first step toward evaluating the possibility of adaptive differentiation in our population, we conducted a reciprocal transplant experiment using genotypes from different microhabitats.

Northern red oak (*Quercus rubra* L.), a widely distributed North American tree species, tends to occur on moist north-facing and east-facing slopes. This species ranges from southern Quebec and Ontario south to northern Florida, and from the eastern edges of Texas, Oklahoma, and Kansas up through Iowa east to southeastern Minnesota (Schopmeyer 1974). Outcrossing rates for northern red oak at our study site were not significantly different from 100% during 1 yr of study (Sork et al. 1992). Moreover, in examining distance of pollen travel based on progeny of five maternal plants, 20%–35% of the progeny resulted from pollen sources greater than 50 m from maternal plants (V. L. Sork, unpublished data).

The study site is located at an 800-ha ecological reserve located at Washington University's Tyson Research Center, St. Louis (38° 31′N, 90° 33′W) on the northeastern end of the Ozark Plateau. This reserve includes oak-hickory forest with northern red oak as a codominant species with white oak, *Quercus alba* (Hampe 1984). Typical for Ozark formation, the hilly study site contains a great deal of microhabitat heterogeneity, with northern red oak occurring most densely on north-facing slopes. The experiment was conducted within a 4-ha plot of oak-hickory forest that contains three microhabitats (Hampe 1984): north-facing slope (NO), which had the greatest inclination (mean = 20°, range = 15°–30°); southwest-facing slope (SW), which had the slightest inclination (mean = 15°, range = 12°–18°); and lower west-facing slope (LW) with intermediate inclination (mean = 13°, range = 10°–15°). The area of each microhabitat is about 1 ha (Hampe 1984).

To test for evidence of local adaptation in red oak seedling populations, we conducted a reciprocal transplant experiment. In fall 1989, we collected open-pollinated acorns from 18 maternal plants located in three adjacent microhabitats (six families per microhabitat): NO, SW, and LW. Because acorns sampled had resulted from open pollination, these 18 families consisted of mixtures of half- and full-sibs. Acorns from each maternal plant were distributed equally among four replicate plots in each of the three microhabitats during fall 1989. Ten acorns from each family were planted into the 12 replicate plots (1 m × 2 m), yielding a total of 180 acorns per replicate plot. Acorns were planted just below the soil surface 10 cm apart in a random array and marked as to their family with a labeled 8 × 1.5-cm stick. The plots were covered with hardware cloth (diameter = 1.25 cm) exclosure cages to prevent seed predation by rodents and herbivory by deer and other mammals.

During March through June 1990, the plots were visited every 5 d to record the date a seedling first emerged from the ground. At the end of summer and early fall, we sampled a total of 604 seedlings, three randomly selected seedlings per maternal genotype per replicate plot (totaling 12 seedlings per genotype per microhabitat). Because some plots contained fewer than three seedlings per maternal genotype, we used the available seedlings, which resulted in sample sizes of 172 seedlings (NO), 215 (SW), and 217 (LW). For each plant, we recorded seedling height. In addition, we measured total leaf area by placing a transparent piece of graph paper (gridded to squares with area of 0.0625 cm^2) over each leaf

of a seedling and counting the number of squares that covered the leaf as well as the number of squares missing because of damage by herbivores. Our estimates of total leaf area and total leaf damage were relatively accurate because only three leaves out of all the plants showed close to 100% herbivory, and most leaves had less than 50% damage. The amount of herbivory incurred by each seedling was calculated by summing leaf area damaged over all leaves. Percentage of leaf area damaged was calculated by dividing the total area damaged by the total leaf area of the seedling. This percentage of total leaf area damaged was used as an index of susceptibility, the inverse of resistance (Rausher and Simms 1989; Simms and Rausher 1989; Marquis 1990).

The data were analyzed using a mixed-model ANOVA with plot as a random effect and maternal microhabitat and planting site microhabitat as fixed effects. We did not include family effects within maternal microhabitat because our goal was not to evaluate differentiation among families. However, our preliminary analyses showed no significant differences among families from the same maternal site, nor did we find significant family-by-site or family-by-replicate-plot interactions. The analyses were done on mean percentage of leaf area damaged per seedling of the three seedlings per parent per plot with square-root arcsine-transformed data. We used family mean values rather than individual seedling values because (1) the individual values were nonnormally distributed and (2) that level of replication is unnecessary for our central hypothesis. To ensure that results from our model were not confounded by other factors, we conducted the analysis with several covariates. The model was done with date of emergence and date of measurement as covariates because time of exposure to herbivores may affect the amount of herbivore damage incurred by a plant. Similarly, seedling height and total leaf area (both natural log transformed) were included as covariates in case plant size affects a plant's chances of being damaged by herbivores.

