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## Landscape genetic structure of bur oak (*Quercus macrocarpa*) savannas in Illinois

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### Abstract

We examined population genetic structure among 14 stands of *Quercus macrocarpa* Mich. (Fagaceae), bur oak, in northeastern and central Illinois using five microsatellite loci. We chose *Q. macrocarpa* because its scattered, pre-settlement distribution in the prairie/savanna landscape of this region allows us to examine the effects of long-term population fragmentation on a wind-pollinated tree species. A pairwise comparison of Wright's  $F_{ST}$  among all pairs of stands showed small but significant differentiation among most stand pairs. However, a Bayesian clustering approach showed no underlying genetic structure. An analysis of molecular variance (AMOVA) showed that over 97% of genetic variation occurred within the stands, while less than 3% occurred among stands. A Mantel test showed no evidence for isolation-by-distance. Therefore, levels of population differentiation among the study stands are low and are not correlated to distance. Despite a dispersed and fragmented distribution that persisted for several thousand years, the savanna bur oaks of this region appear to comprise a single genetic population. The lack of population genetic structure is most likely due to high levels of pollen-mediated gene flow in this species. Our findings suggest that wind-pollinated trees may be quite resilient to the negative genetic consequences of habitat fragmentation.

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### 1. Introduction

Gene flow is a major determinant of genetic structure among populations of sexually reproducing plants. Evolutionary processes, such as genetic drift and migration, along with ecological factors, such as pollen and seed dispersal, contribute to the development of genetic structure within and among populations. When populations become fragmented, for example via anthropogenic disturbance, gene flow may be disrupted, leading to loss of genetic diversity, increased genetic drift within fragments, and increased genetic differentiation among fragments. Ultimately, habitat fragmentation could lead to reproductive isolation, poor recruitment, and reduced fitness through inbreeding or pollen limitation (Young et al., 1996; Lowe et al., 2005; Ghazoul, 2005). Nevertheless, the empirical evidence for the dire genetic consequences of forest fragmentation predicted

from population genetics theory has been mixed. For example, a recent review of the genetic consequences of habitat loss of tropical trees reported that the majority of studies found no significant impact on genetic diversity in fragmented versus control populations, and results for increased genetic differentiation and inbreeding were equivocal (Lowe et al., 2005). In an extensive review of allee effects and plant spacing, Ghazoul (2005) reports negative effects on pollination and reproductive output as a result of fragmentation for a majority of tree species studied, but cites several studies where reduced patch sizes actually result in increased pollination success or seed set (Aizen and Feinsinger, 1994; Dick et al., 2003). Ghazoul (2005) also suggests that there has been a strong bias against reporting results showing non-significant responses to fragmentation, although this bias appears to be declining in recent years.

Fragmentation studies that test for negative genetic effects of fragmentation often fail to find them (e.g. Foré et al., 1992; Young et al., 1993; Latouche-Halle et al., 2004; White et al., 2002; Dick et al., 2003). One reason may be the temporal scale of sampling, especially for long-lived species like trees. In most cases, fragmentation has occurred within the last century, and

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many adult trees sampled were present prior to fragmentation, or are at most one or two generations post-fragmentation. It is not surprising, therefore, that similar levels of allelic richness and gene diversity are found in fragmented versus continuous forest (Céspedes et al., 2003; Dick, 2001; White et al., 1999; Young et al., 1993). To examine *long-term* consequences of habitat loss and fragmentation, it would be extremely useful to identify and study surrogate species whose natural distributions resemble a fragmented landscape, with relatively small groups of individuals that are widely dispersed amidst a large matrix of alternative intervening habitat.

