American Journal of Botany 80(11): 1349–1357. 1993.

ISOZYME VARIATION IN OAKS OF THE APOSTLE ISLANDS IN WISCONSIN: GENETIC STRUCTURE AND LEVELS OF INBREEDING IN *QUERCUS RUBRA* AND *Q. ELLIPSOIDALIS* (FAGACEAE)[1]

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Isozyme variability was examined in populations representing the red oak complex (*Quercus* subg. *Erythrobalanus*) on an island archipelago and adjoining peninsula in Lake Superior, near Bayfield, Wisconsin. A concomitant study of morphometric variation described in the companion manuscript, revealed a continuum in leaf morphology extending from an interior mainland site to the outermost island. The existence of this clinal variation presented an ideal opportunity to examine the genetic structure of a hybrid population along with the putative progenitor species. Dormant leaf bud samples were collected from specimens of *Quercus rubra* L., *Q. ellipsoidalis* Hill, and their putative hybrids from three islands and two locations on the peninsula. Acorns were collected from some of these same trees from one peninsula location and two islands. Twelve putative enzyme loci from six enzyme systems were analyzed. Allele frequency data indicated little differentiation between populations. Mean F_{ST} values for the adult trees and acorns were 0.042 and 0.020. Genetic identities according to Nei ranged from 0.958 to 0.999. Despite these high levels of genetic similarity, the populations appeared to be highly inbred as indicated by positive mean F_{IT} values of 0.183 and 0.373 for the adult trees and acorns. Estimates of migration rate per generation (Nm) for the adult trees was 5.70, a value that is low when compared to estimates for other plant species with similar life history characteristics.

Considerable effort has been directed toward establishing the relationship between life history traits and the electrophoretic diversity within various plant species (Brown, 1979; Hamrick, Linhart, and Mitton, 1979; Gottlieb, 1981; Loveless and Hamrick, 1984; Hamrick and Godt, 1989). Comparisons have been made between plant species with similar ecological and life history attributes to determine whether such species maintain similar levels of genetic variability and/or genetic structure. Hamrick and Godt (1989) used the following eight ecological and life history traits in making such comparisons: taxonomic status, regional distribution, geographic range, life form, mode of reproduction, breeding system, seed dispersal mechanism, and successional status. This and earlier studies (Hamrick, Linhart, and Mitton, 1979; Loveless and Hamrick, 1984; Nevo, Beiles, and Shlomo, 1984) uncovered significant correlations between ecological and life history traits and patterns of genetic diversity and structure. These reviews concur that species such as oaks, which are long-lived, outcrossed, wind-pollinated, and characteristic of the later stages of succession, should maintain higher levels of genetic variation within their populations than between them.

While the genetic structure of herbaceous plant species has been widely studied (Hamrick and Godt, 1989), only limited information is available on woody angiosperm species (Bousquet, Cheliak, and LaLonde, 1987, 1988; Surles, Hamrick, and Bongarten, 1989; Schnabel and Hamrick, 1990; Schwarzmann and Gerhold, 1991). This is unfortunate, because such information is crucial for developing programs to protect and preserve the genetic resources of plant species. Presently, several species of the northern hardwood forests, including *Quercus rubra,* are being subjected to extreme logging pressure (Laursen and DeBoe, 1991). Additionally, it is now thought that certain environmental perturbations, such as global warming and acid rain, could threaten the existence of some tree species (Ledig, 1992). To make responsible decisions concerning the possible impacts of such pressures, we must build a more substantive base of knowledge regarding the population genetic structure of woody angiosperm species.

One of the most determinant factors concerning the population genetic structure of an outcrossing wind-pollinated species such as oaks are the levels of gene flow. While much research has been directed toward understanding the dynamics of gene flow (Levin and Kerster, 1974; Moore, 1976; Slatkin, 1981, 1985; Handel, 1983; Ellstrand, 1988), the actual amount of gene flow via pollen or seed is one of the lesser known parameters concerning natural plant populations (Levin, 1984; Hamrick, 1987). Due to a general lack of suitable markers, direct measurement of gene flow is often difficult (Ellstrand and

[1] Received for publication 17 December 1991; revision accepted 4 June 1993.

The authors thank Dr. Robert Brander, Julie Van Stappen, and their staff at the Apostle Islands National Lakeshore for logistical support and access to the islands; Gary Garten and Dave Buckley for help with collections; Mike Kwantes and Paco Moore for expert computer assistance; Dr. Steve Krebs for insightful discussion and instruction on isozyme analysis; Karen Ludlam for generous technical assistance; and Drs. Steve Tonsor and Tim Holtsford for thoughtful comments on early drafts of this manuscript.

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Marshall, 1985). It is generally agreed that the most efficient method for determining actual gene flow is through the use of isozyme marker genes detected with electrophoretic techniques.