RESULTS AND DISCUSSION

Data from our reciprocal transplant experiment are consistent with the hypothesis that seedling populations occupying different microhabitats show evidence of local adaptation. Seedlings whose mother was native to a given planting site had a lower percentage of leaf area damage compared with nonnative seedlings at all three planting sites (fig. 1; cf. means within rows). Moreover, seedlings always had the lowest percentage of leaf damage when planted in the site native to their maternal parent (fig. 1; cf. means within columns). In other words, when we analyzed percentage of leaf area damaged, native seedlings were less susceptible, or more resistant, than nonnative seedlings, and they showed lower susceptibility and expressed greater resistance when planted in their native site.

These patterns of leaf damage in seedlings are supported statistically by a significant interaction between planting site and maternal source site (table 1), which is necessary evidence for local adaptation. Of the four covariates, the only variable that co-varied with percentage of leaf damage was seedling height (table 1). Taller seedlings had a greater percentage of leaf damage, which suggests that

A. PLANTING SITE: LW

B. PLANTING SITE: SW

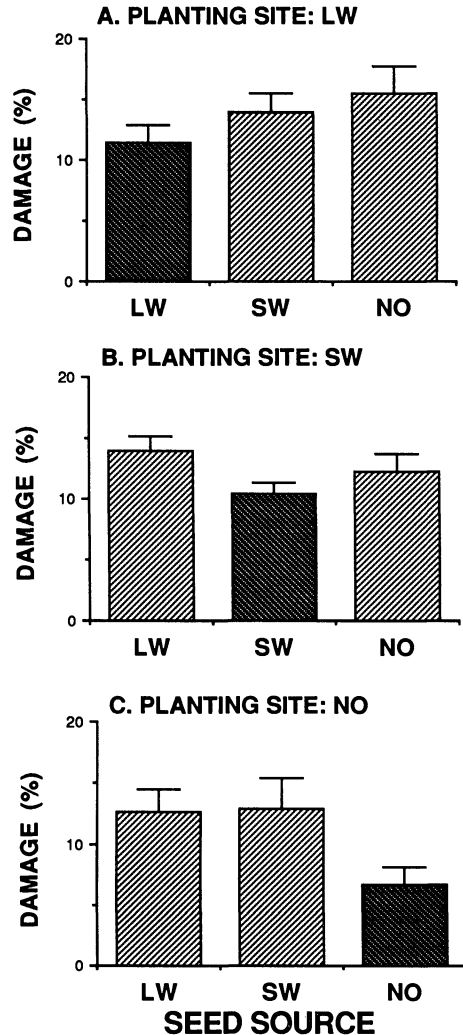## C. PLANTING SITE: NO

SEED SOURCE

FIG. 1.—Mean percentage of leaf damage (+1 SE) of northern red oak seedlings planted in four replicate plots per planting site, given as microhabitats used as planting site (A–C). The location of the source sites for acorn collection is indicated on the X-axis. The three adjacent microhabitats within a Missouri oak-hickory forest were LW, SW, and NO.

plant size affects the likelihood of attack by herbivores. Among the other covariates, seedlings with greater leaf area did not show significantly greater percentage leaf area damaged (df = 1, 167 mean squares [MS] = .0002, F = .09, P > .05), nor did date of emergence (df = 1, 167, MS = .039, F = 3.48, P > .05) or date of measurement (df = 1, 167, MS = .000, F = .01, P > .05). Thus, neither the amount of leaf area nor the amount of time that a seedling was exposed to herbivores had a major impact on percentage of leaf area damaged.

TABLE 1

ANOVA for Percentage of Leaf Area Damage in Reciprocal Transplant Experiment

Source	df	MS	F
Planting site	2	.077	1.05
Seed source	2	.016	1.06
Replicate (planting site)	9	.081	5.32**
Planting site × seed source	4	.062	4.05*
Source × replicate (planting site)	18	.015	1.42
Seedling height	1	.118	10.91**
Error	167	.011	

Note.—The table provides a summary of the ANOVA for mean percentage leaf area damage per maternal family per plot using seedling height as a covariate. Fixed effects were planting site and seed source while replicate plot was a random effect.
* $P < .05$.
** $P < .01$.