Here, we examine population genetic structure in a wind-pollinated, North American oak species, *Quercus macrocarpa* (bur oak). This species offers a unique opportunity to study the long-term effects of habitat fragmentation in a wind-pollinated tree species. Oak savanna and prairie communities were established in this region 5000 years ago, when the post-glacial climatic conditions became comparatively warm and dry, and were maintained by periodic drought and fire until European settlement (Abrams, 1992). The Public Land Survey (PLS) conducted in this region from the 1820's to the 1840's has been used to reconstruct vegetation patterns at the time of European settlement (Bourdo, 1956; Rogers and Anderson, 1979; Bowles et al., 1994; McBride and Bowles, 2001). GIS mapping of PLS notes for approximately 3000 square miles in five of the Illinois counties included in our study has been completed (Bowles et al., 1994; McBride and Bowles, 2001; McBride, unpublished mss). At the time of European settlement, approximately 80% of the landscape was largely treeless prairie, with areas of timber making up less than 20% of the total (McBride and Bowles, 2001). Bur oak is the most fire resistant oak, with deep roots and thick, corky bark (Abrams, 1990, 1992), and was found in areas with the lowest tree densities where fires were most frequent. Rivers served as fire breaks, and continuous forests were confined to their eastern banks and were dominated by other tree species, including red oak (*Quercus rubra*), sugar maple (*Acer saccharum*), and hickory (*Carya* sp.) (Bowles et al., 1994). Thus pre-settlement bur oaks were found at low densities and in isolated patches, separated by a vast expanse of prairie. Bur oak trees over 150 years old established prior to European settlement still occur, therefore, the opportunity exists to characterize pre-settlement population structure.

Previous work on *Q. macrocarpa* using genetic markers and paternity analysis in a single stand (McHenry County, Illinois) showed that this relatively isolated stand received large amounts of pollen from outside sources (Dow and Ashley, 1996, 1998a,b). In this stand, over half of the acorns sampled had been pollinated by trees outside the stand, from distances greater than 150 m away (Dow and Ashley, 1996). Nevertheless, it was not known whether pollen dispersal distances were great enough to effect gene flow across the large distances that separated stands of savanna bur oak in this region.

In this study we explored the landscape genetic structure of *Q. macrocarpa* by examining levels of population differentiation among 14 stands of *Q. macrocarpa* in northeastern and central Illinois. The genetic structure we observe for these pre-settlement stands will allow us to infer historic levels of gene flow across this

region, look for evidence of isolation-by-distance or other spatial structuring of populations, and to compare findings based on local pollination studies (Dow and Ashley, 1996, 1998a,b) to the landscape level genetic patterns examined in this study. The landscape genetic structure of *Q. macrocarpa* in the prairie savanna remnants of this region will reflect the effects of long-term isolation of small stands, and help predict what the genetic effects of habitat fragmentation might be for other wind-pollinated temperate trees over many generations.

## 2. Materials and methods

### 2.1. Study species

*Quercus macrocarpa* Michx., bur oak, is a long-lived, monoecious, wind-pollinated North American tree species with a wide distribution, including most of the northeastern U.S. Although it occurs in mixed forests, it commonly occurs in isolated, single-species stands. In Illinois, *Q. macrocarpa* is most often associated with oak savannas and prairie remnants. Whereas prairies and oak savannas covered much of Illinois' landscape in pre-settlement times, these ecosystems have largely been lost in the past 150 years as the land was converted to agriculture and other anthropogenic disturbances ensued. A few bur oak stands were left intact, and because bur oaks can live over 350 years, trees established prior to European settlement can be found today.

### 2.2. Study sites and sampling

Adult trees were sampled from 14 stands in northeastern and central Illinois (Table 1; Fig. 1). The stands were selected to include stands with varying of degrees of isolation. They included isolated, single-species patches; isolated, mixed species patches; and mixed forests (Table 1). The isolated, mixed species stands did not have other white oak species present and are designated as "isolated stands" in Table 1. Distances between stands ranged from 1.29 km (Urbana Park and Crystal Lake) to 252.75 km (Urbana Park and Mary Beebe Center).

