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ISOZYME VARIATION AS EVIDENCE OF GENE FLOW AND HYBRIDIZATION BETWEEN RED OAKS FOUND IN AN ISLAND ARCHIPELAGO

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By

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ABSTRACT

ISOZYME VARIATION AS EVIDENCE OF GENE FLOW AND HYBRIDIZATION BETWEEN RED OAKS FOUND IN AN ISLAND ARCHIPELAGO

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Isozyme variability was examined in the red oak complex, *Quercus* subg. *Erythrobalanus* found on an island archipelago and vicinity in northeastern Wisconsin. Dormant leaf bud samples were collected from *Quercus rubra* L., *Q. ellipsoidalis* Hill and their putative hybrids from two peninsula locations and on three islands. Acorns were collected from some of these same trees in three of these locations. Twelve putative loci coding for six enzymes were analyzed. Allele frequency data indicated there was little differentiation between populations. Mean F_{ST} values for the adult trees and acorns were 0.042 and 0.020 respectively. Genetic identities according to Nei ranged from .958 to .999. In spite of these high genetic identities, the populations appeared to be experiencing substantial levels of inbreeding as indicated by positive mean F_{IT} values of 0.183 and 0.373 for the trees and acorns respectively. Estimates of migration rate per generation for the adult trees was 5.70.

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Completing this project was a "peak experience" for me. As is often the case, these experiences arise from quality interactions with, and input from exceptional people. Such is the stuff of which we shall one day measure our lives.

It is with extreme gratitude and some pride that I make these acknowledgements. First, I want to thank Dr. Jud Isebrands at the Forestry Sciences Laboratory in Rhinelander, WI, for originally proposing the project, providing substantial funding and considerable logistical support to carry the project. Also at Rhinelander, I want to thank Gary Gartner and Dave Buckley for their help with collections. At the Apostle Islands National Lakeshore, I want to thank Dr. Robert Brander, Julie Van Stappen and their staff for providing access to the populations and for extending a very cooperative atmosphere in which to conduct the fieldwork.

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Everyone in the lab strives to maintain a vigorous, cooperative, non threatening atmosphere in which to work. I thank them all past and present for the unqualified acceptance into such an exceptional group.

I want to thank my thesis committee, Drs. Hancock, Iezzoni, Jensen, and Tonsor for, possibly with no intent to do so, teaching me the power and grace of a positive perspective. Thanks to the 11:30 club for being there, Jim, for taking the gamble which made it all possible, and Karen for love and support on all the days, good and bad.

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INTRODUCTION

Quercus is a wide ranging genus of trees and shrubs comprised of up to 500 species worldwide (Nixon, 1989). In eastern North America, the genus is represented by approximately 50 species rather evenly divided into two reproductively isolated subgenera: *Quercus* subg. *Quercus*, the white and chestnut oaks, and *Quercus* subg. *Erythrobalanus*, the red and black oaks.

Within *Quercus*, a definitive taxonomic structure at the species level has been difficult to achieve. To date, oak classifications have been based almost exclusively on morphological characteristics (Trelease, 1924; Jensen, 1988). However, the apparent ease of hybridization among species (Palmer, 1948), particularly in *Erythrobalanus* (e.g., Jensen, 1977a), renders such an approach problematic, especially where the species ranges overlap. In areas of species sympatry, many trees may have morphologies intermediate between species types. These "hybrid" oaks often defy precise identification (Overlease, 1964; Jensen, 1977a).

Numerous studies have attempted to find alternative characters suitable for differentiation at the species level. These studies have examined wood anatomy (Muller, 1942), phenolic compounds in leaves, young twigs, and male flowers (Li and Hsiao, 1973, 1975, 1976a,b), and scanning electron microscopy of pollen (Solomon, 1983a,b). Although these investigations affirmed the subgeneric discrimination, none of the characters investigated allowed consistent distinctions at

the species level.

Electrophoretic techniques have been employed to establish relationships among the oaks in the eastern United States. Manos and Fairbrothers (1987) evaluated 16 putative isozyme loci among populations of six native red oak species found in New Jersey: *Quercus coccinea* Muenchh., *Q. ilicifolia* Wang., *Q. marilandica* Muenchh., *Q. palustris* Muenchh., *Q. rubra* L., and *Q. velutina* Lam.. The genetic differentiation detected with polymorphic loci did not correlate with previously established taxonomies and, with the exception of *Q. palustris*, no species specific alleles were detected. Thus, isozymes were of little value in clarifying taxonomic structure in these taxa.

Guttman and Weigt (1988) used 18 putative isozyme loci from 12 enzyme systems to study ten species from the subgenus *Erythrobalanus* and eight from subgenus *Quercus*. Unlike that of Manos and Fairbrothers (1987), their study of *Erythrobalanus* did not include *Q. coccinea* and *Q. ilicifolia*.