The distribution of oak populations on the Apostle Islands in Lake Superior presented us with the opportunity to study the population genetics of members of the red oak complex. The islands and adjacent Bayfield Peninsula (BP) (Jensen et al., 1993) support natural populations of only two species of subgenus Erythrobalanus. Morphological characters of leaves and acorns indicate that *Quercus rubra* L. (northern red oak) predominates on the outermost island of the archipelago (Outer Island), while *Q. ellipsoidalis* Hill (northern pin oak) predominates in the peninsula interior. Between these distal populations are populations that can be characterized as being intermediate or hybrid in nature (Jensen et al., 1993). This pattern of morphological variability gave us a system in which to pursue the degree of interaction between *Q. rubra* L. and *Q. ellipsoidalis*. We were interested specifically in the following questions: 1) How is genetic variability distributed within red oaks in a zone of overlap; and 2) How much and how far do genes move among these island populations of oak?

MATERIALS AND METHODS

Collections.—Five populations of oaks were studied in the Bayfield, Apostle Islands National Lakeshore region in northeastern Wisconsin (Jensen et al., 1993). Samples were collected on five separate trips to the region. Two trips were made in June of 1989, and one each in October 1989, March 1990, and May 1990. BP collections were made along the roads that defined the east and north perimeters of the Chequamegon National Forest. Peninsula perimeter (PP) collections were made on the public roads that skirted the perimeter of the BP. At both sites, we sampled the first mature oak of any taxon sighted at 0.4-km intervals along the roads. Collections on Oak Island (OK) were made on a north to south trail that bisected the island. The Stockton Island (STK) and Outer Island (OI) collections were done on trails that ran from southwest to northeast the length of each of the islands. These populations were sampled by walking vigorously for 5 minutes and collecting the first mature oak of any taxon sighted within 20 yards of the trail.

Samples collected in June 1989 consisted of rapidly expanding leaves 15–75 mm in length. On the subsequent trips, three to five pencil-sized branches containing dormant buds and acorns were removed from individual trees using a shotgun. Buds were left on small twigs while acorns and leaves were removed. Samples were placed in labeled zip-lock bags, stored within 3 hours on ice in a cooler, and on return to East Lansing, Michigan, placed in a refrigerator at 2.5 C until enzymes were extracted. Mean sample sizes per locus for trees and acorns at each location are listed in Table 2.

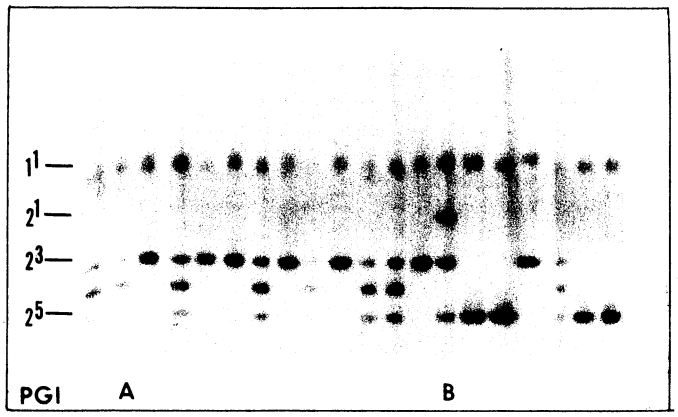
Enzyme extraction—Samples were macerated with pestles in chilled mortars using the Soltis phosphate grinding buffer (Soltis et al., 1983). Prior to grinding, one spatula tip of insoluble polyvinylpolypyrrolidone (PVPP, Sigma #P-6755) completely hydrated with the grinding buffer was added. Approximately 10 mg of dormant bud was ground per specimen. For acorns, 35 to 40 mg of cotyledon tissue, removed from the cap end of the acorn, was ground per sample. Leaves rapidly lost their enzyme activity and had to be prepared within days for electrophoresis, while dormant buds could be stored for up to 2 months before extraction with no appreciable loss in activity. Acorns ground 11 months after harvest also had high enzyme activity.

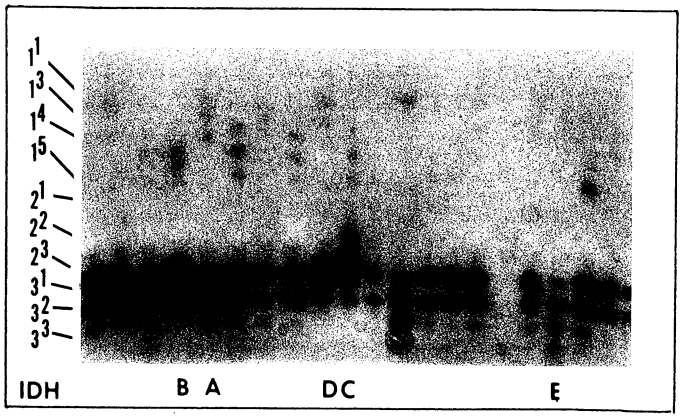
After grinding, the resulting slurry was absorbed through nylon mesh onto 4 × 11-mm wicks cut from #3 Whatman chromatography paper. Wicks sufficient for four electrophoretic examinations were placed in Corning 96 well disposable ELISA plates that were double wrapped in cellophane, bagged in zip-lock bags, and stored at −80 C until electrophoresed. Only enough wicks for one analysis were placed in a plate, so that the wicks were never taken out of the freezer until they were to be analyzed. Well-resolved isozymes were obtained from samples stored up to 14 months under these conditions.

