Patterns of Genetic Variation in Woody Plant Species in the Missouri Ozark Forest Ecosystem Project

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Abstract.—We quantified current patterns of genetic variation of three woody plant species—*Carya tomentosa* (Juglandaceae), *Quercus alba* (Fagaceae), and *Sassafras albidum* (Lauraceae)—distributed throughout the nine Missouri Ozark Forest Ecosystem Project (MOFEP) study sites and evaluated the data in light of the MOFEP experimental design. Genetic variation was estimated using electrophoretically detected isozymes as genetic markers. Results indicate that population level genetic diversity estimates were within the range typical of woody plant species but a comparison of levels of genetic variation of *Q. alba* at MOFEP with other *Quercus* species indicates that levels were somewhat low. Each species showed significant differences among sites for the genetic diversity measures or inbreeding coefficient. *C. tomentosa* and *Sassafras albidum* showed significant differences due to ELT. Unexpectedly high levels of inbreeding coefficients were documented in all three species. Two of the species, *Q. alba* and *Sassafras albidum*, showed a significant impact of year of acquisition on measures of genetic diversity and inbreeding. We found differences among the management treatment classes for *Sassafras albidum* only. We conclude that the pattern of genetic diversity and inbreeding is heterogeneous across MOFEP study sites with land use history of specific sites being at least one of the contributing factors. Nonetheless, this heterogeneity has not created any excessive biases for the experimental design of the management treatment experiment of MOFEP.

"...every human action, every aspect of forest management, has ecological and genetic effects, and we must try to determine what those effects are if we are to maintain healthy ecosystems." (Ledig 1992)

Maintenance of genetic variation has been a major concern to conservation biologists and forest managers because the amount of variation determines the ability of a population to respond to environmental change (Ellstrand and Elam 1993, Fisher 1939). Moreover, the existing quality of genetic variation determines the fitness of populations because it provides the diversity of phenotypic traits that are needed to meet the vicissitudes of current and future environmental conditions (Ledig 1992). Because global warming, atmospheric pollution, and habitat alteration are real threats to living organisms, populations that are genetically depauperate will be less able to respond to environmental changes (Ledig 1992). Unfortunately, consideration of this factor is not always a part of forest management. The Missouri Ozark Forest Ecosystem Project (MOFEP) offers a unique opportunity to examine the current status of genetic variation in Missouri forests.

Genetic variation often is described with three statistical parameters: polymorphism (P), heterozygosity (H), and allelic diversity (A) (Hamrick and Godt 1989, Hamrick *et al.* 1992). Polymorphism is equal to the proportion of total loci that have a common allele with a maximum frequency of 95 percent (P_{95}) or the proportion of

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loci that have two or more alleles in the sample examined with no criteria for the frequency of the common allele (P_{NC}). Heterozygosity can be quantified in two ways: observed heterozygosity (H_O), the proportion of heterozygous individuals per locus averaged across loci; and expected heterozygosity (H_E), which uses the allele frequencies to calculate the expected frequency of heterozygotes when the population is at Hardy-Weinberg equilibrium. H_O is a useful index of heterozygosity because several studies have indicated a positive relationship between fitness components and observed heterozygosity (Mitton and Grant 1984). H_E is a commonly used index of genetic diversity (Hamrick and Godt 1989, Hamrick *et al.* 1992). Allelic diversity is the average number of alleles across all loci (A) or the average considering only polymorphic loci (A_P). All of these measures can be estimated for individual subpopulations and for the entire sampled population. In general, we expect species with low gene flow, high inbreeding, or narrow geographic ranges to have low values for genetic diversity indices and species with high gene flow to have high values (Hamrick and Godt 1989, Hamrick *et al.* 1992). In most studies of forest tree species, the values are relatively high in comparison to annual plants or herbaceous perennial species (Hamrick and Godt 1989, Hamrick *et al.* 1992).

Most population genetic models assume that populations are at equilibrium, that all have equal likelihood of gene flow, and that selection has had no impact on patterns of genetic variation (Hartl and Clark 1989, Slatkin 1985). Yet, populations that exist within a heterogeneous landscape, such as the Missouri Ozarks, may not satisfy these assumptions: gene flow may not be equal among all populations and local selection by microhabitat may create heterogeneity in patterns of diversity. In particular, the assumption that populations are at equilibrium may be questionable for second-growth forests such as those in the Ozarks. The particular land use history of Missouri Ozark forests may have had an especially severe and prolonged impact on genetic variation. Evidence suggests that much of the Ozarks was managed by indigenous groups through deliberate, episodic fires (Guyette and Dey 1997). More recently, in the 1880's, the southern Ozarks experienced extensive clearcutting for timber (Cunningham and Hauser 1989). Photographs of that era indicate a naked landscape extending for large areas (Sork, personnal observation). Subsequent to clearcutting, the site was managed for grazing of sheep and cattle with the use of occasional fires to prevent forest reestablishment (Cunningham and Hauser 1989). The few populations remaining could have suffered a loss of genetic variation if these populations experienced a significant reduction in population size, known as a genetic bottleneck (Hartl and Clark 1989). Typically, genetic bottlenecks are also accompanied by high inbreeding coefficients when the population increases in size. Starting in 1925, the Missouri Department of Conservation purchased sites of land for the purpose of forest management. Thus, the sites of MOFEP have different years of acquisition and land use histories.

The overall goal of this study was to quantify the current patterns of genetic variation of selected woody plant species distributed throughout the Missouri Ozark Forest Ecosystem Project (MOFEP) study sites. As part of MOFEP, this study used its experimental design (Sheriff and He 1997). To represent woody plants, we selected three study species that exhibited a range of pollination and seed dispersal modes and growth forms: *C. tomentosa* (Juglandaceae), *Quercus alba* (Fagaceae), and *Sassafras albidum* (Lauraceae). Genetic variation was estimated using electrophoretically detected isozymes as genetic markers. The specific objectives of this paper were: (1) to quantify the mean amount of genetic variation per population for the three woody plant study species, using several genetic diversity measures and the inbreeding coefficient; (2) to examine whether these measures of genetic diversity differ significantly among sites or between two microhabitat types, using a model independent of the MOFEP design; (3) to explore possible influence of land use history, as indicated by year of land acquisition by the Missouri Department of Conservation, on genetic diversity and inbreeding measures; and (4) to evaluate MOFEP experimental design using pretreatment data on genetic diversity and inbreeding.

MATERIALS AND METHODS

Study Species

We selected two canopy tree species and one understory shrub species to be the focal study species. These three species were chosen because their wide distribution among MOFEP study sites and in the region (Braun 1950) make them ideal representatives of temperate

forest woody species. These species also represent a range of pollination and dispersal modes. The first species is *Carya tomentosa* Nuttell (Juglandaceae) (common name = mockernut hickory), which is a monoecious, canopy tree that is tetraploid (Stone 1961). Pollen is wind dispersed; squirrels and gravity are responsible for seed movement. The second species is *Q. alba* L. (Fagaceae) (common name = white oak), which also is a canopy species. Plants are monoecious, the flowers are wind pollinated, and the seeds are dispersed by gravity, mammals, and birds. The availability of genetic information on *Q. alba* (Sork, unpublished data) and other oak species (Huang 1992, Sork *et al.* 1993) allows us to compare the structure of the MOFEP study site with other locations in the United States. The third species is *Sassafras albidum* (Nuttell) Nees (Lauraceae), which in the Ozarks is generally a shrub or small tree. The plants are generally dioecious with insect-pollinated flowers. Fruiting is largely confined to forest gaps and edges in this shade intolerant species. The seeds are dispersed by birds.