The groupings they discovered were discrepant from both traditional taxonomies and recent multivariate comparisons. Jensen (1977b) used a numerical analysis of 36 morphological characters to evaluate taxonomic relationships in the scarlet oak complex in the eastern United States. This inquiry indicated that *Quercus ellipsoidalis* clustered near *Q. palustris* and also showed relationship to *Q. velutina*. Guttman and Weigt's (1988) data did not confirm this relationship with *Q. velutina*, nor several others proposed by

Jensen (1977b). Guttman and Weigt (1988) attribute these discrepancies to unequal rates of morphological, ecological, and allozymic divergence and the possibility that hybridization between species is preventing the development of a widely accepted, definitive taxonomic structure within the subgenus. Several other authors have mentioned this possibility (Palmer, 1942; Jensen, 1977b; Solomon, 1983b).

Thus, determining a taxonomic structure within the subgenus *Erythrobalanus* becomes partly a matter of identifying hybrids. Because hybridization is a function of the degree of pollen flow between species, understanding the movement of genes within the genus *Quercus* becomes crucial to elucidating the nature of hybrid formation. Inter-specific gene flow is clearly suggested by the wide range of morphologies expressed when two or more red oak species are found in one location (Jensen, 1988).

Commensurate with the recognition of the importance of gene flow, much research has been directed toward understanding its dynamics in both a theoretical and a practical sense (Levin and Kerster, 1974; Moore, 1976; Handel, 1983b; Slatkin, 1981, 1985; Ellstrand, 1988). However, 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 Marshall, 1985). Probably most crucial in designing studies of gene flow is the distinction

between potential and actual gene flow (Levin and Kerster, 1974). Potential gene flow is characterized by the movement of pollen whereas actual gene flow does not occur until fertilization. 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.

Considerable effort has also been directed towards determining the relationship between life history traits and the genetic diversity and structure within various plant species (Brown, 1979; Hamrick et al., 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 study, along with earlier such studies (Hamrick et al., 1979; Nevo et al., 1984; Loveless and Hamrick, 1984), uncovered significant correlations between ecological and life history traits of species and the patterns of genetic diversity and structure they maintain. These reviews concur that species, such as oaks, which are long-lived, outcrossed, wind pollinated and which prevail in the later stages of

succession, generally maintain higher levels of genetic variation within populations than between populations.

Schnabel and Hamrick (1990) used electrophoretic techniques to examine population genetic structure in two species of white oak, *Quercus macrocarpa* Michx. (bur oak) and *Q. gambelii* Nutt. (Gambel oak). Whether considered as species or as individual populations, both *Q. macrocarpa* and *Q. gambelii* were found to maintain levels of allozyme variation which were higher than the overall averages for most other plant species. *Quercus macrocarpa* had an average of 3.58 alleles per locus while *Q. gambelii* had 2.67. The overall genetic diversity for *Q. macrocarpa* was 0.206 with *Q. gambelii* maintaining a level of 0.215.

According to Hamrick and Godt (1989), such high levels of allozyme variation should not be surprising. The life history traits generally associated with *Quercus* species, i.e. broad geographic range, wind pollination, predominantly outcrossing, and a long-lived perennial nature, are generally associated with high levels of allozyme variation.

Despite increased efforts to study population genetic structure in woody angiosperms, (Bousquet et al., 1987, 1988; Surles et al., 1989; Schnabel and Hamrick, 1990), there are still significant gaps in such knowledge. This is particularly true for deciduous trees which make up the northern hardwood forests. Several of these species, quite notable among them *Quercus rubra* (northern red oak), are being subjected to extreme logging pressure. Without an

understanding of what constitutes a healthy genetic structure in a population of such trees, it will be difficult to determine what level of harvest is sustainable.

Coincident with this economic pressure, there is also concern about the effects of certain environmental perturbations, such as global warming and acid rain, on the genetic health of forest tree species, . To make responsible decisions concerning the possible impacts of such pressures, a more substantive base of knowledge must be built. In particular we need to have a better understanding of the population genetic structure of tree species such as the northern red oak.

Populations of oaks on the Apostle Islands in Lake Superior presented us with the opportunity to address the issues noted above. The islands and adjacent Bayfield Peninsula are inhabited by natural populations of two species of subgenus *Erythrobalanus*. Leaf and acorn morphological characters indicate that *Quercus rubra* L. (northern red oak) predominates on the outer most island of the archipelago (Outer Island), while *Q. ellipsoidalis* Hill (northern pin oak) predominates in the peninsula interior. Between these distal populations exist populations which can be characterized as being intermediate or hybrid in nature (Jensen et al., manuscript in review). This pattern of morphological variability presented us with a system to answer the following questions: 1) Do species specific alleles exist for *Quercus rubra* or *Q. ellipsoidalis* which would allow for the

unambiguous identification of these species and their hybrids? 2) What levels of genetic variation are contained within these small populations of adult trees and their acorns? 3) How much and how far do genes move among these island populations of oak?