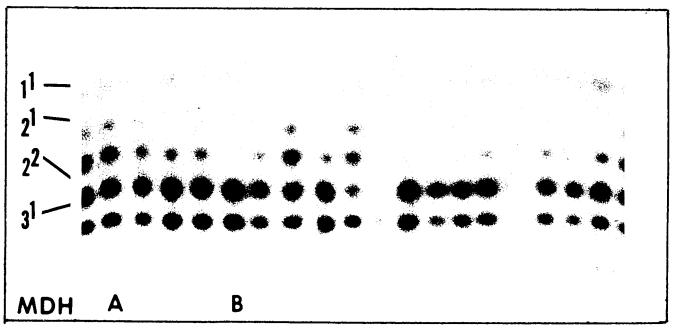
Electrophoresis—Experiments with a number of gel/electrode buffer systems revealed that the pH 8.3 lithium borate, tris-citrate system (Scandalios, 1969), and the morpholine-citrate pH 6.1 system (Clayton and Tretiak, 1972) clearly resolved the largest number of enzymes. Ten-millimeter-thick 6.1 gels were typically run for 7 hours at 55–65 milliamps, and approximately 250 volts. Six-millimeter-thick 8.3 gels were run for 6 hours at 50 milliamps or until 300 volts was reached. Slices from the 8.3 Scandalios system were stained for phosphoglucose isomerase (PGI) (Vallejos, 1983) and leucine amino peptidase (LAP) (Soltis et al., 1983). Slices from the 6.1 morpholine-citrate system were stained for 6-phosphogluconate dehydrogenase (6-PGDH) (Conkle et al., 1982), isocitrate dehydrogenase (IDH) (Soltis et al., 1983), malate dehydrogenase (MDH) (Vallejos, 1983), and shikimate dehydrogenase (SKDH) (Soltis et al., 1983). All staining assays were conducted as cited except for LAP. The substrate used was L-leucine-B-napthylamide HCL (Sigma #L0376) which was dissolved directly in the buffer solution. Once the bands stained clearly, the slices were rinsed in 1% acetic acid solution and fixed in a 50% ethanol solution. Slices were then bagged in zip-lock bags and refrigerated at 4 C for later analysis.

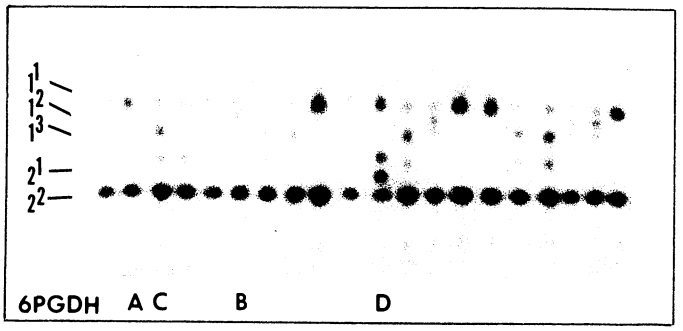
Bands were read in the conventional manner with those loci migrating farthest from the origin being designated as number one, the next farthest number two, etc. Within a locus the fastest allele was named one, the next two, etc. (Figs. 1, 2). Because we made no controlled crosses to analyze segregation of the isozyme banding patterns, all allele and loci designations are putative.

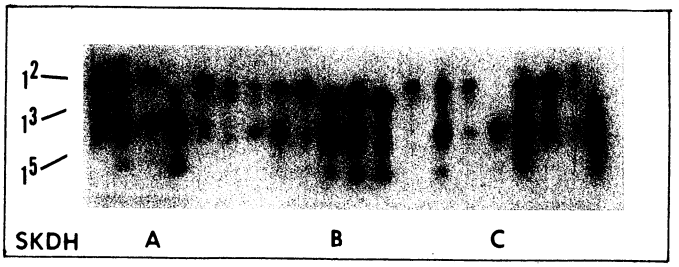
Our initial experiments indicated that resolution became increasingly poor as leaves expanded, while dormant buds gave consistently good results. Thus, we report results from only dormant bud or acorn samples. The acorns analyzed in this study were collected from adult trees that were also analyzed electrophoretically. Five acorns were electrophoresed from each of these parent trees.