Study Site

The Missouri Ozark Forest Ecosystem Project study area incorporates one wildlife area and four State forests that are located in the Ozark Mountains of south-central Missouri: Deer Run State Forest (Reynolds County), Paint Rock, Cardavera, and Carr Creek State Forests (Shannon County), and Peck Ranch Wildlife Area (Carter County). In the MOFEP design, these forests are divided into nine sites, each of which is assigned to one of three forest management treatments (even-aged, uneven-aged, and no harvest; Kurzejeski *et al.* 1992, Brookshire *et al.* 1977, Sheriff and He 1997). The entire MOFEP study area is divided into stands that are classified according to ecological land type (ELT).

Before 1880, most of these Ozark forests were dominated by continuous *Pinus echinata* (shortleaf pine) communities, but intensive harvesting (1880 to 1920) followed by repeated burning and grazing altered the landscape to produce the oak-hickory and oak-pine communities found there today (Cunningham and Hauser 1989). In the Ozarks, *Q. alba* shares the canopy with other species of oaks, including *Q. stellata*, *Q. velutina*, *Q. coccinea*, and with *Pinus echinata*, and *C. tomentosa* (Kurzejeski *et al.* 1992). Forest stands that were sampled range in elevation from 182 to 275 m and were within 37°00' N and 37°15' N, and 91°07' W and 91°00' W (U.S. Topographic Maps, 7.5 minute series: Fremont Mo., Van Buren North Mo., Stegall Mountain Mo., Powder Mill Ferry Mo., and Exchange Mo). Distances between populations range from 0.2 km to 24 km.

Sampling Procedure

Sampling for this study was conducted in 1993 and 1994. In July 1993, leaf samples from *C. tomentosa* and *Sassafras albidum* were collected. In June and July 1994, leaf samples were collected from *Q. alba*. In both years, we sampled 36 stands located throughout the MOFEP study area. Using a hierarchical sampling design, we collected mature leaf tissue from nine sites, two microhabitats within each site, (ELT 17, north- and northeast-facing slopes, and ELT 18, south- and southwest-facing slopes), and two forest stands within each ELT. Within a stand, 48 juvenile and adult trees were sampled within an area approximately 1 ha in size. We consider this sample of individuals within a stand as representative of the local population. Trees were sampled along two to three transects within the midslope region and were separated by at least 7 to 10 m to minimize sampling of related individuals. From each individual, the diameter at breast height (d.b.h.) was measured and two to three leaves were collected. Leaves were kept on ice for up to 3 days until they were transported to the University of Missouri-St. Louis and stored in a -70°C freezer.

Laboratory Analysis

All leaf samples were analyzed using standard horizontal starch gel electrophoresis procedures (Kephart 1990, Sork *et al.* 1992). Mature leaf tissue was ground into a fine powder in a mortar using liquid nitrogen and a pestle. Protein from each sample was extracted using a phosphate extraction buffer (Mitton *et al.* 1979), fortified with 10 percent (w/v) polyvinylpyrrolidone to inactivate phenolics, which tend to bind proteins. The crude extract was absorbed onto 4 mm x 6 mm Whitman #3 chromatography paper wicks and stored at -70°C until electrophoresis. Gels used in electrophoresis consisted of 10 percent starch (Sigma S-4501).

After surveying 20 enzymes on various combinations of five gel/electrode buffer systems, we selected various buffer combinations and enzymes for each species (table 1). For analyses

Table 1.—*List of enzymes, gel/electrode buffer systems, and number of alleles observed for 36 subpopulations of three woody plant species found in the MOFEP study area in southern Missouri. Numbers for gel/electrode buffer systems refer to recipes in Soltis* et al. *(1983) or modifications of those recipes. System 8 modifications followed Rieseberg and Soltis (1989), while system 6 gel buffer contained 0.055 M citric acid and 0.190 M tris.*

Enzymes (Abbreviation-locus)	Gel/electrode buffer system	Number of alleles
	Carya tomentosa	
Aspartate aminotransferase (AAT-2)	7	3
Diaphorase-2 (DIA-2)	7	2
Fluorescent esterase (FES-1)	8	2
Leucine aminopeptidase (LAP-1)	6	1
Malate dehydrogenase (MDH-1)	4	1
Menadione reductase-1 (MNR-1)	6	1
Menadione reductase-2 (MNR-2)	6	2
Peroxidase (PER-1)	6	2
Peroxidase (PER-3)	6	2
Phosphoglucoisomerase-1 (PGI-1)	6	1
Phosphoglucoisomerase-2 (PGI-2)	6	4
Phosphoglucoisomerase-3 (PGI-3)	6	4
Shikimate dehydrogenase (SKDH-1)	4	2
	Quercus alba	
Colormetric esterase (CES)	6	3
Fluorescent esterase (FES-1)	8	3
Fluorescent esterase (FES-2)	8	1
Fluorescent esterase (FES-3)	8	1
Fluorescent esterase (FES-4)	8	3
Fructose 1,6-diphosphatase (F16)	4	3
Malate dehydrogenase (MDH-1)	4	1
Menadione reductase-1 (MNR-1)	6	3
Peroxidase (PER-1)	6	3
Peroxidase (PER-3)	6	3
Phosphoglucoisomerase (PGI-1)	8	1
Phosphoglucoisomerase (PGI-2)	8	4
Shikimate dehydrogenase (SKDH-1)	4	2
	Sassafras albidum	
Aspartate aminotransferase (AAT-2)	7	3
Aspartate aminotransferase (AAT-3)	7	3
Diaphorase-2 (DIA-1)	7	2
Diaphorase-2 (DIA-2)	7	2
Menadione reductase-1 (MNR-2)	6	1

done on all 36 populations of each species, we selected a subset of putative loci: 9 loci for *C. tomentosa*, 10 loci for *Q. alba*, and 5 loci for *Sassafras albidum*. Although we could have increased the number of loci in the first two species, we concentrated our efforts on loci that are polymorphic in at least one population because those loci are more useful in identifying patterns of genetic differences among populations. This decision reduced the time and expense of running additional gels and staining for apparently monomorphic loci for more than 1,700 genotypes per species. However, we acknowledge that the bias toward polymorphic loci biases genetic diversity measures upward.

Statistical Analysis

We calculated standard measures of genetic diversity using BIOSYS-1 (Swofford and Selander 1981) for *Q. alba* and *Sassafras albidum* and our own programs for the tetraploid species, *C. tomentosa*. The measures we used were: percent of polymorphic loci (P_{95} and P_{NC}), number of alleles per polymorphic locus (A_p), and observed and expected heterozygosities (H_O and H_E) for polymorphic loci. We calculated inbreeding coefficients for each individual population based on the formula: $F_{IS} = 1 - (H_O/H_E)$.

We tested whether these five measures of genetic diversity and the inbreeding coefficient differed among site or ELT, using a two-way fixed effects analysis of variance model. For this analysis, we treated sites as fixed effects because they were not randomly sampled from the Ozarks but represent the only sites available for this study. We transformed P_{95}, P_{NC}, H_O, and H_E using an arc sine square root transformation to satisfy the equal variances assumption of ANOVA. Even after transformations, P_{95} and P_{NC} did not satisfy the normality assumption well because the data were not continuously distributed, but the data did approximate a normal distribution with one mode around the mean. We present the results anyway both here and with models described below because ANOVA can be quite robust. Nonetheless, our discussion will emphasize other measures of genetic diversity.

