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Genetic Structure Inside a Declining Red Oak Community in Old-Growth Forest

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Abstract

Problems with oak regeneration have been documented in the last 50 years at numerous sites in the Midwestern United States. We applied nuclear microsatellites to examine the demographic and fine-scale spatial genetic structure of red oaks in two old-growth stands in Indiana. Oaks in one stand have declined in numbers over the past several decades whereas oaks in the other, smaller stand have increased. Large amounts of genetic variation were maintained within stands, and there was slight but significant differentiation among stands. There was significant but weak isolation by distance genetic structure within the large stand, likely reflecting family structure. No significant differences exist in allele frequencies or in levels of genetic diversity between cohorts that remain well represented within each stand, even between medium-sized adults and those antedating European settlement of the area. However, a virtual absence of smaller size classes in the forest interior of the large stand represents the early stages of a genetic bottleneck in what had been the core habitat of this stand. Whether future generations of this old-growth stand will retain the present genetic character depends on the oaks regenerating at the forest margins, absent any major changes in disturbance regimes. Similar demographic and genetic dynamics are likely occurring in a large number of remnant oak forests across the Midwest.

Despite the present widespread distribution of oaks in temperate zone forests, concerns have arisen from the past 50 years of research that oak regeneration is faltering in many forests (reviewed in Abrams 1992, 2003; Lorimer 1993). Increasingly, stands are comprising large oak trees with few seedlings or saplings in the understory, where more shade-tolerant species, such as maple (*Acer* spp.), maintain dense thickets of advanced regeneration. These patterns have been described in a range of forest types, from small woodlots in Indiana (Parker et al. 1985) to large expanses of the southern Appalachian Mountains (Lorimer 1993). The causes of the reproductive failure appear to be several (Lorimer 1993) and include changes in disturbance regimes, such as post–European settlement suppression of fire, which had served to keep both canopies and understories open and suitable for oak regeneration. Compounding these effects are elevated rates of herbivory and predation or ineffective dispersal of oak regeneration by deer and rodents (Frelich and Lorimer 1985; Goheen and Swihart 2003) and competition with invasive plant species (Huebner 2003).

It is unclear what effect these changes are having on oak genetic resources in the Midwestern United States, especially in the old-growth forests that have not been well studied genetically. This is despite the importance of the genus for timber and ecosystem function. Do the cohorts of the largest-size oaks in old-growth forest carry novel genetic variation that has not passed into the smaller size classes? Is the demographic structure of these stands likely to provide for the in situ maintenance of variation currently present in the old-growth stands?

A few studies have examined the internal organization of genetic variation in old-growth forests in other taxa, and several conclude that the abundance of contemporary regeneration is key to whether genetic variation is being lost. In some within-stand studies where abundant regeneration was present, no significant genetic differentiation was found between old and young trees (*Acer saccharum*: Fore et al. 1992; Simon et al. 1995; *Pinus taeda*: Roberds and Conkle 1984). In the absence of regeneration, partial harvest of old-growth can result in a loss of alleles (Buchert et al. 1997; Rajora et al. 2000), but stands that regenerate by natural means often show no significant reduction in genetic diversity relative to other old-growth stands (Gomory 1992; Neale 1985; Rajora 1999;

Rajora and Pluhar 2003; Schmidtling and Hipkins 1998). Replanted stands can contain less genetic diversity than old-growth stands (Gomory 1992; Rajora 1999) but can also display no detectable difference (Rajora and Pluhar 2003).

Here, we report on the genetic composition of red oaks in remnant old-growth forest in Indiana. We applied microsatellite markers to explore the internal (spatial and temporal) organization of the genetic diversity in two adjacent stands (20.6 ha and 2.6 ha in size) isolated during European settlement in the 1800s. Oaks are failing to regenerate in the large stand (Parker et al. 1985) but are rebounding in the small stand (Aldrich et al. 2005). We compare genetic diversity within and between stands, within and between size classes within a stand, and describe the fine-scale spatial genetic structure in the large stand.