Several stands were pre-settlement remnants (Mary Beebe Center, Royal Grove, Sibley Grove, and Gerth's Grove) composed solely of trees 150–300 years old (Dow and Ashley, 1998a; Gleason, 1913; LaGesse et al., 1998; McClain et al., 1998). Individuals sampled in the remaining stands included a mix of pre-settlement and post-settlement trees, but all sampled trees represented naturally regenerated, mature trees (average DBH 67.55 cm). Twenty to 30 individuals were randomly sampled from each stand. Additionally, two stands were exhaustively sampled (Burnham Prairie and Goose Lake Prairie) and 115 trees were sampled in third stand (Cranberry Slough), as these three sites were part of another study (Craft and Ashley, 2006, submitted for publication). Four or five mature or newly erupted leaves were sampled per tree. All leaves were initially stored on ice then stored at  $-80^{\circ}\text{C}$  until DNA extraction was performed. DBH measurements and GPS coordinates were taken for each tree. GPS coordinates were used to determine distances among the study sites.

Table 1  
Location and type of study sites

Study site	Label	Latitude and longitude	Location (County)	Population type
Burnham Prairie Nature Preserve	B.P.	41.64N 87.54W	Cook	Isolated stand
Burton Woods Forest Preserve	B.W.	41.84N 88.38W	Kane	Mixed forest
Cranberry Slough Forest Preserve	C.S.	41.72N 87.86W	Cook	Mixed forest
Crystal Lake Park	C.L.	40.12N 88.20W	Champaign	Isolated stand
Gerth's Grove	G.G.	40.71N 88.31W	Livingston	Isolated stand
Goose Lake Prairie Natural Area	G.L.	41.35N 88.30W	Grundy	Isolated stand
Lone Grove Forest Preserve	L.G.	41.82N 88.56W	Kane	Mixed forest
Mary Beebe Girl Scout Center	M.B.	42.38N 88.54W	McHenry	Mixed forest
Middlefork Savanna Nature Preserve	M.S.	42.25N 87.88W	Lake	Isolated stand near mixed forest
The Morton Arboretum	M.A.	41.82N 88.05W	DuPage	Mixed forest
Royal Grove	R.G.	40.21N 87.97W	Champaign	Isolated stand
Sibley Grove Nature Preserve	S.G.	40.58N 88.37W	Ford	Isolated stand
Turtle Pond	T.P.	40.68N 88.29W	Livingston	Isolated stand
Urbana Park	U.P.	40.12N 88.20W	Champaign	Isolated stand

### 2.3. DNA genotyping

Approximately 1 g of leaf material was ground to a fine powder in liquid nitrogen. Extractions were performed according to Keim et al. (1989), followed by additional

purification using the Qiagen DNA Mini Kit. This second step was performed to remove contaminants such as tannins, pigments, and other polymerase chain reaction inhibitors. DNA was stored in sterile, deionized water and diluted to approximately 0.2–0.4  $\mu\text{g}/\mu\text{l}$ . Five microsatellite primers were