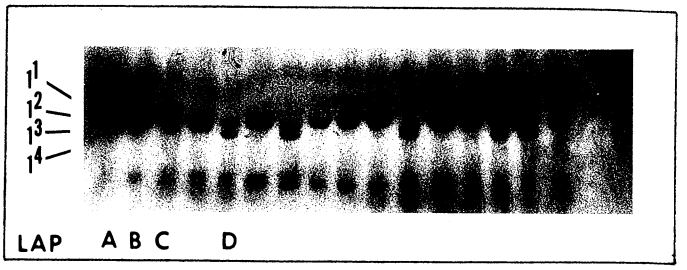


Fig. 1. Electrophoretic patterns for three of the six enzyme systems used in this study. Numbers to the left of the photographs indicate putative loci. Superscript numbers indicate putative alleles of each locus. PGI was scored as a dimeric enzyme encoded by two loci. Locus one was considered monomorphic. Lane A was heterozygous for alleles three and five of locus two. Lane B was heterozygous for alleles one and five of locus two. MDH was scored as a dimeric enzyme encoded by three loci. In this photograph all individuals were homozygous for allele one of locus one. Lane A was heterozygous for alleles one and two of locus two. Allele one of locus three has co-migrated with allele two of locus two. Lane B was homozygous for allele two of locus two. Allele one of locus three has co-migrated with allele two of locus two. SKDH was scored as a monomeric single locus enzyme. Lane A was homozygous for allele two. The second band was thought to be a plastid form of the enzyme and not scored. Lane B shows a heterozygote for alleles three and five. The faint bottommost band was considered a plastid form of allele five and not scored. Lane C was homozygous for allele five.

Fig. 2. Electrophoretic patterns for the remaining three of six enzyme systems used in this study. Labeling remains the same as on the previous page. IDH was scored as a dimeric enzyme encoded by three loci. Lane A was heterozygous for alleles one and three of locus one. Lane B was heterozygous for alleles four and five of locus one. Lane C was heterozygous for alleles one and two of locus two. Lane D was homozygous for allele one of locus three. Lane E was heterozygous for alleles two and three of locus three. 6PGDH was scored as a dimeric enzyme encoded by two loci. Lane A was homozygous for allele one of locus one. Lane B was heterozygous for alleles one and two of locus one. Lane C was heterozygous for alleles one and three of locus one. Lane A was homozygous for allele two of locus two. Lane D was heterozygous for alleles one and two of locus two. LAP was scored as a monomeric, single locus enzyme. Only the uppermost locus was scored for this study. Lane A was homozygous for allele two. Lane B was homozygous for allele three. Lane C was homozygous for allele one. Lane D was heterozygous for alleles two and four.

Statistical analysis—Allele frequencies, average heterozygosities (direct and estimated), percent loci polymorphic, mean alleles per locus, *F*-statistics, and genetic identity according to Nei (1978) were calculated using the BIOSYS-1 program, release 1.7, which is adapted for the personal computer (Swofford and Selander, 1989).

Direct or observed average heterozygosities were calculated by adding the number of heterozygous individuals at each locus in the population, dividing this number by the total number of individuals in the population, and averaging this value over loci. Calculations of average-estimated heterozygosity were based on Hardy-Weinberg

TABLE 1. Allele frequencies at 12 putative enzyme loci for five adult and three acorn populations of *Quercus* studied on the Apostle Islands and vicinity.

		BP		PP		OK		STK		OI	
Locus	Allele	Adlt	Acor	Adlt	Acor	Adlt	Acor	Adlt	Acor	Adlt	Acor
PGI-1	1	1.00	—	1.00	—	1.00	—	1.00	—	1.00	—
PGI-2	1	0.000	—	0.014	0.000	0.000	0.000	0.000	—	0.000	0.000
	2	0.030	—	0.000	0.011	0.000	0.027	0.000	—	0.000	0.007
	3	0.790	—	0.688	0.819	0.583	0.818	0.656	—	0.667	0.691
	4	0.010	—	0.036	0.011	0.046	0.009	0.021	—	0.028	0.026
	5	0.170	—	0.261	0.160	0.370	0.145	0.323	—	0.306	0.276
LAP-1	1	0.340	—	0.176	0.457	0.186	0.474	0.427	—	0.225	0.244
	2	0.300	—	0.485	0.283	0.539	0.386	0.302	—	0.250	0.411
	3	0.180	—	0.250	0.207	0.186	0.096	0.177	—	0.425	0.256
	4	0.180	—	0.088	0.054	0.088	0.044	0.094	—	0.100	0.089
6PGD1	1	0.930	—	0.623	0.693	0.641	0.696	0.670	—	0.706	0.710
	2	0.040	—	0.098	0.034	0.141	0.107	0.085	—	0.147	0.117
	3	0.030	—	0.279	0.273	0.217	0.196	0.245	—	0.147	0.173
6PGD2	1	0.000	—	0.007	0.022	0.010	0.009	0.010	—	0.000	0.000
	2	1.00	—	0.986	0.978	0.990	0.982	0.990	—	0.975	0.994
	3	0.000	—	0.007	0.000	0.000	0.009	0.000	—	0.025	0.006
IDH-1	1	0.448	—	0.341	0.389	0.255	0.219	0.372	—	0.306	0.253
	2	0.000	—	0.000	0.000	0.000	0.000	0.032	—	0.000	0.019
	3	0.354	—	0.333	0.211	0.431	0.430	0.351	—	0.361	0.474
	4	0.000	—	0.024	0.000	0.010	0.000	0.000	—	0.000	0.000
	5	0.198	—	0.302	0.400	0.304	0.351	0.245	—	0.333	0.253
IDH-2	1	0.000	—	0.000	0.000	0.020	0.000	0.064	—	0.050	0.058
	2	0.000	—	0.101	0.133	0.059	0.175	0.021	—	0.000	0.168
	3	1.00	—	0.899	0.867	0.922	0.825	0.915	—	0.950	0.744
IDH-3	1	0.020	—	0.087	0.167	0.069	0.069	0.022	—	0.000	0.094
	2	0.950	—	0.884	0.813	0.931	0.879	0.957	—	1.00	0.906
	3	0.030	—	0.029	0.021	0.000	0.052	0.022	—	0.000	0.000
MDH-1	1	0.980	—	0.980	0.989	0.980	0.991	0.990	—	1.00	0.980
	2	0.020	—	0.020	0.011	0.020	0.009	0.010	—	0.000	0.020
MDH-2	1	0.000	—	0.014	0.011	0.000	0.000	0.000	—	0.289	0.006
	2	1.00	—	0.986	0.989	1.00	1.00	1.00	—	0.711	0.994
MDH-3	1	0.950	—	0.958	0.989	0.980	0.983	1.00	—	1.00	0.977
	2	0.050	—	0.042	0.011	0.020	0.017	0.000	—	0.000	0.023
SKDH1	1	0.048	—	0.030	0.000	0.010	0.000	0.000	—	0.000	0.000
	2	0.269	—	0.523	0.716	0.539	0.552	0.510	—	0.556	0.636
	3	0.029	—	0.152	0.136	0.088	0.208	0.198	—	0.167	0.130
	4	0.029	—	0.053	0.034	0.029	0.000	0.031	—	0.056	0.026
	5	0.606	—	0.235	0.114	0.314	0.240	0.260	—	0.222	0.208
	6	0.019	—	0.008	0.000	0.020	0.000	0.000	—	0.000	0.000