MATERIALS AND METHODS

Collections We studied five populations of oaks in the Bayfield, Apostle Islands National Lakeshore region in northeastern Wisconsin (Figure 1). 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 of 1990. Bayfield Peninsula collections (BP) were made along roads which defined the east and north perimeters of the Chequamegon National Forest. At approximately 0.4 km intervals, the first mature oak sighted was sampled. Peninsula perimeter collections (PPC) were made on the public roads which skirted the perimeter of the Bayfield peninsula. As in the national forest, we sampled the first mature oak sighted at 0.4 km intervals along the roads. Collections on Oak Island (OK) were made on a north to south trail which bisected the island. After five minutes of vigorous walking we sampled the first mature oak sighted within 20 yards of the trail. A similar sampling strategy was used for the other island collections. The Stockton Island (STK) and Outer Island (OI) collections were done on a trail which ran from southwest to northeast the length of the island.

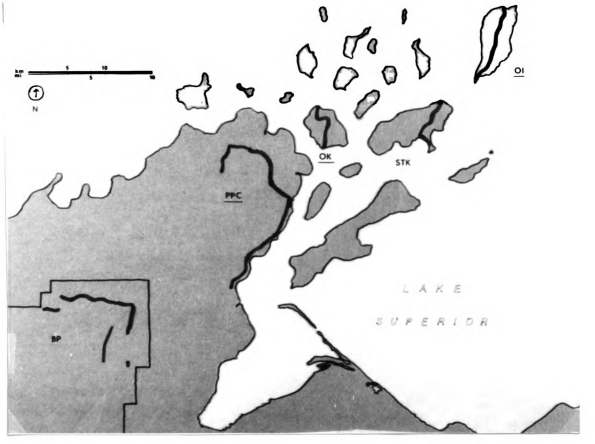

Figure 1. Map of the Apostle Islands and adjoining Bayfield Peninsula in northeastern Wisconsin. The five populations studied are initialed, Bayfield Peninsula (BP), Peninsula Perimeter (PPC), Oak Island (OK), Stockton Island (STK), and Outer Island (OI). Underlined initials indicate populations from which acorns were collected. Heavy lines represent the transects sampled.

Samples collected in June, 1989 consisted of rapidly expanding leaves 15-75mm in length. On the subsequent trips dormant buds and acorns were collected. In all cases, pencil sized branches were removed from individual trees. Buds were left on small twigs while acorns and leaves were removed. Samples were placed in labeled zip-lock bags and stored within three hours in a cooler. On return to East Lansing, MI, the plant material was placed in a cooler at 2.5° C until enzymes were extracted. Leaves rapidly lost their enzyme activity and had to be prepared immediately for electrophoresis, while dormant buds could be stored up to two months before extraction with no appreciable loss in activity. Acorns ground 11 months after harvest also had high enzyme activity.

Enzyme Extraction All 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 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.

After grinding, the resulting slurry was absorbed through nylon mesh onto 3 x 4 x 11 mm wicks cut from Whatman chromatography paper. Wicks sufficient for four electrophoretic examinations were prepared at this time. These wicks were placed in Corning 96 well disposable Elisa plates which were double wrapped in cellophane, bagged in

ziplock bags and stored at -80°C until electrophoresed. Only enough wicks for one analysis were placed in a plate, thus the wicks were never taken out of the freezer until they were to be analyzed. Well resolved isozymes were obtained from wicks 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) resolved the largest number of enzymes clearly. Ten millimeter thick 6.1 gels were typically run for seven hours at 55-65 milliamps, and approximately 250 volts. Six millimeter thick 8.3 gels were run for six hours at 50 milliamps or until 300 volts was reached. Slices from the 8.3 Scandalios system were stained for phosphoglucose isomerase (PGI) and leucine amino peptidase (LAP). Slices from the 6.1 morpholine-citrate system were stained for 6-phosphogluconate dehydrogenase (6-PGDH) (Conkle, 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 then fixed in a 50% ethanol solution. Slices were then bagged in zip lock bags and refrigerated at 4°C for later analysis.

During our initial experiments, we found that resolution became increasingly poor as leaves expanded, while dormant buds gave us consistently good results. Thus, we discontinued using leaves, and all results reported herein were obtained using dormant bud or acorn samples. The acorns analyzed in this study were collected from adult trees which were also analyzed electrophoretically. Five acorns were electrophoresed from each of these parent trees.

Isozyme 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. (Figures 2,3). Because we made no controlled crosses to analyze segregation of the isozyme banding patterns, all allele and loci designations are putative.