Materials and Methods

Study Site and Sampling

The Davis-Purdue Research Forest (DPRF) is an old-growth deciduous hardwood forest located in east-central Indiana in Randolph County (SE 1/4 of section 23, Township 21N, Range 12E). Oaks had an importance value of 41.2% in the 1976 canopy, with roughly half of these red oaks (Parker and Leopold 1983; for site descriptions, see Parker et al. 1985). The establishment of agriculture on the property during the 1800s left four remnant stands, of which we studied the largest (20.6 ha, S_{20}) and the smallest (2.3 ha, S_2), separated by roughly 400 m. Purdue University acquired the property in 1917 and suppressed exogenous disturbances thereafter. Beginning in 1926, professor Burr N. Prentice (Purdue Department of Forestry) mapped, measured, and tagged all stems $\geq$ 4.0 inches (10.2 cm) diameter at breast height (dbh) in both stands (Prentice 1927). Later censuses included 1976 (large stand only), 1986–1987, 1992, and 1998.

Aldrich et al. (2003b) revealed only limited genetic differentiation among the three red oak (*Quercus* sect. *Lobatae*) species present at the site—namely, *Q. rubra* L. (northern red oak), *Q. shumardii* Buckl. (Shumard oak), and *Q. palustris* Muench. (pin oak). This is explained by overlap in morphological traits used to identify species, recent speciation, and extensive hybridization. Hybridization is thought to be common in the red oak group (Jensen 1995), and all pairwise hybrids between these three species have been described based on morphology (Laughlin 1963, 1964; Morsink and Pratt 1984; Palmer and Steyermark 1935). Consequently, we have treated the red oak community collectively.

For genotyping, we selected adult trees haphazardly from the small 2.3 ha stand (n = 62, or 37% of total 168 stems in the whole patch) and at a comparable sampling density (n = 96) from a 9.5 ha sampling plot falling within the northern half of the large 20.6 ha stand with roughly half of its area in core interior habitat and half in edge habitat. We collected cambium tissue from the trunk of adult trees using a chisel and hammer, placed samples on ice in the field, and froze them at Purdue University until DNA extraction.

Molecular Genetics

For DNA extractions, we ground sample tissue under liquid nitrogen with a wood pulverizer (CertiPrep 6750 Freezer/Mill, Spex) and then with a metal bead in an agitating rotor (FastPrep Bio101, Savant); details are in Aldrich et al. (2002). We adjusted template concentrations to 5–10 ng/μl for polymerase chain reactions.

Red oak microsatellite markers are described in Aldrich et al. (2002, 2003a), including primer sequences and recommended annealing temperatures. We examined their linkage relationships in an open pollinated mapping population (unpublished data) and selected a set of twelve that appear to be unlinked: red oak markers: *quru-GA-0A01*, *−0A03*, *−0C19*, *−0I21*, *−0M07*, *−1C06*, *−1C08*, *−1F02*, *−1G13*, *−1H14*, and *−2G07*, along with the European white oak locus *ssrQpZAG9* (Steinkellner et al. 1997).

We conducted polymerase chain reaction amplifications (25 μl volume) as follows: 1× *Ex Taq* Buffer (Panvera, proprietary except 2.0 mM $MgCl_2$), 100 μM dNTP each, 72 nm each forward and reverse primer, 0.01 U/μl Takara *Ex Taq* Polymerase (Panvera), and 0.2–0.4 ng/μl DNA. Polymerase chain reaction profiles were 94 °C, 1 min; 40–50 cycles of 94°C, 30 sec; T_a, 45 sec; 72 °C, 1.5 min; 72 °C, 10 min. We analyzed fragment sizes on an eight-capillary CEQ2000XL genotyper (Beckman Coulter).

Data Analysis

For the 158 total red oak stems genotyped in the study, we examined their diameter distribution in the full data set and identified three categories with balanced sample sizes within and across stands (Table 1; S = small, 10 cm $\leq$ dbh $<$ 28 cm; M = medium, 28 cm $\leq$ dbh $<$ 81 cm; and L = large, dbh $\geq$ 81 cm). In the large stand, we further divided the large size class red oaks into the "relict" trees that already were large (L_R, dbh $\geq$ 53 cm) by the 1926 census (and hence were likely to have been established prior to fragmentation) and those "nonrelict" trees that were smaller (L_{NR}, 10 $\leq$ dbh $<$ 53) in 1926.

Some of the red oak size classes were poorly stocked, with stems either entirely missing or rare (see Aldrich et al. 2005 for detailed demography of both stands). In the 9.5 ha plot of the large stand, no stems were in the juvenile class (dbh $<$ 10 cm), and adult stems in the small size class (S_{20}-S) were rare. In the small stand, only a few stems were in the large size class (S_2-L). Although we did not directly determine the genetic composition of these groups, we discuss here the implications of their near absence for the future gene pool.