expectations. For each locus, allele frequencies were inserted into a Hardy-Weinberg equation derived for the number of alleles at that locus. These values were then averaged over the number of loci within the population.

The percentage of polymorphic loci was calculated using a 95% criterion, i.e., a locus was considered polymorphic only if the most common allele occurred at a frequency of 0.95 or less in the population. The number of loci in a population that fit this criterion was divided by the total number of loci in the population to generate this percentage. Mean alleles per locus were calculated by summing all the alleles across loci in a population and dividing by the total number of loci.

F-statistics (fixation indices; Wright, 1951, 1965) were calculated according to Nei (1977). This procedure measures the deviation of genotype frequencies from Hardy-Weinberg expected frequencies in a subdivided population. Deviation in heterozygosity from the level expected under Hardy-Weinberg equilibrium is partitioned into three components, F_{IS}, F_{ST}, and F_{IT}. F_{IS} describes the inbreeding in individuals relative to the subpopulations to which they belong. $F_{IS} = (h_s - h_o)/h_s$, where h_o is equal to the frequency of heterozygous individuals in an island population and h_s is equal to the expected frequency of heterozygous individuals in an equivalent random mating island population. F_{ST} is the proportion of the deviation from equilibrium contained within subpopulations and represents a measure of the differentiation between populations. $F_{ST} = (h_T - h_s)/h_T$, where h_T = the expected frequency of heterozygous individuals in an equivalent random mating total population. F_{IT} is a measure of the reduction in heterozygosity of an individual in relation to the whole population. F_{IT} can be viewed as the total heterozygote deviation from Hardy-Weinberg equilibrium. It is comprised of both the deviation due to nonrandom mating within island populations (F_{IS}), and to the

TABLE 2. Mean sample size per locus, percent polymorphic loci, mean and effective number of alleles per locus, and mean heterozygosity, direct and expected, for five adult and three acorn populations of *Quercus* in the Apostle Islands and vicinity.[a]

Population	Mean sample size/locus	% Polymorphic loci	Mean # alleles/ locus	Effective # alleles/locus	Mean heterozygosity	
					Direct	Expected
Bayfield	49.9 (0.3)	63.6	2.7 (0.5)	1.60	0.198 (0.069)	0.242 (0.085)
Peninsula Perimeter	66.3 (1.8)	63.6	3.2 (0.4)	1.79	0.232 (0.073)	0.342 (0.083)
Perimeter-Acorns	43.1 (1.9)	63.6	2.8 (0.3)	1.61	0.187 (0.061)	0.291 (0.075)
Oak Island	50.5 (0.6)	63.6	2.9 (0.4)	1.71	0.249 (0.084)	0.303 (0.085)
Oak-Acorns	53.2 (2.6)	63.6	2.7 (0.3)	1.64	0.181 (0.058)	0.296 (0.077)
Stockton Island	47.9 (0.4)	54.5	2.7 (0.3)	1.74	0.245 (0.074)	0.296 (0.090)
Outer Island	19.0 (0.3)	63.6	2.4 (0.3)	1.76	0.327 (0.090)	0.322 (0.089)
Outer-Acorns	79.5 (3.2)	63.6	2.9 (0.3)	1.72	0.203 (0.065)	0.313 (0.080)
Mean	51.2 (16.4)	62.5 (3.0)	2.8 (0.21)	1.70 (0.07)	0.228 (0.045)	0.301 (0.027)

[a] Numbers in parentheses indicate standard error.

heterozygote deviation due to the subdivision of the population (F_{ST}); $F_{IT} = (h_T - h_o)/h_T$.