We used year of acquisition by the Missouri Department of Conservation as a measure of impact of land use history. Although we do not know the exact chronology of land use history of each site, once the MDC purchased a site, the forests were protected from fire and were managed to promote tree regeneration. Thus, year of acquisition represents the number of years of forest management. Because this variable created unequal sample sizes, we conducted a nonparametric Kruskal-Wallis k-sample test using the F-values generated by an ANOVA model based on ranks. Each species was analyzed separately.

To address our final goal of the pre-treatment study, we examined our genetic diversity and inbreeding measures, using the experimental design of the MOFEP project (Brookshire *et al.* 1997, Sheriff and He 1997). The ANOVA model included block and management treatment as the main effects. Both terms were tested over the interaction term. The model was run separately for each species. For the sake of simplicity, we did not include ELT in this model because variation due to ELT was tested in the ANOVA model above when we examined site and ELT. However, in future analyses of post-treatment data, ELT can be incorporated into the model.

In our statistical models, we indicate levels of significance, which start at 0.10. While we will cautiously interpret any findings with a probability level greater than 0.05, we want to minimize risk of Type II error (that is, the probability that we accept our null hypothesis of no differences when differences truly exist). To evaluate the risk of Type II error, we conducted a power analysis on all variables with nonsignificant treatment effects within the MOFEP experimental design, using the block by treatment interaction as the error term. For this analysis, we set alpha = 0.05. In general, to avoid a Type II error, one needs a high degree of power.

RESULTS

Variation Across Site and ELT

The average levels of genetic variation and inbreeding per population differed across the three species (table 2). *C. tomentosa*, a tetraploid species, had higher values of the five genetic diversity measures and inbreeding coefficient than the other two species. *S. albidum* had higher values for all five genetic diversity indices and the inbreeding coefficient than *Q. alba*. We caution about comparisons among species because the differences may be due, at least in part, to the differences in number of loci

Table 2.—*Species means (with 1 standard error in parentheses) for population measures of genetic variation and inbreeding. Thirty-six populations were sampled for each species with equal sampling of the nine MOFEP sites. The measures of genetic variation were: polymorphism at 95 percent level (P_{95}); polymorphism with no criterion (P_{NC}); allelic diversity of polymorphic loci (A_P); observed heterozygosity (H_O); expected heterozygosity based on Hardy-Weinberg expectations (H_E); and inbreeding coefficient (F_{IS}).*

	No. loci	Mean no. inds. per population	P_{95}	P_{NC}	A_P	H_O	H_E	F_{IS}
Carya tomentosa	9	41.0	0.710 (0.019)	0.811 (0.009)	3.26 (0.109)	0.315 (0.008)	0.484 (0.011)	0.348 (0.009)
Quercus alba	10	47.3	0.331 (0.012)	0.497 (0.013)	1.17 (0.004)	0.098 (0.002)	0.105 (0.002)	0.059 (0.018)
Sassafras albidum	5	42.6	0.689 (0.028)	0.856 (0.025)	2.55 (0.037)	0.188 (0.011)	0.259 (0.010)	0.267 (0.038)

sampled. Nonetheless, H_O, H_E, and F_{IS} should be not be too biased by sample size because these values are based on means across polymorphic loci. Thus, for the two diploid species, we cautiously conclude that the *S. albidum*, an understory shrub, may have higher genetic diversity and higher inbreeding than *Q. alba*, a canopy tree.

The ANOVA models, which analyzed variation within the six genetic diversity and inbreeding measures across sites and between ELT's, indicated slightly different patterns among the three species (table 3). *S. albidum* was the species that indicated the most genetic diversity variables with significant differences across sites. For example, when we compare the pattern of variation in H_E across sites for the three species (fig. 1), we see that *S. albidum* had much greater variation. In terms of the inbreeding coefficient, *C. tomentosa* and *Q. alba* showed significant variation due to site (table 3, fig. 1). An examination of the variation in mean F_{IS} for *S. albidum* (fig. 1) revealed that this species also varied greatly across sites but that the standard errors were sufficiently high to prevent statistical significance. In general, the three species did not show the same pattern across sites for either H_E or F_{IS} (fig. 1).

In general, we observed a significant effect due to ELT in very few models (table 3). Both *C. tomentosa* and *S. albidum* were significantly different for H_E (table 3), although in opposite directions (fig. 2). *C. tomentosa*, the only species that showed significant differences between ELT's for the inbreeding coefficient (table 3), showed greater inbreeding on south- and west-facing slopes (fig. 2). *C. tomentosa* and *Q. alba* tended to have higher values of H_E and F_{IS} on south- and west-facing slopes, while *S. albidum* showed the opposite pattern to those two species (fig. 2).

Impact of Year of Acquisition

The nonparametric analyses of the effect of year of acquisition on genetic diversity and inbreeding measures indicated that two of the species, *Q. alba* and *S. albidum*, showed significant differences for at least some of the measures (table 4). *Q. alba* had three variables that were either significant or almost significant, and *S. albidum* had two variables that had P-values less than 10 percent (table 4). A comparison of the pattern in H_E across years shows that *Q. alba* and *C. tomentosa* had a similar trend of decreasing values with year (fig. 3). In spite of some dramatic differences in mean inbreeding across years for *Q. alba* (fig. 3), *S. albidum* is the only species that showed a trend toward significance for this variable (table 4). Interestingly, *Q. alba*, a canopy tree, and *S. albidum*, an understory tree, showed opposite patterns across years (fig. 3).

Analysis of MOFEP Design

In general, our analyses revealed that none of the three species show strong differences among management treatment classes (table 5). For *C. tomentosa*, A_P is significant but the overall model is not unless we remove block. This result is due to reduced allelic diversity in