In this article, we report levels of genetic diversity (mean values for the 12 loci) within each stand along with comparisons between size classes (cohorts) within each stand. Diversity measures included average number of alleles per locus (allelic richness, A_r), effective number of alleles per locus (A_e; Kimura and Crow 1964), observed heterozygosity (H_o), unbiased expected heterozygosity (H_e), and panmictic index (f; Weir and Cockerham 1984). We performed permutation tests (C++ program; Glaubitz et al. 2003) to test for significant differences in genetic diversity measures between

Table 1. Comparisons of levels of genetic diversity (based on 12 microsatellite loci) between size classes within a stand for the large stand (20.6 ha) and small stand (2.3 ha)

Stand	dbh class	n	n_g	A_r	A_e	H_o	H_e	f
Large stand								
S_{20}	All sizes	96	85.8	15.83	6.62	0.759	0.796	0.047
	S	—	—	—	—	—	—	—
	M	48	43.8	12.92	6.51	0.756	0.794	0.049
	L	48	41.9	13.58	6.28	0.762	0.798	0.045
	L_{NR}	26	23.8	11.58	5.92	0.752	0.795	0.055
	L_R	20	18.2	9.75	5.85	0.777	0.799	0.029
Small stand								
S_2	All sizes	62	49.2	12.00	5.97	0.783	0.794	0.014
	S	32	24.6	9.83	5.44	0.778	0.786	0.010
	M	30	24.6	10.17	5.76	0.784	0.797	0.017
	L	—	—	—	—	—	—	—

The following comparisons were made and found not significant ($P > .05$): S_{20}-M versus S_{20}-L; S_{20}- L_{NR} versus S_{20}- L_R; S_{20}-M versus S_{20}- L_R; S_2-S versus S_2-M. All measures of diversity were tested. Dashes (—) denote stems in poorly stocked size classes that were absent or rare.

dbh = stem diameter at breast height (cm) in 1998; n = number of stems genotyped; n_g = mean number of stems assayed per locus; A_r = mean number of alleles per locus (allelic richness); A_e = effective number of alleles per locus; H_o = observed heterozygosity; H_e = unbiased expected heterozygosity; f = panmictic index; S = small stem, $x < 28$; M = medium stem, $28 \leq x < 81$; L = large stem diameter, $x \geq 81$ cm; L_{NR} = large stem that was < 53 cm in 1926 census; L_R = large relict stem in 1998 that was ≥ 53 cm in the 1926 census.

P values (all > .05) for within-stand pairwise comparisons were obtained via permutation of individuals among the two size classes.

pairs of size classes. All diversity measures were tested in this manner. Observed differences were tested against distributions derived from 10,000 random permutations of individual multilocus genotypes among the two samples. In addition, the program GDA (Genetic Data Analysis; Lewis and Zaykin 2001) was used to estimate 95% confidence limits for all three F statistics at the stand level, based on 10,000 bootstrap resamples of loci. Also, to more fully examine potential associations between tree ages and the retention of rare alleles, we tested for an association between the mean number of rare alleles (frequency < 0.05 in full study) per locus per stem and the diameter of the tree (dbh, cm).

We also examined differentiation in allele frequencies between the two stands as a whole and between size classes (cohorts) within and between stands. This was done using the program GENEPOP 1.2 (Raymond and Rousset 1995a), accepting default settings for burn-in length, number of batches, and number of iterations per batch. For each test, GENEPOP calculated a global P value for an overall departure from null hypothesis expectations across loci using Fisher's method (Raymond and Rousset 1995b).

We used the program SPAGeDi 1.1 (Hardy and Vekemens 2002) to estimate F_{ST} (i.e., θ; method of Weir and Cockerham 1984; Weir 1996, chap. 5) for each pairwise comparison, testing the significance of each F_{ST} estimate via permutation of multilocus genotypes among the two samples in question (one-tailed test based on 20,000 permutations). At the stand level, the significances of f and F were also tested: f by permuting genes among individuals within stands and F by permuting genes among individuals in the total population.