Effective number of alleles per locus (A_{ep}) was calculated according to Weir (1989), where $A_{ep} = 1/(1 - H_{ep})$. H_{ep}, the genetic diversity per locus is equal to $1 - \Sigma p_i^2$. Here p_i equals the frequency of the i^{th} allele in each population. This analysis was done to "weight" the alleles present in the population. Because the calculation is based on the frequency of the allele in the population rather than mere presence, more "weight" is given to alleles with a higher frequency.

The number of migrants exchanged per generation (Nm) was also estimated where N equals the effective population size and m equals the proportion of migrants exchanged between populations per generation. Nm was calculated using the F_{ST} value described previously. According to Wright (1931), $F_{ST} = 1/(1 + 4Nm)$.

RESULTS

Twelve putative enzyme loci were consistently scoreable and subsequently employed in this study (Fig. 1, 2). Eleven of the 12 loci were polymorphic; however, no alleles were found to be unique to any of the populations of adult trees or acorns except for the rare Allele 1 of PGI-2 (Table 1). All populations, both adult and acorn, had 63.6% polymorphic loci except for the STK adults which were polymorphic for 54.5% of their loci (Table 2).

Mean number of alleles per locus ranged from 2.4 of OI adults to 3.2 for the PP adults (Table 2). The effective number of alleles per locus ranged from 1.60 in the BP adult population to 1.79 in the PP adult trees (Table 2).

Mean heterozygosity in each of the populations was lower than expected according to Hardy-Weinberg predictions, except among the OI adult trees (Table 2). This overall deficiency in heterozygotes was reflected by positive mean F_{IT} in both adults and acorns, although there was considerable variation across loci. The majority of the deviation from equilibrium resided among individuals within populations (F_{IS}). Mean F_{IT} in adult trees equaled 0.183, while the mean F_{IS} component was 0.147 (Table 3). Similarly, the acorn mean F_{IT} value was 0.373, while the mean F_{IS} component totaled 0.360 (Table 4).

Mean migrant per generation estimates (Nm) for adults and acorns were substantially different. The mean Nm estimate for adult populations was 5.70 compared to a value of 12.25 for the acorns (Table 4). Values for the individual loci in adults ranged from 0.84 at the MDH2 locus to 49.75 at the MDH1 locus (Table 3). The acorns showed more variation—values for individual loci ranged from 7.10 at the IDH1 locus to 249.75 at MDH3 (Table 4).

TABLE 3. Deviations from Hardy-Weinberg equilibrium among individuals (F_{IS}), among populations (F_{ST}), and total deviation (F_{IT}), with migrants per generation (Nm), according to Wright (1931), for 11 loci from *Quercus* adults.

Locus	# of alleles	F_{IS}	F_{IT}	F_{ST}	Nm
PGI-2	5	−0.023	−0.003	0.020	12.25
6-PGDH1	3	−0.111	−0.057	0.049	4.85
6-PGDH2	3	−0.017	−0.009	0.008	31.0
LAP1	4	0.452	0.447	0.046	5.18
IDH1	5	0.070	0.081	0.012	20.58
IDH2	3	0.542	0.555	0.029	8.37
IDH3	3	0.651	0.659	0.025	9.75
MDH1	2	−0.019	−0.014	0.005	49.75
MDH2	2	−0.383	−0.065	0.230	0.84
MDH3	2	0.088	0.106	0.020	12.25
SKDH1	6	0.130	0.179	0.056	4.21
Mean	3.45	0.147	0.183	0.042	5.70

TABLE 4. Deviations from Hardy-Weinberg equilibrium among individuals (F_{IS}), among populations (F_{ST}), and total deviation (F_{IT}), with migrants per generation (Nm), according to Wright (1931), for 11 loci from *Quercus* acorns.

Locus	# of alleles	F_{IS}	F_{IT}	F_{ST}	Nm
PGI-2	4	−0.028	−0.007	0.020	12.25
6-PGDH1	3	0.087	0.093	0.007	35.46
6-PGDH2	3	−0.017	−0.012	0.005	49.75
LAP1	4	0.523	0.536	0.028	8.68
IDH1	4	0.105	0.135	0.034	7.10
IDH2	3	0.870	0.871	0.008	31.0
IDH3	3	0.793	0.796	0.016	15.38
MDH1	2	−0.016	−0.014	0.002	124.75
MDH2	2	−0.009	−0.006	0.004	62.25
MDH3	2	−0.019	−0.018	0.001	249.75
SKDH1	4	0.555	0.562	0.016	15.38
Mean	3.09	0.360	0.373	0.020	12.25

Values for Nei's unbiased genetic identity revealed the adult and acorn populations to be closely related in each pairwise comparison (Table 5). Identities ranged from 0.958 for the comparison of the BP adult population to the PP acorn population, to 0.999 for the comparison of the PP adult population to OK adults (Table 5). Pairwise comparisons of the BP adult population to the other four populations of adults and three populations of acorns showed the Bayfield adults to be the most genetically disparate of the populations studied. The BP adult identity values, which ranged from 0.958 to 0.979, contained six of the lowest seven pairwise genetic identity values (Table 5).