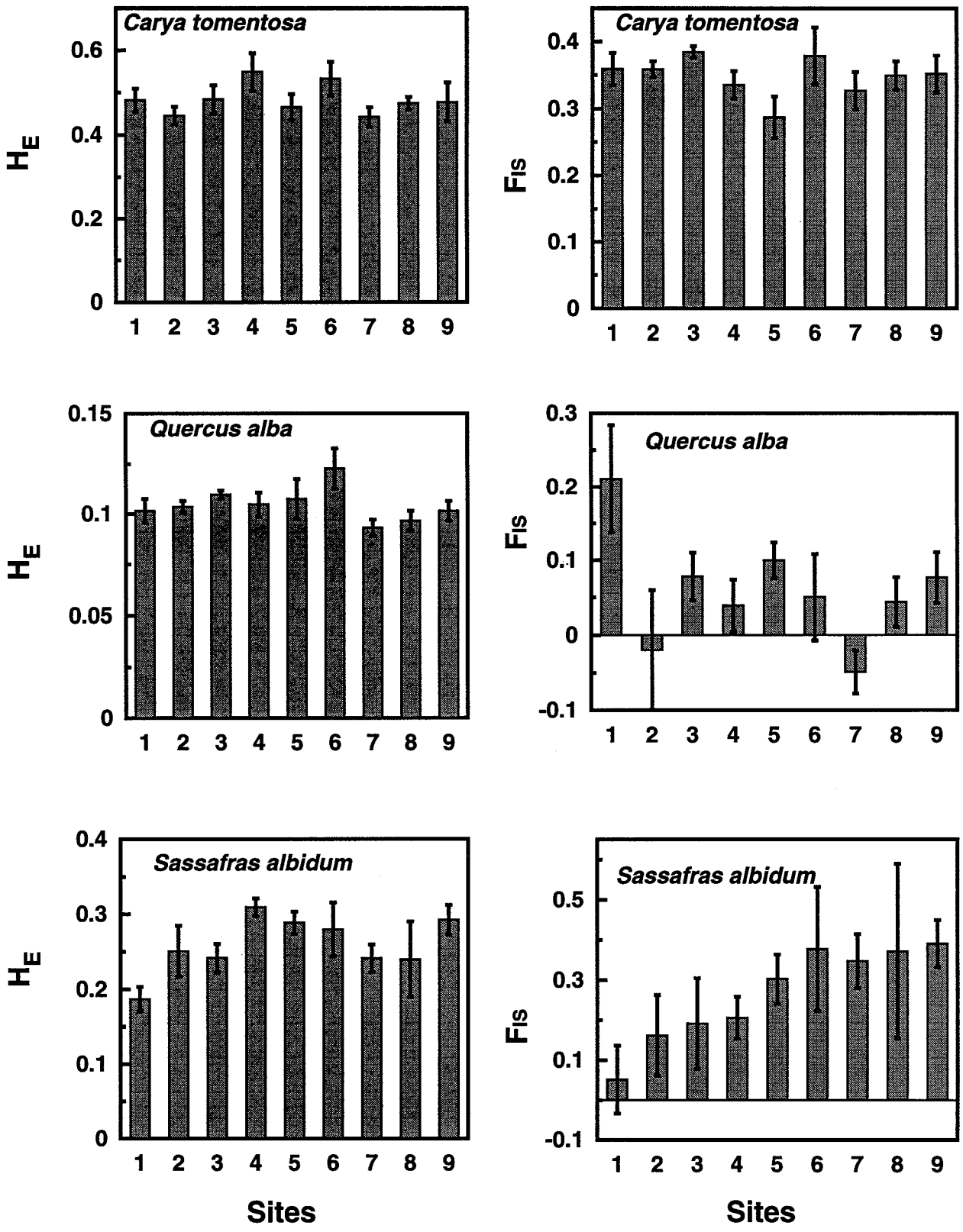

Figure 1.—*Mean (+/- 1 SE)* H_E *and* F_{IS} *of four populations per each of nine sites of MOFEP study area in south-central Missouri for* Carya tomentosa, Quercus alba, *and* Sassafras albidum.

Table 3.—*Summary of F-values from six separate two-way fixed ANOVA models estimating the effect of site and ELT on five measures of genetic diversity and the inbreeding coefficient (see table 2 for description of variables). A total of 36 populations distributed throughout MOFEP study plot are used. The degrees of freedom for the error term of each source of variation is 18.*

Source	DF	P_{95}[a]	P_{NC}[a]	A_P	H_O[a]	H_E[a]	F_{IS}
			Carya tomentosa				
Model	17	0.90	2.68*	0.88	1.72	1.53	7.89***
Site	8	0.98	2.18†	0.79	1.36	1.41	5.21**
ELT	1	0.05	0.27	0.29	1.83	4.63*	5.02*
Site x ELT	8	0.92	3.49*	1.05	2.07	1.26	10.93***
			Quercus alba				
Model	17	0.91	1.55	1.33	2.03†	1.34	1.90†
Site	8	1.28	1.25	0.73	2.89*	1.85	2.59*
ELT	1	0.08	0.54	0.00	0.12	0.19	0.03
Site x ELT	8	0.64	1.97	2.10†	1.40	0.98	1.45
			Sassafras albidum				
Model	17	1.64	2.86*	1.00	0.87	3.19**	1.27
Site	8	2.93*	5.65***	1.34	0.80	3.23*	1.22
ELT	1	2.30	1.71	0.11	1.64	10.26**	0.03
Site x ELT	8	0.27	0.22	0.77	0.84	2.28†	1.47

[a] variables were arc sine square root transformed

† $P < 0.10$ * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

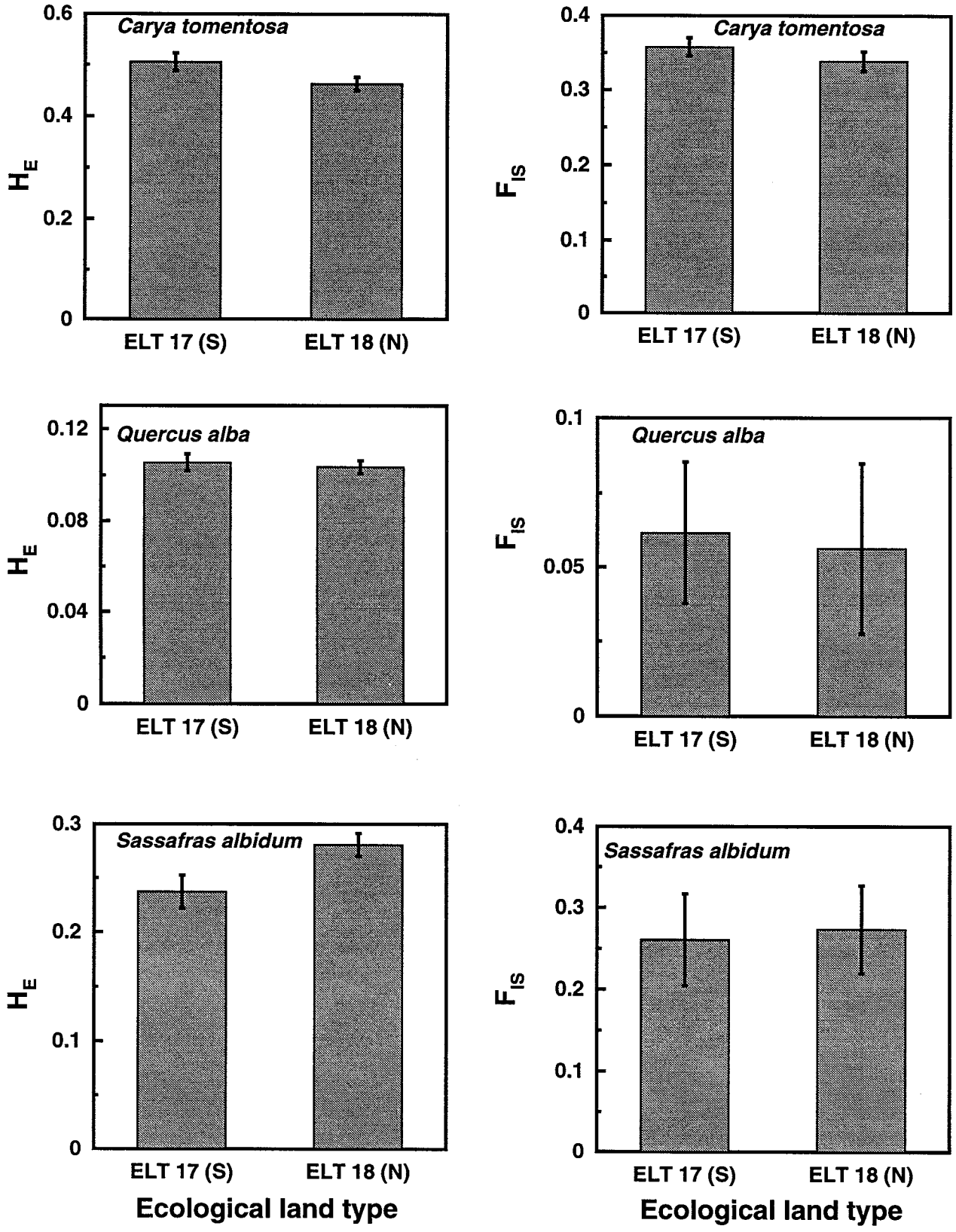

Figure 2.—*Mean (+/- 1 SE)* H_E *and* F_{IS} *of subpopulations found in each of two ELT 17 (south- to west-facing slope) and ELT 18 (north- to east-facing slope) in the MOFEP study area for* Carya tomentosa, Quercus alba, *and* Sassafras albidum.