Statistical Analysis Allele frequencies, average heterozygosities (direct and estimated), percent loci polymorphic, mean alleles per locus, F-statistics and genetic identity according to Nei (1978b) were calculated using the BIOSYS-1 program, release 1.7, which is adapted for the PC (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

Figure 2. Electrophoretic patterns for three of the six enzyme systems used in this study. Numbers to the right 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 is heterozygous for alleles three and five of locus two. Lane B is heterozygous for alleles one and five of locus two. MDH was scored as a dimeric enzyme encoded by three loci. In this photo all individuals are homozygous for allele one of locus one. Lane A is heterozygous for alleles one and two of locus two. Allele one of locus three has comigrated with allele two of locus two. Lane B is homozygous for allele two of locus two. Allele one of locus three has comigrated with allele two of locus two. SKDH was scored as a monomeric single locus enzyme. Lane A is 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 bottom most band was considered a plastid form of allele five and not scored. Lane C is homozygous for allele five.

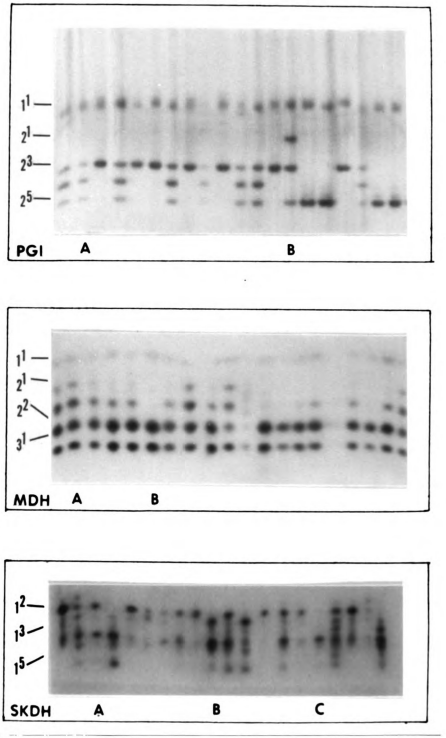

Figure 2.

Figure 3. 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 is heterozygous for alleles one and three of locus one. Lane B is heterozygous for alleles four and five of locus one. Lane C is heterozygous for alleles one and two of locus two. Lane D is homozygous for allele one of locus three. Lane E is heterozygous for alleles two and three of locus three. 6PGDH was scored as a dimeric enzyme encoded by two loci. Lane A is homozygous for allele one of locus one. Lane B is heterozygous for alleles one and two of locus one. Lane C is heterozygous for alleles one and three of locus one. Lane A is homozygous for allele two of locus two. Lane D is 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 is homozygous for allele two. Lane B is homozygous for allele three. Lane C is homozygous for allele one. Lane D is heterozygous for alleles two and four.

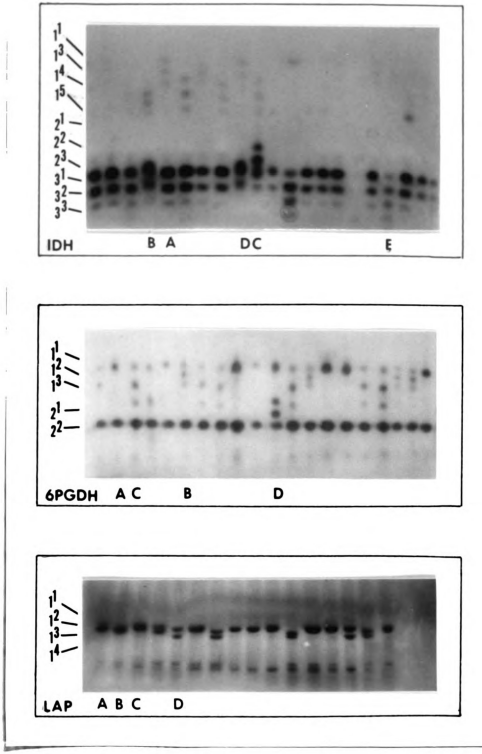
1¹
1³
1⁴
1⁵
2¹
2²
2³
3¹
3²
3³
IDH
B A
DC
E
1¹
1²
1³
2¹
2²
6PGDH
A C
B
D
1¹
1²
1³
1⁴
LAP
A B C
D

Figure 3.

heterozygosity were based on Hardy-Weinberg 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 which 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 number of loci in the population.

F-statistics (fixation indices; Wright 1951, 1965b) were calculated according to Nei (1977c). 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}, the proportion of the deviation from equilibrium contained within subpopulations, can be

utilized as a measure of the deviation 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 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. 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 (Table 1). All populations, both adult and acorn, had 63.6 percent polymorphic loci except for the STK adults which were polymorphic for 54.5 percent of their loci (Table 2).