DISCUSSION

Identification of species and their hybrids—Our survey of 21 enzyme systems on seven buffer systems yielded no species-specific alleles except for the rare PGI-2[1]. This was not surprising considering results from similar studies. Manos and Fairbrothers (1987) found that among the systems they examined, aside from identifying *Quercus palustris,* isozymes did not separate species within *Erythrobalanus.* Guttman and Weigt (1989) attributed the lack of allozyme divergence among the red oaks to extensive hybridization between the species. Because neither of these earlier studies examined populations of *Quercus ellipsoidalis,* we had hoped to find an allele unique to that species; this was not the case.

Whittemore and Schaal (1991) used variations in chloroplast DNA and nuclear ribosomal DNA to study interspecific gene flow in five species of white oak native to the eastern United States. Their study revealed a species-specific length variant of nuclear ribosomal DNA that distinctly separated three species of white oak. Such variants could prove to be useful in making an unambiguous identification of red oak species and their hybrids.

Genetic variability in Apostle region oak populations—We found the levels of allozyme variation in the Apostle region oaks to be high when compared to other vascular plant species. Hamrick and Godt (1989) found that species have, on average, 50.4% of their loci polymorphic, 1.96 alleles per locus, and 1.21 effective alleles per locus. Among the Apostle region oaks we found mean values of 62.5% of the loci polymorphic, 2.8 alleles per locus, and 1.7 effective alleles per locus. Other oak studies have found alleles per locus to vary from 1.4 to 2.5, and percent polymorphism from 30 to 65 (Manos and Fairbrothers, 1987; Guttman and Weigt, 1989; Schnabel and Hamrick, 1990; Schwarzmann and Gerhold, 1991). The life history characteristics of oaks would lead one to predict high levels of allozyme diversity. In particular, Hamrick and Godt found that geographic distribution and breeding system correlate strongly with variability, particularly at the species level. Thus, geographically widespread, outcrossing, wind-pollinated species such as oak are expected to have higher levels of genetic variation than plant species as a whole.

The oak populations of Manos and Fairbrothers (1987) contained the least diversity of any oak study. Guttman and Weigt (1989) attributed the low value of Manos and Fairbrothers to the limited range of the species over which they sampled. The implication here is that by limiting the range over which the species is sampled, the degree

TABLE 5. Unbiased genetic identity values according to Nei (1978), for *Quercus* adults and acorns from the Apostle Islands and vicinity.

Population	1	2	3	4	5	6	7	8
1 Bayfield Peninsula Adults								
2 Peninsula Perimeter Adults	0.972							
3 Peninsula Perimeter Acorns	0.958	0.988						
4 Oak Island Adults	0.971	0.999	0.976					
5 Oak Island Acorns	0.971	0.990	0.993	0.986				
6 Stockton Island Adults	0.979	0.995	0.990	0.993	0.993			
7 Outer Island Adults	0.967	0.987	0.976	0.984	0.976	0.988		
8 Outer Island Acorns	0.967	0.996	0.987	0.996	0.993	0.993	0.986	

of genetic variability is commensurately lowered. However, we collected individuals from a much more restricted range than any of the other studies and found levels of variability that were comparable to or higher than those previously reported. This indicates that the amount of variation present in red oak species may relate more closely to how many individuals are sampled within a population, rather than the breadth of the geographical area considered.

The levels of heterozygosity we observed in the oaks and acorns in the Apostles region were generally lower than the expected Hardy-Weinberg equilibrium values (Table 2). In only one instance did the observed mean value exceed the expected; among the OI adult trees there was a small excess of heterozygotes. This observation was mirrored by the positive mean F_{IT} values for both adult and acorn populations, although the individual values varied greatly from locus to locus (Tables 3, 4). It is not known why the loci were so variable, unless they were differentially affected by selection.

Guttman and Weigt (1989) also described a general deficiency of heterozygotes among adult trees. Using their data we calculated the mean levels of expected and direct heterozygosity from the ten species of red oak they studied. These values equaled 0.178 and 0.103, respectively. Although our expected (0.301) and direct (0.228) values were higher, the difference between our two values (0.073) was very similar to the difference between the values from Guttman and Weigt's data (0.075). Schnabel and Hamrick (1990) and Schwarzmann and Gerhold (1991) also reported a deficiency in heterozygotes, but the differences between expected and direct values were much smaller (<0.005).