Table 4.—*Summary of F-values from Kruskal-Wallis non-parametric tests using year of acquisition as the class variable and six dependent genetic variables (see table 2 for description of variables). Models were run separately for each species. (Results for* Q. alba *are taken from Koop 1996.)*

	DF	P_{95}	P_{NC}	A_P	H_O	H_E	F_{IS}
Carya tomentosa	3, 32	0.52	1.47	0.67	1.03	1.27	0.46
Quercus alba	3, 32	2.59†	1.41	0.37	2.59†	5.43**	0.96
Sassafras albidum	3, 32	1.48	12.46***	1.97	1.54	1.87	2.89†

† P < 0.10 * P < 0.05 **P < 0.01 *** P < 0.001

the sites assigned to the even-aged management class (fig. 4). *Q. alba* does not have any genetic variability that is significantly different across treatment classes (table 5); however, mean inbreeding coefficients across treatment classes show a great deal of variation (fig. 4), with the even-aged treatment showing the lowest values. These means are significantly different across treatments in an ANOVA model that treats treatment class and site nested within treatment as fixed effects (ANOVA, df = 2,27, F = 4.65, P < 0.05), which illustrates the potential for Type II error of this experiment. *S. albidum* has two variables with P-values less than 10 percent: P_{95} and P_{NC} (table 5). In this case, polymorphism is highest in the even-aged treatment and lowest in the control treatment (fig. 4). In general, the pattern of variation across treatment classes does not show the same trend across species.

The power analysis of our treatment effect revealed that for most variables our power was very low. For almost all variables tested, the power was less than 0.30. The exceptions were: for *Q. alba*, the power of detecting a treatment effect of F_{IS} was 0.60, and for *S. albidum*, the power of detecting a treatment effect for H_E = 0.65, for H_O = 0.78, and for P_{95} = 0.87.

Table 5.—*Summary of F-values from six separate two-way mixed ANOVA models estimating the effect of species and future management treatment on five measures of genetic diversity and inbreeding coefficient (see table 2 for description of variables). As described in table 3, the following variables were transformed:* P_{95}, P_{NC}, H_O, H_E.

Source	DF	P_{95}	P_{NC}	A_P	H_O	H_E	F_{IS}
			Carya tomentosa				
Model	8, 27	1.04	1.27	0.79	1.01	1.17	1.27
Block	2, 4	0.52	0.73	2.08	5.29†	1.93	0.90
Treatment	2, 4	0.06	1.63	15.75*	0.02	0.31	0.46
Error (B X T)	4, 27	1.61	1.17	0.16	0.55	1.10	1.52
			Quercus alba				
Model	8, 27	1.49	0.98	0.57	2.66*	1.92†	2.35*
Block	2, 4	2.16	0.71	1.35	0.59	3.48	0.84
Treatment	2, 4	1.60	0.50	0.73	0.18	0.80	2.76
Error (B X T)	4, 27	1.03	1.22	0.56	3.84*	1.22	1.68
			Sassafras albidum				
Model	8, 27	3.53**	7.11***	1.49	0.82	1.87	1.10
Block	2, 4	4.26†	8.92*	2.76	3.25	6.64*	7.96*
Treatment	2, 4	4.51†	6.19†	1.05	3.68	2.94	0.43
Error (B X T)	4, 27	1.31	1.66	1.03	0.37	0.65	0.42

† P < 0.10 * P < 0.05 ** P < 0.01 *** P < 0.001

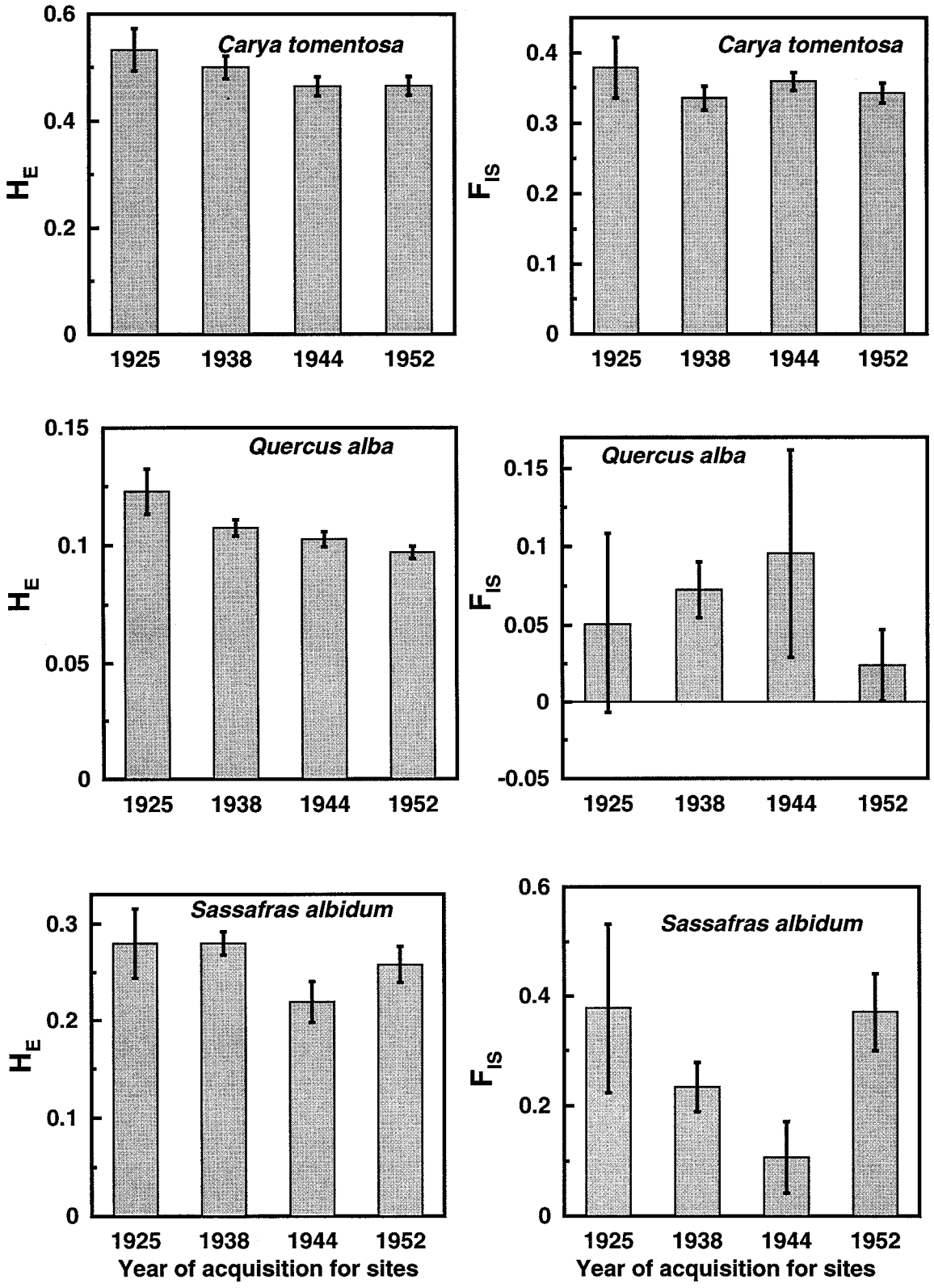

Figure 3.—*Mean (+/- 1 SE) H_E and F_{IS} of populations found in sites with different years of acquisition by the Missouri Department of Conservation for* Carya tomentosa, Quercus alba, *and* Sassafras albidum. *Site 6 was purchased in 1925, sites 3-5 were purchased in 1938, sites 1 and 2 were purchased in 1944, and sites 7-9 were purchased in 1952.*