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

Both adult and acorn populations exhibited an overall deficiency in heterozygotes. Direct mean heterozygosities were less than the expected values for all adult and acorn populations except among the OI adult trees (Table 2). On an individual locus basis, 6 of the 11 loci showed some deficiency of heterozygotes in the adult population (Table 3). Of the remaining five, only two of these differed substantially from equilibrium, 6-PGDH1 with a value of -0.057 and MDH2 with a value of -0.065 (Table 3). In the acorn populations five loci showed slight excesses in heterozygotes, however none deviated substantially from equilibrium (Table 4).

The overall deficiency in heterozygotes was reflected by

Table 1. *Allele frequencies at 12 putative enzyme loci for five adult (Adlt) and three acorn (Acor) populations of Quercus subg. Erythrobalanus studied on the Apostle Islands and vicinity.*

		BP		PPC		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	.000	-	.014	.000	.000	.000	.000	-	.000	.000
	2	.030	-	.000	.011	.000	.027	.000	-	.000	.007
	3	.790	-	.688	.819	.583	.818	.656	-	.667	.691
	4	.010	-	.036	.011	.046	.009	.021	-	.028	.026
	5	.170	-	.261	.160	.370	.145	.323	-	.306	.276
LAP-1	1	.340	-	.176	.457	.186	.474	.427	-	.225	.244
	2	.300	-	.485	.283	.539	.386	.302	-	.250	.411
	3	.180	-	.250	.207	.186	.096	.177	-	.425	.256
	4	.180	-	.088	.054	.088	.044	.094	-	.100	.089
6PGD1	1	.930	-	.623	.693	.641	.696	.670	-	.706	.710
	2	.040	-	.098	.034	.141	.107	.085	-	.147	.117
	3	.030	-	.279	.273	.217	.196	.245	-	.147	.173

Table 1. *Continued.*

		BP		PPC		OK		STK		OI	
Locus	Allele	Adlt	Acor	Adlt	Acor	Adlt	Acor	Adlt	Acor	Adlt	Acor
6PGD2	1	.000	-	.007	.022	.010	.009	.010	-	.000	.000
	2	1.00	-	.986	.978	.990	.982	.990	-	.975	.994
	3	.000	-	.007	.000	.000	.009	.000	-	.025	.006
IDH-1	1	.448	-	.341	.389	.255	.219	.372	-	.306	.253
	2	.000	-	.000	.000	.000	.000	.032	-	.000	.019
	3	.354	-	.333	.211	.431	.430	.351	-	.361	.474
	4	.000	-	.024	.000	.010	.000	.000	-	.000	.000
	5	.198	-	.302	.400	.304	.351	.245	-	.333	.253
IDH-2	1	.000	-	.000	.000	.020	.000	.064	-	.050	.058
	2	.000	-	.101	.133	.059	.175	.021	-	.000	.168
	3	1.00	-	.899	.867	.922	.825	.915	-	.950	.744
IDH-3	1	.020	-	.087	.167	.069	.069	.022	-	.000	.094
	2	.950	-	.884	.813	.931	.879	.957	-	1.00	.906
	3	.030	-	.029	.021	.000	.052	.022	-	.000	.000

Table 1. *Continued.*

		BP		PPC		OK		STK		OI	
Locus	Allele	Adlt	Acor	Adlt	Acor	Adlt	Acor	Adlt	Acor	Adlt	Acor
MDH-1	1	.980	-	.980	.989	.980	.991	.990	-	1.00	.980
	2	.020	-	.020	.011	.020	.009	.010	-	.000	.020
MDH-2	1	.000	-	.014	.011	.000	.000	.000	-	.289	.006
	2	1.00	-	.986	.989	1.00	1.00	1.00	-	.711	.994
MDH-3	1	.950	-	.958	.989	.980	.983	1.00	-	1.00	.977
	2	.050	-	.042	.011	.020	.017	.000	-	.000	.023
SKDH1	1	.048	-	.030	.000	.010	.000	.000	-	.000	.000
	2	.269	-	.523	.716	.539	.552	.510	-	.556	.636
	3	.029	-	.152	.136	.088	.208	.198	-	.167	.130
	4	.029	-	.053	.034	.029	.000	.031	-	.056	.026
	5	.606	-	.235	.114	.314	.240	.260	-	.222	.208
	6	.019	-	.008	.000	.020	.000	.000	-	.000	.000