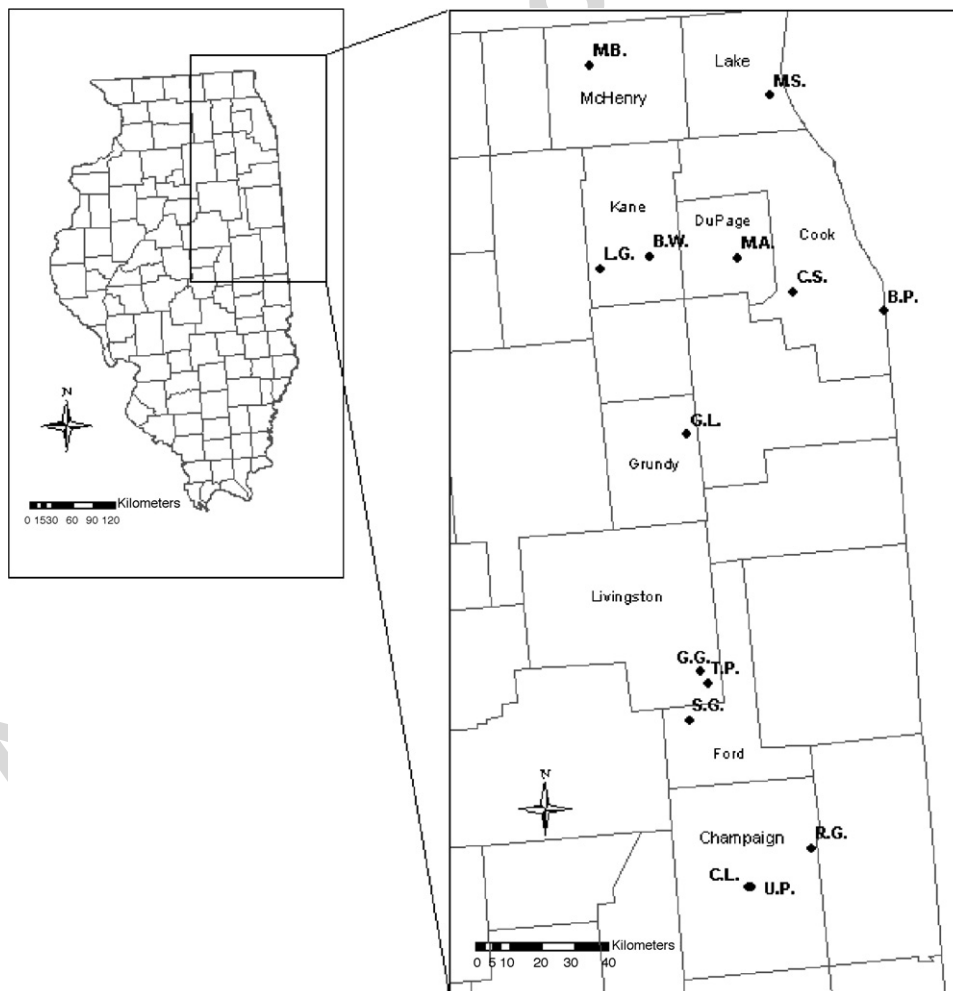


Fig. 1. Site locations in Illinois. See Table 1 for abbreviations and further descriptions.

used for this study. Three of these primers, MSQ13, MSQ4, and MSQ16, were developed for *Q. macrocarpa* by Dow et al. (1995) and two, QpZAG9 and QpZAG110, were developed for *Q. petraea* by Steinkellner et al. (1997). Polymerase chain reactions (PCR) were performed using 0.2–0.4 µg genomic DNA, 100 µM dNTP (Perkin-Elmer), 0.15–0.6 µM primer, 2.0–3.0 mM MgCl<sub>2</sub>, 1.0 µg/µl bovine serum albumin, PCR buffer (Promega), and 0.04 µl Taq polymerase (Promega). One primer of each pair was end-labeled with a fluorescent tag (Cy-3, Fam, or Tet) (IDT DNA Technologies). Polymerase chain reactions consisted of a five min 94 °C preheat followed by 38 cycles of 94 °C denaturation for 30 s, 50–54 °C annealing for 30 s, 72 °C extension for 30 s, and one 72 °C extension for 5 min. PCR products were then mixed with a fluorescent size standard, ROX 350 (Genescan), and run on an MJ BaseStation automated sequencer (MJ Research, Massachusetts, U.S.A.). All microsatellite alleles were scored using Cartographer software (MJ Research).

#### 2.4. Genetic analysis

Expected and observed heterozygosities were generated using GENEPOP (Raymond and Rousset, 1995). Additionally, we used GENEPOP to implement the private allele method for estimating the number of migrants per generation (Slatkin, 1985a,b); to test for deviations from Hardy–Weinberg equilibrium among the stands; to estimate the genic differentiation, or the homogeneity of allele distribution, among the 14 stands; and to estimate Weir and Cockerham (1984) estimator of genetic differentiation,  $\theta$ .