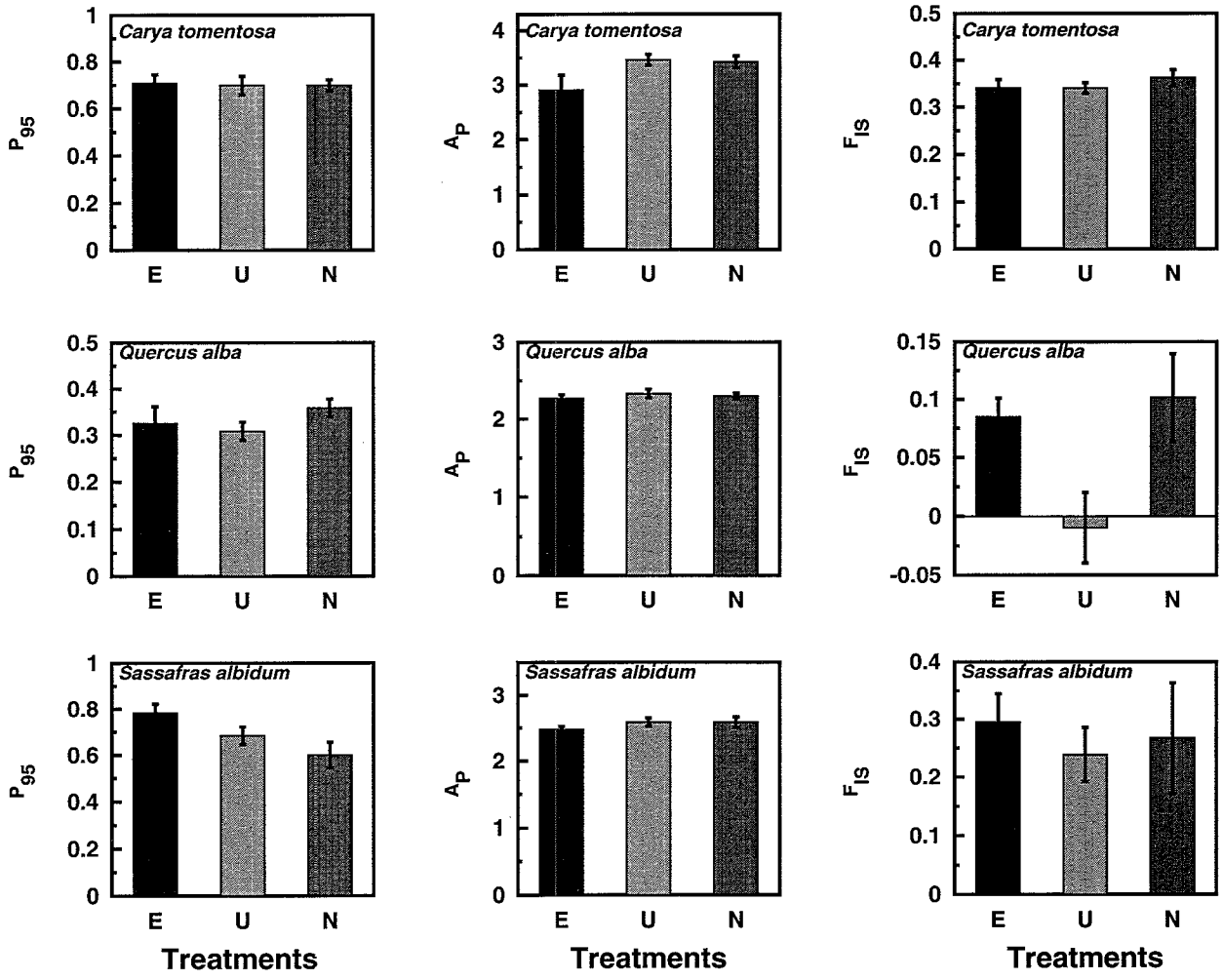

Figure 4.—*Mean (+/- 1 SE) P_{95}, A_P, and F_{IS} of 12 populations per each of three management treatments (even-aged (E), uneven-aged (U), and no harvest (N)), which are part of the MOFEP experimental design.*

DISCUSSION

The genetic variation of the three study species is within the range of expectations for woody plant species as indicated by a summary of the literature by Hamrick and Godt (1989), who estimated that the average values of P (no criteria) across all loci to be 50.0 percent, A to be 1.79, and H_E to be 0.149. Both *C. tomentosa* and *S. albidum* have values much higher than these means, but a precise comparison is not feasible for either of these species. For *C. tomentosa*, a comparison is not valid because the values of Hamrick and Godt (1989) are based on diploid data. Tetraploids, like *C. tomentosa*, should have much higher values than would diploid species because they have twice as many alleles. In fact, several studies have reported higher genetic diversity for tetraploids than for their diploid congeners: *Avena* sp. (Allard *et al.* 1993), *Antennaria* sp. (Bayer 1989), *Prunus* sp. (Beaver *et al.* 1995), and *Heuchera* sp. (Ness *et al.* 1989). A notable exception is a tetraploid variety of *Turnera ulmifolia*, which has less diversity than diploid congeners, possibly due to founder events (Shore 1991). A comparison with the literature is hampered for *S. albidum* because of the small number of loci for which we could obtain electrophoretic resolution. Because the five loci we used were all highly polymorphic, they may yield a higher estimate of diversity than if we had included more loci. For both of these species, it would be useful to sample populations at sites outside the Ozarks to make comparisons about whether the values we report here are typical or atypical for the species.

A useful way to compare genetic diversity of Ozark woody plants with the published literature is through *Q. alba*. Our estimates of P, A, and H are based on averages across polymorphic loci that should give higher estimates than the approach of Hamrick and Godt (1989). Nonetheless, our estimate of P (49.7 percent) is close to their average (50 percent). Moreover, we observed allelic diversity of 1.17 and expected heterozygosity of 0.105, which are much lower than the values stated above. We infer that the lower than expected levels of genetic diversity found in *Q. alba* may indicate that Ozark woody plants have slightly less genetic variation than similar species found elsewhere. An analysis of other woody plant species in the Ozarks will be necessary before we can conclude whether reduced genetic diversity is restricted to *Q. alba* or applies to other species.

The pattern of genetic variation of populations within each species is very heterogeneous across the MOFEP landscape. The null hypothesis for outcrossing long-lived species is that measures of P, H, and A will vary randomly across populations. For *C. tomentosa* and *Q. alba*, that expectation is generally true: both species had only one measure that was significant or almost significant. In contrast, P_{95}, P_{NC}, and, H_E were significantly different across sites for *S. albidum*. Differences in genetic diversity across MOFEP sites may be due to uneven gene flow within this landscape or they could be due to independent founder events. Our analyses also revealed that microhabitat was associated with variation in genetic diversity in *C. tomentosa* and *S. albidum*. In the case of *C. tomentosa*, northern and northeastern aspects had greater expected heterozygosity, while in *S. albidum* the pattern was the opposite. These sets of findings about the distribution of genetic variation across sites and ELT's indicate that genetic diversity can be influenced by landscape features. They also indicate that the manner in which landscape influences genetic diversity is species-specific.