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.*

POPULATION	MEAN SAMPLE SIZE/LOCUS	%POLYMORPHIC LOCI	MEAN # ALLELES/LOCUS	EFFECTIVE # ALLELES/ LOCUS	MEAN HETEROZYGOSITY DIRECT	EXPECT
BAYFIELD	49.9 (0.3)[1]	63.6	2.7 (0.5)	1.60	.198 (.069)	.242 (.085)
PENINSULA PERIMETER	66.3 (1.8)	63.6	3.2 (0.4)	1.79	.232 (.073)	.342 (.083)
PERIMETER-ACORNS	43.1 (1.9)	63.6	2.8 (0.3)	1.61	.187 (.061)	.291 (.075)
OAK ISLAND	50.5 (0.6)	63.6	2.9 (0.4)	1.71	.249 (.084)	.303 (.085)
OAK-ACORNS	53.2 (2.6)	63.6	2.7 (0.3)	1.64	.181 (.058)	.296 (.077)
STOCKTON ISLAND	47.9 (0.4)	54.5	2.7 (0.3)	1.74	.245 (.074)	.296 (.090)
OUTER ISLAND	19.0 (0.3)	63.6	2.4 (0.3)	1.76	.327 (.090)	.322 (.089)
OUTER-ACORNS	79.5 (3.2)	63.6	2.9 (0.3)	1.72	.203 (.065)	.313 (.080)
MEAN	51.2 (16.4)	62.5 (3.0)	2.8 (0.21)	1.70 (0.07)	.228 (.045)	.301 (.027)

(1) Numbers in parentheses indicate standard deviation.

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	FIS	FIT	FST	Nm
PGI-2	5	-0.023	-0.003	0.020	12.25
6-PGDH1	3	-O.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	FIS	FIT	FST	Nm
PGI-2	4	-0.028	-0.007	0.020	12.25
6-PGDH1	3	O.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

positive mean F_{IT} values. The majority of this total deviation from equilibrium resided among individuals within populations (F_{IS}). Of the total variation in adult trees, F_{IT}, which equaled 0.183, the F_{IS} component comprised 0.147 (Table 3). Similarly, the acorn F_{IT} value, 0.373 was comprised primarily of the F_{IS} component which 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).

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 PPC acorn population, to 0.999 for the comparison of the PPC 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).

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								
2 Peninsula Perimeter	.972							
3 Peninsula Perimeter Acorns	.958	.988						
4 Oak Island	.971	.999	.976					
5 Oak Island Acorns	.971	.990	.993	.986				
6 Stockton Island	.979	.995	.990	.993	.993			
7 Outer Island	.967	.987	.976	.984	.976	.988		
8 Outer Island Acorns	.967	.996	.987	.996	.993	.993	.986	

DISCUSSION

Identification of species and their hybrids. This survey of 21 enzyme systems on 7 buffer systems yielded no species specific alleles. This was not surprising considering results from similar studies. Manos and Fairbrothers (1987) concluded that among the systems they examined, aside from distinguishing *Quercus palustris*, isozymes would be of little use in making species distinctions within *Erythrobalanus*. Guttman and Weigt (1988) attributed the lack of allozyme divergence among the red oaks to extensive hybridization between the species. Because neither of these studies examined populations of *Quercus ellipsoidalis*, we had hoped to find an allele unique to that species. Our findings confirmed Manos and Fairbrother's conclusion that, at least with the systems examined, isozymes will be of little use in making species distinctions within *Erythrobalanus*, *Q. ellipsoidalis* inclusive.

Recently, 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 the existence of a species specific length variant of nuclear ribosomal DNA which distinctly separated three species of white oak. It is possible that such variants could prove to be useful for 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 quite high when compared to other plant species. Hamrick and Godt (1989) reviewed the literature and found at the species level, on average, plants have 50.4 percent 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 percent of the loci polymorphic, 2.8 alleles per locus, and 1.7 effective alleles per locus.

The life history characteristics of oaks would lead one to predict these higher levels of allozyme diversity. In particular, Hamrick and Godt (1989) 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 predicted to have higher levels of genetic variation than plant species as a whole.

The mean numbers of alleles per locus found in our populations are somewhat higher than those reported for red oak species by Manos and Fairbrothers (1987) and Guttman and Weigt (1988), who found mean numbers of alleles of 1.37 (range = 1.2-1.5) and 2.1 (range = 1.8-2.4) respectively. Our populations had a mean value of 2.8 alleles per locus with a range of 2.4-3.2 (Table 2). When we considered adult trees alone, we generated the same alleles per locus values.

Schnabel and Hamrick (1990) reported genetic variability measures for two species of white oak, *Quercus macrocarpa*

Michx. (bur oak) and *Q. gambelii* Nutt. (Gambel oak). The values they describe for mean alleles per locus are also lower than those we report. For *Q. macrocarpa* the mean numbers of alleles per locus was 2.12 (range = 1.8-2.4) while *Q. gambelii* had a mean value of 2.14 (range = 2.12-2.2).