Finally, we examined fine-scale spatial genetic structure within the large stand only (x-y coordinates were not available for the small stand). We used SPAGeDi to estimate relatedness (Hamilton 1964a,b) according to Queller and Goodnight (1989) between all possible pairs of genotyped trees within the large stand. As a parameter, relatedness (r) measures the expected proportion of allele sharing between two individuals as a result of identity by descent such that $r = 0.5$ for full sibs and $r = 0.25$ for half sibs. For every 20 m pairwise distance class, SPAGeDi computed an average of the pairwise estimates of r and then obtained 95% confidence intervals for these averages based on 20,000 permutations of multilocus genotypes among locations (x-y coordinates).

Results

Levels of Genetic Diversity

The oak populations displayed high levels of genetic diversity overall (Table 1), in each case exhibiting heterozygosities > 0.75 regardless of the stand or size class (not including the poorly stocked size classes). Levels of diversity were comparable in the two stands. Total inbreeding in the study was low ($F = 0.047$) as was stand-level inbreeding ($f = 0.034$) and differentiation ($\theta = 0.013$) according the GDA analysis. Inbreeding at individual loci in the study ranged from $f = -0.053$ to 0.172; null alleles may be present at the two loci where $f > 0.1$.

There were no significant differences ($P > .05$) in levels of genetic diversity between the well-stocked size classes within a stand. This held for all comparisons (S_{20}-M versus S_{20}-L; S_{20}- L_{NR} versus S_{20}- L_R; S_{20}-M versus S_{20}- L_R; S_2-S versus S_2-M). No association was found between the mean number of rare alleles (frequency < 0.05) per locus per stem and the stem dbh ($r = 0.042$).

Genetic Differentiation Between and Within Stands

We detected significant differences in allele frequencies between stands but not within stands (Table 2). All interstand comparisons of allele frequencies were significant for all of the size classes compared. There were no detectable differences in overall allele frequencies between well-stocked size classes within a stand according to the pairwise GENEPOP tests. This held even for the oldest cohort (the relict trees or S_{20}-L_R), which was not significantly differentiated from other well-stocked cohorts in the large stand. Identical results were obtained from analysis of pairwise F_{ST}.

Fine-Scale Spatial Genetic Structure Within the Large Stand

Average genetic relatedness was a decreasing function of distance class in the large stand (Figure 1). The average

Table 2. Tests of genetic differentiation across all 12 loci for all pairwise comparisons among size- and stand-specific cohorts

Comparison	X^2	(*P* value)	F_{ST}	(*P* value)
Between stands (S_{20} versus S_2)	Infinity	(.000)	0.0130	(0.000)
Within stand, between size class				
S_{20}-L versus S_{20}-M	20.2	(.685)	−0.0010	(.630)
S_2-M versus S_2-S	30.4	(.172)	0.0040	(.161)
Between stand, within size class				
S_{20}-M versus S_2-M	84.3	(.000)	0.0130	(.001)
Between stand, between size class				
S_{20}-L versus S_2-M	Infinity	(.000)	0.0110	(.002)
S_{20}-L versus S_2-S	63.4	(.000)	0.0090	(.003)
S_{20}-M versus S_2-S	97.7	(.000)	0.0140	(.001)
"Relict forest" versus other classes				
S_{20}-L_R versus S_{20}-L_{NR}	27.1	(.299)	−0.0010	(.512)
S_{20}-L_R versus S_{20}-M	28.0	(.261)	−0.0020	(.658)
S_{20}-L_R versus S_2-M	Infinity	(.000)	0.0116	(.017)
S_{20}-L_R versus S_2-S	53.4	(.001)	0.0116	(.012)

S_{20} = large stand; S_2 = small stand; L = large stem diameter in 1998, $x \geq 81$ cm; M = medium stem, $28 \leq x < 81$; S = small stem, $x < 28$; L_R = large relict stem in 1998 that was < 53 cm in the 1926 census; L_{NR} = large stem in 1998 that was < 53 cm in 1926 census.

Chi-square statistics and associated *P* values are from exact tests of genic differentiation (24 degrees of freedom for all tests). *P* values for the pairwise F_{ST} estimates were obtained via permutation of individual genotypes among the two samples being compared (one-tailed tests for positive F_{ST}).

relatedness estimate, *r* (Queller and Goodnight 1989), was significantly greater than zero for distance classes ≤ 80 m. The highest average relatedness value ($r = 0.079$, with $P = .0000$) was found for the shortest distance class (0–20 m), indicating that pairs of individuals within 20 m of each other have, on average, a level of relatedness slightly higher than that expected of half cousins (Blouin 2003). At distances ≥ 240 m, stems were, on average, no more genetically similar than expected of a random assemblage. Overall, we can convincingly reject the null hypothesis that geographic position carried no information on genetic relatedness. However, the low maximum average *r* estimate of 0.079 for the 0–20 m distance class suggests that parent-offspring clusters are heavily diluted with more distantly related individuals.