Our analysis of heterogeneity across sites and ELT's was also extended to the population levels of inbreeding as measured by $F_{IS} = 1 - H_O/H_E$. We caution that estimates of inbreeding based on this formula will differ from inbreeding estimates based on the F-statistic formulas (e.g., Davis *et al.* 1991; Weir and Cockerham 1984; Wright 1951, 1965). Therefore, we will not compare the values reported here to those of the literature. However, we will point out that all three species have significant inbreeding coefficients using F-statistic formulas of Davis and others (1991): *C. tomentosa* $F_{IS} = 0.316$, *S. albidum* $F_{IS} = 0.234$ (Sork, unpublished data), and *Q. alba* $F_{IS} = 0.103$ (Koop 1995). Thus, each of these species demonstrates significant inbreeding, and it is worthwhile to investigate whether inbreeding is heterogeneous within the MOFEP landscape.

Inbreeding can be the result of self-pollination or mating with relatives. Because *C. tomentosa* and *Q. alba* are known to be outcrossing species that rarely self-pollinate and *S. albidum* is dioecious, which prevents self-pollination, we conclude that any inbreeding we observe is most likely due to mating with relatives. Mating with relatives is possible when seed dispersal is sufficiently restricted that half-sibs become established within the vicinity of the mother and grow up with some likelihood of mating with each other. *C. tomentosa* showed significant variation in F_{IS} due to site, ELT, and the interaction term. *Q. alba* showed significant effects due to site only. Thus, of the three species, *C. tomentosa* showed the most heterogeneity in its level of inbreeding. Of the three species, *C. tomentosa* also has the most restricted seed dispersal because it has large nuts that are likely to be dispersed by terrestrial rodents who do not carry nuts far (Sork, personal observation). In contrast, *Q. alba* is dispersed by both birds and rodents, and *S. albidum* has berries that are dispersed mostly by birds. It is possible that restricted seed dispersal is a contributing factor to overall high level of inbreeding of *C. tomentosa* and to the high heterogeneity in inbreeding. However, one must be cautious in drawing conclusions about dispersal mode because this study does not replicate species within a mode. Thus, the high heterogeneity in F_{IS} of *C. tomentosa* may be due to its restricted seed dispersal mechanism or to one or more other species-specific factors.

One obvious factor of the MOFEP landscape that might contribute to the heterogeneity in genetic diversity or inbreeding is the land use history of these sites. Our analysis of year of acquisition by the Missouri Department of Conservation was the approach we used to address this question. Although the various sites probably differ in many others ways (e.g., soils, Kabrick *et al.* 1997), year of acquisition represents the time at which each site began to be managed for forest instead of grassland. In effect, year of acquisition is probably a year of

release for any woody plant species that were suppressed by fire and grazing, and it represents a shift in the type of local ecosystem. Our findings indicate that this shift is associated with different levels of local genetic diversity in two of the species we examined. For *Q. alba*, we found significant differences for H_E such that the populations that were managed for forest longer have greater genetic diversity. *C. tomentosa*, a co-occurring canopy species, showed the same trend, but it was not significant. Such data provide preliminary evidence of a relationship between management and genetic diversity.

Our findings also revealed a highly significant difference in inbreeding level across year of acquisition for *S. albidum*. This pattern does not correlate directly with year of acquisition as we observed for heterozygosity in the two canopy species. However, this pattern does show an inverse relationship with *Q. alba* (see fig. 3) that suggests that land use history may be influencing inbreeding as well. Thus, factors that may promote inbreeding for *S. albidum* may be the same factors that retard inbreeding for the other species. The lack of an overall pattern in the extent to which genetic diversity measures and inbreeding levels vary across sites and year of acquisition illustrates that not all woody plant species will respond similarly to management regimes. In all likelihood, *C. tomentosa* and *Q. alba* were suppressed by the grassland management regime. In contrast, *S. albidum*, which does well in open areas, may not have been suppressed under those conditions. For each of the woody plant species, the impact of management may have been slightly different. Nonetheless, the data are highly suggestive that the change in management contributed to the creation of a mosaic of patterns of genetic diversity for many species.

The final goal of our study was to evaluate the MOFEP experimental design using our pre-treatment data. Of greatest concern is whether the genetic patchiness of the MOFEP landscape might have any impact on the analysis of management treatments that are currently being implemented. Of the five genetic diversity parameters, H_E is the most important one to monitor because it is the best measure of genetic diversity due to its wide use across studies. Moreover, this measure shows more continuous variation than P and A, which makes it more amenable to ANOVA. None of the species had significant treatment effects for this variable although we found that *S. albidum* has two genetic parameters (P_{95}, P_{NC}) that were almost significant. If we use these parameters in the post-treatment analyses, these results will need to be taken into account.

As we evaluate the models for significant treatment effects, we need to address the concern of falsely accepting a null hypothesis (Type II error). For example, when we visually analyze the means across data (fig. 4), *Q. alba* shows some dramatic differences in F_{IS} across management treatments and *S. albidum* shows some differences. At least two factors can contribute to a Type II error. The first is that the power of our test is weak. Indeed, results from the power analysis indicated that we had low power on most of the variables we examined. The best way to increase power without adding new sites is to reduce the variance within site by sampling more stands per site. We will consider this approach in the post-treatment study.

A second factor that can contribute to a Type II error would be an inappropriately designed ANOVA model. Given this possibility, we analyzed the *Q. alba* F_{IS} data using a different ANOVA model (eliminate block and consider site as a fixed effect nested within treatment) and found that management treatment is significant. We discuss this example to point out the MOFEP experimental design may create a Type II error for some variables and some species. Not only does the randomized block have very few degrees of freedom for the error term (df = 4) but the blocks do not always help the model because there may be significant heterogeneity within them. In the post-treatment analysis of data, it will be important to examine the data with several models and with an awareness of means and variances across treatment classes to avoid overly conservative analysis of findings. Nonetheless, after considering the results of all of the ANOVA models (table 5) and the patterns in the data (fig. 4), we conclude that differences among pre-treatment classes do not reflect strong biases that will hamper the impact of future management treatments or interpretation of post-treatment data.

The fact that the Ozark landscape reflects a mosaic of genetic patterns illustrates the benefits of evaluating an ecosystem before initiating an experimental treatment. Few studies actually measure the pre-existing conditions of an experiment. It is fascinating that land use history may have influenced patterns of genetic

variation across the MOFEP landscape. This factor is probably applicable to most other regions of North American eastern deciduous forest. All regions have a history of varying land use by humans. Many areas have gone from forest to agriculture back to forest during post-settlement periods, and, during presettlement, indigenous groups may have used fire for management. When we study the population genetics of forest tree species, our findings indicate that land use history may have a profound effect on the levels of genetic diversity even within the same geographical region and within gene flow distances. It is appropriate that the MOFEP study has conducted extensive pre-treatment analyses. The findings reported here and in other MOFEP studies indicate that interpretation of future results can be strongly influenced by the history of a site as well as heterogeneous pre-existing conditions.

CONCLUSIONS

1. The level of genetic diversity observed for *Q. alba* is less than expected for woody plant species. All three species showed significant heterogeneity in genetic diversity and/or inbreeding among sites. *S. albidum* also showed differences in genetic diversity across ELT's. Because we also found significant differences in year of acquisition for genetic diversity measures in two of the species, we conclude that some of the heterogeneity in diversity and inbreeding may be attributable to different land use histories across sites. The overall reduced levels of genetic diversity in *Quercus alba* may also be attributable to land use history that could have created a genetic bottleneck.