Manos and Fairbrothers (1987) reported a mean percentage of polymorphic loci of 29.7 (range = 18.8-37.5), while Guttman and Weigt (1989) found a mean value of 65.0 (range = 55.6-72.2). Schnabel and Hamrick (1990) reported mean values for *Q. macrocarpa* of 59.0 (range = 50.0-65.0) and for *Q. gambelii* of 54.0 (range = 52.0-57.0). Our mean value, 62.5 (with a range of 54.5-63.6) is considerably higher than that reported by Manos and Fairbrothers, somewhat higher than those of Schnabel and Hamrick, and very similar to that of Guttman and Weigt.

Guttman and Weigt (1989) attribute the differences between their mean values for polymorphic loci and alleles per locus and those of Manos and Fairbrothers (1987) to the limited range of the species over which Manos and Fairbrothers sampled. The implication here is that by limiting the range over which the species is sampled, the degree of genetic variability is commensurately lowered. However, measured amounts of genetic variability seem to relate more closely to how adequately populations are sampled. In this study, individuals were collected from a more restricted range than in any of these other studies, yet the variability found is comparable to that reported by Guttman & Weigt and Schnabel

and Hamrick. Thus, the variation present in red oak species appears to be adequately represented in populations of the size studied in the Apostles region.

Levels of gene flow among the Apostle region oaks. 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 adult oaks, 5.70 migrants per generation, is low when compared with other species which are widely distributed, long lived, outcrossing, and wind pollinated in nature. 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 et al. (1989) reported Nm levels from another conifer, *Pinus flexilis* James (limber pine), as 11.1 migrants per generation.

These lower than expected Nm values from the oaks may be related to the mode of seed dispersal they utilize. Acorns are dispersed by gravity and animals, while conifer seeds are winged and disperse by the wind. According to Hamrick (1987), large-seeded, gravity dispersed species have an average of 0.161 migrants per generation, while animal dispersed species range from 0.171-0.292 migrants per generation, depending on the mechanism of animal dispersal. Species with winged seed dispersal mechanisms have an average migration rate of 4.313 migrants per generation. It is possible that the reduction in the overall migration rate in the Apostle region oaks could be

due in part to the reduced distribution of the animal or gravity dispersed acorns as contrasted to the winged, wind dispersed seed of the conifers.

Additional evidence for low levels of dispersal can be seen at the empirical level by reviewing our allele frequency data at the most divergent loci (Table 1). If there were high levels of gene flow, allele frequencies in acorn populations would be expected to deviate from their parental frequencies in the direction of adjacent island populations. However, we noted no consistent patterns between adult and acorn frequencies. For example, at the PGI-2 locus, allele four is present among only the BP adults, but is found in all three acorn populations. This appears to be strong evidence for gene flow. In contrast, at PGI-2, allele one is present among the PPC adults, but was not noted in any other population, adult or acorn. Similarly, at the LAP-1 locus, the frequency of allele two in the acorns appears to be unaffected by the frequencies in the surrounding adult populations despite the fact that there is considerable variation in frequencies for this allele among the adults. In fact, the major trend we noted among the acorns was a nearly two fold increase in homozygosity over the adult populations as revealed by F_{IT} values (Tables 3,4). This F_{IT} value for the acorns (0.373) indicates that substantial inbreeding is occurring within these populations which is inconsistent with the notion that substantial gene flow is occurring among these populations.

The levels of heterozygosity we observe in the oaks and

acorns in the Apostles region are generally lower than the expected Hardy-Weinberg equilibrium values (Table 2). In only one instance does the observed value exceed the expected: among the OI adult trees there is a negligible excess of heterozygotes. This observation is mirrored by the overall positive F_{IT} values for both adult and acorn populations (Tables 3,4).

Guttman and Weigt (1988) also described a deficiency of heterozygotes. Using their data we calculated the mean expected and direct levels of 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) also reported a deficiency in heterozygotes, but the differences between expected and direct values are much smaller (0.005 and 0.003) for the two species.

The heterozygote deficiency we and others have noted in the oaks is indicative of considerable inbreeding. As we mentioned previously, this was particularly evident in the acorn population. The F_{IT} value for the acorns, 0.373, was more than twice the value for the adult trees 0.183. The mean migrants per generation value of 12.25 for the acorn population would seem to be large enough to offset any tendency towards inbreeding. However, when this value is compared to the Nm rates Hamrick (1987) presented in his

review, 12.25 migrants per generation is still low for wind pollinated, outcrossed plant species.

The heterozygote deficiency observed among the adult and acorn populations of the Apostles region is quite congruous with the low migration rates, however, some elements of the data seem to contradict this conclusion. Measures of unbiased genetic identities (following Nei, 1978b) revealed that oak populations in the Apostles region had high genetic identities (Table 5). These genetic identity measures showed only one population to be slightly distinct. The BP adult population contained six of the seven lowest pairwise identity values.