Discussion

These remnant old-growth red oak populations have retained high levels of genetic variation despite the genetic bottlenecks that seem to have accompanied European settlement of the area. For example, 87% of Indiana was forested in 1816 (Petty and Jackson 1966) whereas today much of northern Indiana is used for agriculture with occasional, spatially isolated second-growth and less frequent old-growth stands (Spetich et al. 1997). In addition to the bottleneck in numbers resulting from the initial harvests, the process of removing the biggest and best trees (i.e., high-grading) from the forest remnants continues (McGuire et al. 1999; Nyland 1992), potentially further depleting the gene pool of important

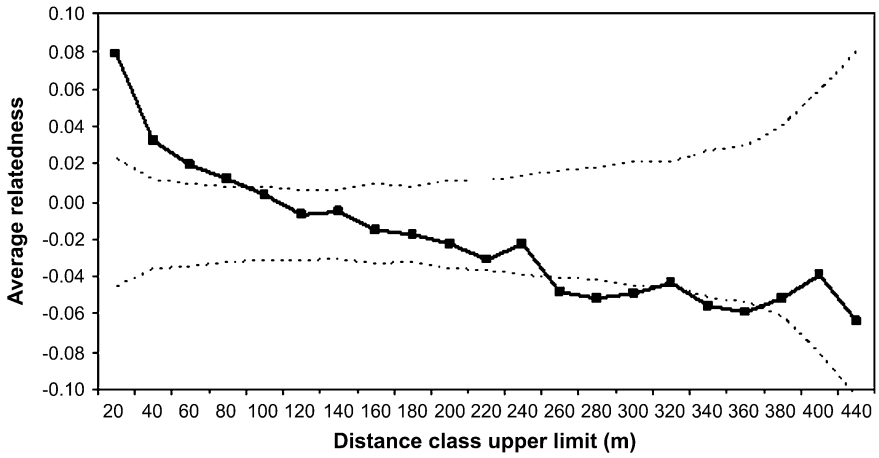

Figure 1. Fine-scale spatial genetic structure within the 9.5 ha plot of the large stand (20.6 ha, S_{20}) evaluated using the average relatedness coefficient (Queller and Goodnight 1989) for all pairs of trees within each 20 m distance class. Dotted lines indicate 95% confidence limits under the null hypothesis (based on 20,000 permutations of spatial locations among individuals).

Table 3. Fine-scale spatial genetic structure in oak populations (*Quercus*)

Species	Stand history	Structure	Intensity	Distance	Reference
Q. acutissima	No human disturbance	None	—	—	Chung et al. 2002
Q. acutissima	Disturbed	IBD	Weak	< 50 m	Chung et al. 2002
Q. chrysolepis	Mature	Clonal, IBD	Strong, weak	< 10 (4) m	Montalvo et al. 1997
Q. laevis	Old-growth	Clonal, IBD	Weak, weak	< 5 m, < 10 m	Berg and Hamrick 1994
Q. macrocarpa	Mature	IBD	Weak	< 10 m	Geburek and Tripp-Knowles 1994
Q. petraea	Ancient, native	IBD	Weak	< 80 m	Cottrell et al. 2003
Q. petraea	Natural regeneration	IBD	Moderate	< 50 m	Bacilieri et al. 1994; Streiff et al. 1998
Q. petraea	Replanted	IBD	Strong	< 80 m	Cottrell et al. 2003
Q. robur	Ancient, native	IBD	Weak	80 m	Cottrell et al. 2003
Q. robur	Natural regeneration	IBD	Weak	30 m	Bacilieri et al. 1994, Streiff et al. 1998
Q. robur	Replanted	IBD	Moderate	< 160 m	Cottrell et al. 2003
Q. rubra	Ecological reserve	IBD	Weak	< 5 m	Sork et al. 1993
Q. rubra[a]	Old-growth (DPRF)	IBD	Weak	< 80 m	This study
Q. variabilis	No human disturbance	None	—	—	Chung and Chung 2002

IBD = isolation by distance.