Levels of gene flow among the Apostle region oaks—The low levels of heterozygosity found in the oak populations might be the result of limited gene flow and high levels of inbreeding. Our mean estimates of migrants per generation (Nm) for the Apostle region oaks were high for plant species as a whole. However, the level we estimated from the adults (5.70) is on the low end of the spectrum for species that are widely distributed, long-lived, outcrossing, and wind-pollinated. Hamrick (1987) presented migration rates from plant species fitting into various life history categories based on breeding system. The wind-pollinated, outcrossed category was represented by four conifers with Nm estimates ranging from 5.3 to 37.8. Schuster, Alles, and Mitton (1989) reported Nm from another conifer, *Pinus flexilis* James (limber pine), as 11.1 migrants per generation.

The low Nm values of oaks may be related to their mode of seed dispersal. Acorns are dispersed by gravity and animals, while conifer seeds are winged and disperse by the wind. According to Hamrick (1987), species with winged seed dispersal mechanisms have an average migration rate that is at least 20 times greater than gravity- and animal-dispersed species.

Additional evidence for low levels of dispersal among the Apostle region oaks can be seen at the empirical level, as no consistent patterns were observed across islands between adult and acorn allele frequencies (Table 1). If there had been high levels of pollen flow, allele frequencies in acorn populations would be expected to deviate from their parental frequencies in the direction of adjacent island populations. However, in most cases acorn frequencies were most closely aligned with their own adult population. In fact, the acorns were almost twice as homozygous as the adult populations, as revealed by the F_{IT} values (Tables 3, 4). This F_{IT} value for the acorns (0.373) indicates that substantial inbreeding is occurring within these populations.

While this heterozygote deficiency was quite congruous with low migration rates, other elements of the data seemed to imply the opposite. Measures of unbiased genetic identities revealed the oak populations in the Apostles region to be quite similar, a pattern often indicative of high levels of migration (Table 5). High levels of gene flow were also indicated by the low F_{ST} values calculated for these populations (Tables 3, 4). Among both the acorns and adults, the levels of genetic variation between populations (F_{ST}) were the smallest component of the overall deviation from Hardy-Weinberg equilibrium. A similar pattern was observed by Manos and Fairbrothers (1987), Schnabel and Hamrick (1990), and Schwarzmann and Gerhold (1991).

Another line of evidence indicative of high levels of gene flow among the Apostle region oaks involved patterns of morphological variation (see companion manuscript—Jensen et al., 1993). Using principle component analysis techniques, we found evidence of a morphological cline that paralleled the geographic layout of the region. For principle component one, the continuum of leaf morphologies extended from typical *Quercus ellipsoidalis* on the left to typical *Q. rubra* on the right. Trees from the BP population clustered to the left, OI, STK, and PP trees clustered to the right of center, while OK trees were found near center. Despite the fact that the positioning of the populations on the cline was not always perfect, the morphological evidence indicated that substantial gene flow had occurred from the mainland into the islands.

Therefore, the isozyme and morphological data indicate two seemingly contradictory trends. Low levels of allozyme heterozygosity indicated that these populations are currently experiencing little gene flow, while high genetic identities and morphological patterns suggested that considerable hybridization and gene flow has occurred, at least in a historical context. One possible explanation for the lower than expected levels of heterozygosity among our oak populations concerns the logging history of the Apostles region tree communities. Significant episodes of logging have occurred on these islands and the mainland. The last major logging activity in the islands ended in the 1930s while logging continues to this day in the Chequamegon National Forest on the BP. In all the populations we sampled, there were a few remnant stands of virgin trees. It is likely that some of the secondary growth stands we sampled were the products of "bottlenecked" regeneration from the patchy remnant stands of unlogged trees. If this was the case, these secondary stands were regenerated from a restricted parent pool, and thus are more similar in genetic constitution (more homozygous) than those stands found on less disturbed sites. Such "bottleneck" regeneration may have contributed to the increased level of homozygosity we noted in the Apostles region oaks.

It may also be only in exceptional years that the BP trees, which hold the most diverse set of genes for the

region, contribute to the pollen pool, and thus serve to increase levels of heterozygosity. In two seasons of collecting in the Apostles region, no acorns were found among the BP adult trees. These BP trees exist in a sand barrens area. The soil is sandy, xeric, and quite disturbed by logging activity. The trees are reduced in size and appear quite stressed. The lack of acorns noted among these trees may be paralleled by a lack of pollen production. That is, in response to marginal survival conditions, the BP trees may be diverting energy from sexual reproduction to mere survival or asexual reproduction. Only in an exceptional year, i.e., one with above average rainfall, mild winter, minimal browse damage, etc., will these trees flower and shed pollen, thus adding to the genetic variability noted in the region.

In conclusion, we suspect that these populations are currently experiencing high levels of inbreeding due to "bottlenecked" regeneration, or diminished pollen input from the region's most genetically diverse population. In a historic or more episodic context, the populations may have experienced higher levels of pollen-mediated gene flow which would account for the high genetic identities. From a management perspective, the lower than expected levels of gene flow suggest that proximally located populations of oaks must be maintained. Such a strategy would be critical for the preservation of the species genetic resources by allowing for periodic mixing of adaptively significant alleles.

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