High levels of gene flow are indicated by the low F_{ST} values calculated for these populations (Tables 3,4). Among both the acorns and adults, F_{ST} is the smallest component of the overall deviation from Hardy-Weinberg equilibrium. F-statistics reveal the majority of the deviation from Hardy-Weinberg equilibrium resides within individuals within populations (F_{IS}). This finding is in complete agreement with the review by Hamrick and Godt (1989). Plant species which are wide spread in distribution, long lived, outcrossing, and wind pollinated maintain the majority of their genetic variation within individuals within populations (F_{IS}). Such species also maintain low levels of variation between populations (F_{ST}). In this respect our results are similar to those of Manos and Fairbrothers (1987) and Schnabel and Hamrick (1990), who reported similar patterns of variation.

Another line of evidence indicative of high levels of gene flow among the Apostle region oaks involved patterns of morphological variation (Jensen et al., manuscript in review). Using principle component analysis techniques, the authors found evidence of a morphological cline which mimicked the geographic layout of the region (Fig. 1). For principle component one, the continuum of leaf morphologies extends from typical *Quercus ellipsoidalis* on the left to typical *Q. rubra* on the right. Trees from the BP population cluster to the left, OI, STK and PPC trees cluster to the right of center while OK trees are found near center. Despite the fact that the positioning of the populations on the cline is not perfect, i.e. PPC to the right of center, the morphological evidence indicates gene flow has occurred from the mainland into the islands.

Our data, which are contradictory in nature, suggest two trends. Low levels of allozyme heterozygosity indicate that currently, these populations are experiencing little gene flow. In contrast, high genetic identities and morphological patterns suggest that considerable hybridization and gene flow must have occurred in an historical context.

One possible explanation for the higher than expected levels of homozygosity among these populations concerns the logging history of the Apostles region tree communities. Our sampling strategy took no account of the history of the region. Significant episodes of logging have occurred on these islands and the mainland. The last major logging

activity in the islands ended in the 1930's while logging continues to this day in the Chequamegon National Forest on the Bayfield Peninsula. In all the populations we sampled, there were remnant stands of virgin trees. On Oak Island we sampled through a small stand of virgin oaks containing two trees in excess of 200 years of age. It is likely that some of the secondary growth stands we sampled were the products of "bottlenecked" regeneration from 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. Consequently, sampling through sites which experienced such "bottleneck" regeneration may have contributed to the increased level of homozygosity we noted in the Apostles region oaks.

An alternative explanation involves the interaction of canopy development and pollen flow. When the islands were originally being vegetated, there was very little vegetative canopy. In this environment, pollen movement would be largely unrestricted and effective pollinations could occur over much greater distances. Given these circumstances it is likely that little inbreeding occurred among the oaks. As the canopy closed, pollen movement became more restricted. Restricted pollen movement may have contributed to increased levels of near neighbor mating and consequently to increased levels of inbreeding we see today. In this scenario, periodic episodes of logging would serve to "open" the canopy. These openings

would allow for increased levels of pollen mediated gene flow and thus the potential for an increase in heterozygosity.

Finally, it may 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. Interestingly, in the two seasons of collecting in the Apostles region we never found one acorn 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. Perhaps this is the normal survival strategy utilized by these trees. 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.

CONCLUSIONS

Results from this isozyme analysis of the red oak complex in the Apostle Islands region revealed two apparently contradictory trends concerning these populations. Both acorn and adult tree populations were found to be deficient in heterozygotes when compared to Hardy-Weinberg expectations, and acorns were more than twice as inbred as the adult trees.

This observation is consistent with lower than expected migration rate estimates from these populations when compared to other long lived, widely distributed, outcrossing, wind pollinated species such as conifers. In contrast, the populations had very high genetic identities. These high genetic identities, mirrored by low F_{ST} values, indicate there is little genetic differentiation between these populations. Such characteristics are typically the result of high levels of gene flow.

In answer to this seeming contradiction, we suspect that these populations are currently experiencing high levels of inbreeding due to "bottlenecked" regeneration, canopy closure, or diminished pollen input from the most genetically diverse population. In an 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.

Although considerable inbreeding is occurring in these populations, the two-fold difference in homozygosity between the acorns and adult trees suggests that selection is reducing the amount of homozygosity between the acorn and adult stage of development. It is also likely that these populations experience periodic episodes of long distance gene flow which serve to increase their levels of heterozygosity. From a management perspective, proximally located populations of oaks must be maintained to allow for periodic mixing of adaptively significant alleles.

More certainly we are left with the need to acquire more vital information. We have yet to learn over what distance an effective pollination can occur between oaks. How heterogeneous is the red oak subgenus at large? Do different red oak populations have a distinct genetic structure or could the red oak complex of the eastern United States be considered one large heterogeneous population? Such questions must be answered in order to make responsible decisions concerning the management of natural stands of red oak.

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