[a] Includes pure and hybrid forms of *Q. rubra*, *Q. shumardii*, and *Q. palustris*.

variation. Although we cannot speak to any genetic diversity already purged from the DPRF stands, the remaining trees are highly diverse genetically, and the younger cohorts that are well stocked are still genetically representative of trees that had existed during settlement of the area. Because nonindustrial private forest owners control almost 60% of the forested land in the United States (Smith et al. 2001), wise management of these remnant woodlots should be included in a stewardship plan for the red oak gene pool.

The small observed genetic differentiation between the two stands can be explained by genetic structure that existed before fragmentation of the forest in the 1800s. Interstand genetic differentiation ($\theta = 0.013$), though statistically significant, was low, and it fell within other interstand estimates published for *Q. rubra* ($G_{st} = 0.009$, Schwarzmann and Gerhold 1991; $F_{ST} = 0.092$, Sork et al. 1993). The presettlement forest may have been composed of genetic patches of familial substructure that, upon fragmentation, were transformed into significant though slight genetic differences among stands.

It is unlikely that postfragmentation genetic drift associated with small effective breeding populations has played a prominent role in the differentiation of the stands. Life expectancy of red oaks is about 100 to 150 years with peak reproduction occurring sometime between 25 and 50 years (Burns and Honkala 1990). Given the average diameter growth rate for red oaks at the DPRF (0.444 cm/year), the average-size red oak that we genotyped (small stand, mean 31.7 cm; large stand, 81.5 cm) established over 70 and 180 years ago in the small and large stands, respectively. Thus, only one or two generations have accrued in the large stand since the European settlers fragmented this landscape and only slightly more generations in the small stand, providing little opportunity for the erosion of genetic diversity by random drift.

Compared to the oaks in nine other published studies (Table 3), the red oaks in the large DPRF stand displayed typical patterns of fine-scale spatial genetic structure. Oak populations usually exhibit weak genetic correlations between near-neighbor stems, typically within 50 m (< 80 m at the DPRF), though reports ranged from 0 to 160 m. Spatial genetic structure is negligible and at times negative beyond these distances. These patterns are consistent with the isolation by distance (IBD) genetic structure predicted by Wright (1943, 1946) to accrue in a continuous population with limited dispersal of progeny from parents, though several other factors can generate and erode spatial genetic structure in oak populations and thus deserve consideration.

Additional factors beyond limited primary dispersal from parents may have contributed to the fine-scale IBD genetic structure at the DPRF. First, the constituent species of a hybrid swarm could contribute to spatial genetic correlations within a stand if the species are distributed nonrandomly, though we detected only a weak association between genetic relatedness and species identity (Mantel test, $p = 0.1556$). Second, differential survivorship of genotypes across habitats can produce spatial genetic correlations within a stand (Epperson 1990b), and it is known that *Q. palustris* is tolerant of wet soils, whereas *Q. rubra* prefers well-drained soils (Burns and Honkala 1990). Third, some oaks exhibit clonal genetic structure (Berg and Hamrick 1994), though the oak species at the DPRF are not known for this, despite their ability to resprout from stumps (Burns and Honkala 1990).

Fourth, episodic recruitment following disturbance could introduce spatial genetic structure if several progeny of relatively few trees recruit into a canopy opening or if stands are replanted using few acorn sources. Stands whose oaks are not regenerating will gradually loose their spatial aggregation (Aldrich et al. 2003c) and should lose their IBD genetic structure through random attrition (Hamrick et al. 1993). Our literature review (Table 3) confirmed these expectations, that oaks in old-growth stands generally exhibit less pronounced IBD genetic structure than that found in naturally regenerated or replanted stands.

Finally, secondary dispersal by animals deserves additional consideration as a source of IBD genetic structure.

Secondary dispersal might diminish family structure unless seeds are transported together in a fruit or to a cache or larder. Darley-Hill and Johnson (1981) reported that blue jays (*Cyanositta cristata*) moved 54% of pin oak (*Q. palustris*) acorns over a kilometer on average, which should dissipate IBD genetic structure except that acorns were moved in groups of one to five (mean of 2.2) per foraging bout. Squirrels are another important agent of oak dispersal. The North American red squirrel (*Tamiasciurus hudsonicus*) is replacing the native gray squirrel (*Sciurus carolinensis*) in many parts of its range. Differences in their foraging (described by Goheen and Swihart 2003) will likely become manifest as differences in red oak genetic structure. Gray squirrels are scatter-hoarders, dispersing few acorns to each of many sites, which should reduce the amount of IBD genetic structure in a stand. Red squirrels are larder-hoarders, dispersing numerous acorns to each of few sites. The codispersal of sibs would tend to increase IBD genetic structure.