2. The pre-treatment analysis of management effects shows that pre-treatment differences among management treatments are minimal. Findings of our study indicate that assignment of sites to the three management treatments will not hamper post-treatment analysis and interpretation of data. These findings demonstrate the importance of conducting pre-treatment examination of regions before initiating large-scale experiments on components of the ecosystem.

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LITERATURE CITED

Allard, R.W.; Garcia, P.; Saenz de Miera, L.E.; Perez de la Vega, M. 1993. Evolution of multilocus genetic structure in *Avena hirtula* and *Avena barbata*. Genetics. 135: 1125-1139.

Bayer, R.J. 1989. Patterns of isozyme variation in western North American anternnaria (Asteraceae: Inuleae) II. Diploid and polyploid species of section Alpinae. American Journal of Botany. 76: 679-691.

Beaver, J.A.; Iezzoni, A.F.; Ramm, C.W. 1995. Isozyme diversity in sour, sweet, and ground cherry. Theoretical and Applied Genetics. 90: 847-852.

Braun, L. 1950. Deciduous forests of eastern North America. New York City, NY: McGraw-Hill.

Brookshire, Brian L.; Jensen, Randy; Dey, Daniel C. 1997. The Missouri Ozark Forest Ecosystem Project: past, present, and future. In: Brookshire, Brian L.; Shifley, Stephen R., eds. Proceedings of the Missouri Ozark Forest Ecosystem Project symposium: an experimental approach to landscape research; 1997 June 3-5; St. Louis, MO. Gen. Tech. Rep. NC-193. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 1-25.

Cunningham, R; Hauser, C. 1989. The decline of Missouri Ozark forest between 1880 and 1920. In: Proceedings of pine hardwood mixtures: a symposium of management and ecology of the Type. Gen. Tech. Rep. SE-58. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southeast Forest Experiment Station: 34-37.

Davis, S.K.; Strassmann, J.S.; Hughes, C.; Pletscher, L.S.; Templeton, A.R. 1991. Population structure and kinship in *Polistes* (Hymenoptera, Vespidae): an analysis using

ribosomal DNA and protein electrophoresis. Evolution. 44: 1242-1253.

Ellstrand, N.C.; Elam, D.R. 1993. Population genetics of small population size: implications for plant conservation. Annual Review of Ecology and Systematics. 23: 217-242.

Fisher, R.A. 1939. The genetical theory of natural selection. Oxford: Clarendon Press. 272 p.

Guyette, R.; Dey, D.C. 1997. Historic shortleaf pine (*Pinus echinata* Mill.) abundance and fire frequency in a mixed oak - pine forest (MOFEP, site 8). In: Brookshire, Brian L.; Shifley, Stephen R., eds. Proceedings of the Missouri Ozark Forest Ecosystem Project symposium: an experimental approach to landscape research; 1997 June 3-5; St. Louis, MO. Gen. Tech. Rep. NC-193. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 136-149.

Hamrick, J.L.; Godt, M.J. 1989. Allozyme diversity in plant species. In: Brown, A.H.D.; Clegg, M.T.; Kahler, A.L.; Weir, B.S., eds. Plant population genetics and germ resources in crop improvement. New York City, NY: Sinauer: 43-63.

Hamrick, J.L.; Godt, M.J.; Sherman-Broyles, S.L. 1992. Factors influencing levels of genetic diversity in woody plant species. New Forests. 6: 95-124.

Hartl, D.L.; Clark, A.G. 1989. Principles of population genetics, 2d ed. Sunderland, MA: Sinauer Associates, Inc. 682 p.

Huang, S. 1992. Macrogeographic and microgeographic genetic structure in populations of northern red oak, *Quercus rubra* L. in midwestern USA. St. Louis, MO: University of Missouri-St. Louis. 151 p. Ph.D. dissertation.

Kabrick, J.M.; Larsen, D.R.; Shifley, S.R. 1997. Analysis of pre-treatment woody vegetation and environmental data for the Missouri Ozark Forest Ecosystem Project. In: Brookshire, Brian L.; Shifley, Stephen R., eds. Proceedings of the Missouri Ozark Forest Ecosystem Project symposium: an experimental approach to landscape research; 1997 June 3-5; St. Louis, MO. Gen. Tech. Rep. NC-193. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 150-168.

Kephart, S.R. 1990. Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. American Journal of Botany. 77: 693-712.

Koop, A.L. 1995. Genetic variation and structure in *Quercus alba* L. in a Missouri ozark landscape. St. Louis, MO: University of Missouri-St. Louis. 122 p. M.S. thesis.

Kurzejeski, E.; Clawson, R.L.; Renken, R.B.; Sheriff, S.L.; Vangilder, L.D.; Hauser, C.; Faaborg, J. 1993. Experimental evaluation of forest management: the Missouri Ozark Forest Ecosystem Project. In: McCabe, Richard E.; Glidden, Kelly A., eds. Transactions of the 58th North American Wildlife and Natural Resources conferece; 1993 March 19-24; Washington, DC. Washington, DC: Wildlife Management Institute: 599-609.

Ledig, T.F. 1992. Human impact on genetic diversity in forest ecosystems. Oikos. 63: 87-108.

Mitton, J.B.; Grant, M.C. 1984. Relationships among protein heterozygosity, growth rate, and developmental stability. Annual Review of Ecology and Systematics. 15: 479-499.

Mitton, J.B.; Linhart, Y.B.; Sturgeon, K.B.; Hamrick, J.L. 1979. Allozyme polymorphism detected in mature needle tissue of ponderosa pine. Journal of Heredity. 70: 86-89.

Ness, B.D.; Soltis, D.E.; Soltis, P.S. 1989. Autopolyploidy in *Heuchera micrantha* (Saxifragaceae). American Journal of Botany. 76: 614-626.

Rieseberg, L.H.; Soltis, D.E. 1989. Assessing the utility of isozyme number for determining ploidal level evidence from *Helianthus* and *Heliomeris* (Asteraceae). Aliso. 12: 277-286

Sheriff, S.R.; He, Z. 1997. The experimental design of the Missouri Ozark Forest Ecosystem Project. In: Brookshire, Brian L.; Shifley, Stephen R., eds. Proceedings of the Missouri Ozark Forest Ecosystem Project

symposium: an experimental approach to landscape research; 1997 June 3-5; St. Louis, MO. Gen. Tech. Rep. NC-193. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station: 26-40.

Shore, J.S. 1991. Tetrasomic inheritance and isozyme variation in *Turnera ulmfoia* vars. *elgans* Urb. and *intermedia* Urb. (Turneraceae). Heredity. 66: 305-312.

Slatkin, M. 1985. Gene flow in natural populations. Annual Review of Ecology and Systematics. 16: 393-430.

Soltis, D.E.; Haufler, C.H.; Darrow, D.C.; Gastony, G.J. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. American Fern Journal. 73: 9-27.

Sork, V.L.; Idol, J.; Noyes, R.; Wiener, E. 1992. Mating system in three species of North American oaks. American Journal of Botany. 79: 187.

Sork, V.L.; Huang, S.; Wiener, E. 1993. Macrogeographic and fine-scale genetic structure in a North American oak species, *Quercus rubra* L. Annales des Sciences Forestieres 50 (Suppl.). 1: 128-136.

Stone, D.E. 1961. Ploidal level and stomatal size in the American hickories. Brittonia. 13: 293-302.

Swofford, D.L.; Selander, R.B. 1981. BIOSYS-I: A Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity. 72: 281-283.

Weir, B.; Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution. 38: 1358-1370.

Wright, S. 1951. The genetical structure of populations. Annuals of Eugenics. 15: 323-354.

Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution. 19: 395-420.