Our results do not show significant differences in levels of damage by insect herbivores among red oak seedlings from different source sites (table 1; mean percentage of damage was 12.64%, 12.35%, and 12.17% for LW, SW, and NO source microhabitats, respectively). Thus, seedlings originating from acorns collected from any one microhabitat did not show performance superior, in terms of percentage of leaf area damaged, to that of seedlings from the other microhabitats.

Northern red oak seedlings did not suffer different levels of leaf damage by insect herbivores among the three microhabitats (table 1). The means for percentage of damage were 13.61%, 12.35%, and 10.99% for LW, SW, and NO planting microhabitats, respectively. Thus, we conclude that, within the year in which this experiment was conducted, there is little evidence that selection pressure by leaf herbivores differs across microhabitats. However, the interaction between planting site and source site indicates that, if genotypes become established away from the maternal microhabitat, they will suffer greater herbivore damage and thus could be selected against.

An unanticipated result from our study is the significant variation in resistance across replicate plots within a microhabitat (table 1). This result might be due to environmental variation within a microhabitat, which, in turn, affects seedling performance or it might be due to patchy distribution in type and abundance of insect herbivores. This variation across replicate plots illustrates the heterogeneity even within researcher-defined microhabitats.

While our results support the hypothesis that northern red oak seedlings at our study site are locally adapted to microhabitats, this type of experiment cannot provide a mechanism. Several non–mutually exclusive mechanisms could be evaluated. One possibility is that insect herbivore communities differ in abundance; virulence, and/or species composition across microhabitats and represent different selective pressures on the oaks within each. This could result in plants possessing specific adaptations for defense against insects that occur in their respective microhabitats. In order to examine this mechanism, it would be ideal to identify specific characters that provide resistance (e.g., leaf toughness or

tannin content) and then measure genetic differentiation in those traits across subpopulations.

Another possible mechanism is that seedlings are physiologically better adapted to the source site and, thus, are more able to allocate resources to herbivore-resistant traits. Seedlings in different microhabitats may perform differently and, thus, may be more resistant to insects when planted in their own microhabitat than when planted in others. However, when we co-varied percentage of leaf damage with seedling height and total leaf area, two measures of seedling performance, we removed the variation due to these factors and yet still found a significant site-by-source interaction.

Finally, one other factor that could contribute to our findings is a site-specific maternal seed source-by-planting-site interaction maternal effect. In other words, an environmental maternal effect that is only expressed when acorns are planted in their maternal environment. While this site-specific maternal effect is impossible to rule out, it does not seem plausible for the following reasons. First, one of the most common characters affected by maternal environment is seedling height (Roach and Wulff 1987). However, a separate statistical analysis of seedling height for our population did not show a significant interaction between maternal seed source and planting site (V. L. Sork and K. A. Stowe, unpublished data). Second, if maternal effects were strong we would expect to find a significant maternal seed source effect, which we did not (table 1). Finally, those characters that may be responsible for this resistance, for example, secondary chemistry, leaf hairiness, leaf toughness, and so on, seem unlikely to be transmitted from the endosperm to the seedling. Thus, a site-specific maternal effect seems a much less likely explanation of our findings than genetic differentiation for the quantitative character of resistance.

In conclusion, this study provides evidence in support of the hypothesis that our population of *Quercus rubra* L., a widely outcrossing angiosperm tree species, is locally adapted with respect to the quantitative character of herbivore resistance (or conversely, susceptibility to herbivores). This unique finding indicates that natural selection may occur on a local scale not usually examined for long-lived species. In this case, it appears that selection due to variation among microhabitats occurring throughout the life span of *Q. rubra* L. was sufficient to create genetic structure; however, further studies would be needed to demonstrate the extent to which our findings are due to the impact of herbivores. Other differences due to soil, moisture, or nutrient conditions or to changes in competitive, predator-prey, mycorrhizal, or pathogenic interactions can all contribute to differential selection across adjacent microhabitats (see, e.g., Mopper et al. 1991). Nonetheless, our finding that native seedlings show lower leaf damage in their own microhabitat demonstrates genetic differentiation among adjacent subpopulations in spite of high levels of gene flow across those microhabitats.

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