As for pollination, evidence suggests that the DPRF breeding populations of red oaks have remained large, judged here indirectly and only for the well-stocked classes. We found little evidence of elevated inbreeding (positive f) that might arise through biased fecundity distributions or consanguineous matings (*Q. rubra* is considered an obligate outcrosser; Schwarzmann and Gerhold 1991). We did not observe the pattern of decreasing heterozygote deficit (decreasing f) with age that would be expected had there been selection over time against inbred individuals (Mitton et al. 1997). Inbreeding as measured by heterozygote deficit was slightly greater in the large stand ($f = 0.047$) than in the small stand ($f = 0.014$) but, according to bootstrap tests, was not significantly different from zero overall. Ecological evidence also suggests a large breeding population, as the stands still contain numerous large red oaks, many of which regularly produce acorns in great numbers (Aldrich, personal observation).

Lack of genetic differentiation among size classes within a stand indicates that much of the genetic variation present in the oldest red oaks has passed successfully between generations. Studies of old-growth pine (*Pinus*) and maple (*Acer*) have found similarly (*Pinus ponderosa*: Linhart et al. 1981; *Pinus taeda*: Roberds and Conkle 1984; *Acer saccharum*: Fore et al. 1992; Simon et al. 1995). If oaks on adjacent farms have contributed heavily to the smaller DPRF size classes, then they must have essentially the same genetic composition as the large DPRF red oaks.

One aspect that distinguishes our findings from these studies of pine and maple is the reproductive failure of the oaks and the ensuing implications for the genetic variation. For example, maples are shade tolerant and generally able to thrive following canopy closure arising from cessation of forest disturbances. In fact, the intrusion of maples into the subcanopy and eventually the canopy of the DPRF appears to be a primary factor in the decline of the oaks, which are less tolerant of shade (Aldrich et al. 2003c, 2005; Parker et al. 1985). Aldrich et al. (2005) surveyed red oaks in the center of the large stand in 2001 and found none smaller than 10 cm diameter, even though the nearby small stand contained 98 stems (42.4 stems/ha) in this size class. Combined with the rarity of small adults (dbh < 28 cm), red oaks in the core of the large stand are experiencing the incipient stages of a severe genetic bottleneck.

There is little evidence of the demographic failure abating in the core habitat of the large stand (Aldrich et al. 2005). Although the genetic diversity in the old-growth oaks has been passed on to the medium-size trees in the same stand, the local breeding pool may become depleted once the currently medium- and large-size trees senesce. In the absence of natural disturbance or management intervention, the genetic erosion currently evident in the juvenile and small adult size classes is likely to propagate into the canopy classes. And while it is known that oak pollen can move large distances (Streiff et al. 1999), recent improvements in estimation of pollen dispersal (Austerlitz and Smouse 2001a,b; Smouse et al. 2001) show that the local breeding pool is often dominated by relatively few oaks, on the order of four or five individuals (Sork et al. 2002), suggesting that random drift may be more important in oak populations than previously thought. Further reductions in pollen donor density at the DPRF could lower acorn production, as was seen in blue oak (*Q. douglasii*; Knapp et al. 2001), which would compound the problems already evident from low survivorship of small stems in the shaded core habitat.

Moreover, the core habitat gene pool composition is increasingly dependent on input from oaks in other habitats, reminiscent of a source-sink (Pulliam 1988). These sites differ in genetic composition to a lesser (small DPRF stand) or greater degree (presumably other, more distal woodlots), and high rates of gene flow into the core may eventually displace the relict variation currently maintained in the canopy classes. The DPRF sites that would likely serve as a genetic source for the core habitat are mostly edge habitat—namely, the extreme margins of the large stand and the small stand, which is itself mostly edge habitat. Forest edges often experience greater environmental stochasticity and lower adult survivorship rates (reviewed in Murcia 1995); note the rarity of large red oaks in the small DPRF stand. Thus, the core habitat is likely to be supported by a shifting genetic base, and it is unlikely that future generations of DPRF oaks will retain the present genetic character. The same scenario (increasing dependence on volatile edge habitat) is likely being played out in numerous remnant oak stands throughout the Midwest.

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