Genetic differentiation among stands was calculated using Wright's pairwise  $F$ -statistic (Goudet, 2001). Significance was tested for all pairwise  $F$ -statistics after a standard Bonferroni correction. Analysis of molecular variance (AMOVA) was also performed using the program Arlequin (Schneider et al., 2000) to assess the distribution of molecular variance within and among the stands. Significance of these values was tested using a non-parametric permutation according to Excoffier et al. (1992).

Mantel tests were performed using Arlequin software (Schneider et al., 2000) to assess isolation-by-distance by testing the correlation between a matrix of geographic distances with a matrix of genetic distances. The matrix of geographic distances was constructed by converting GPS coordinates of latitude and longitude to distances in kilometers between all pairs of stands.

To test for underlying genetic structure among the stands that may not be detected using classical population genetic approaches, a Bayesian approach described by Pritchard et al. (2000) was used, implemented by the program *structure*. An advantage of the method employed by *structure* is that it is an individual-based analysis that can reveal cryptic population structure, such as unrecognized barriers to dispersal. This program employs a model-based clustering approach, which uses a Markov-Chain Monte Carlo (MCMC) method for assigning individuals to genetic clusters ( $K$  groups) based on their multilocus genotype. The model assumes there are  $K$

populations, defined by the user, each with a characteristic set of allele frequencies at each locus. The model then compares individual genotypes within the data set and assigns genotypes to  $K$  groups, assuming Hardy–Weinberg equilibrium within each cluster, while simultaneously estimating population allele frequencies per locus. These clusters are generated with minimal within-cluster linkage disequilibrium and each genotype may exist simultaneously in more than one genetic cluster if admixture is indicated. To infer the number of genetic clusters, we conducted 14 independent runs of the Gibbs sampler, using a burn-in period of 40,000 runs and collected data for 10<sup>6</sup> iterations. These 14 runs represented 14 possible populations:  $K=1$ , one genetic cluster,  $K=2$  two genetic clusters, etc. All individuals were assigned to  $K$  populations ( $1 \leq K \leq 14$ ) based solely on their multilocus genotype. We ran the data using no information regarding population identification (USEPOPINFO = 0).

### 3. Results

Consistent with previous studies (Dow et al., 1995), *Q. macrocarpa* showed high levels of microsatellite variation (Table 2). All loci were highly polymorphic, with 7–17 alleles per locus and a mean expected heterozygosity of 0.864 (Table 2). The mean observed heterozygosity was 0.917, which revealed a small but statistically significant heterozygote excess. This slight heterozygote excess has been found in other oak species (Craft et al., 2002; Dutech et al., 2005) and may be due to the high degree of outcrossing observed in oaks. Private alleles were rare. Half the stands had no private alleles, while the other stands had one or two (Table 2). The mean frequency of private alleles was 0.0356, resulting in an estimate of 2.8 migrants per generation (Slatkin, 1985a,b; Barton and Slatkin, 1986). However, the presence of private alleles may be a sampling artifact, i.e., these alleles may occur in other stands, but were not sampled because they occur at low frequencies. Weir and Cockerham's (1984) estimator of genetic differentiation,  $\theta$ , averaged across stands is shown in Table 3. The global  $\theta$  across all loci and stands was 0.0271, indicating very little genetic structure among the stands. The pairwise comparisons of  $\theta$  among stands showed small but significant differences after Bonferroni correction for 80 of the 91 pairwise comparisons (Table 4).

AMOVA analysis showed that 97.31% of the genetic variation is contained within the stands, while only 2.69% occurred among stands (Table 5). The global fixation index,  $F_{ST}$ , was 0.0269, which was highly significant ( $P < 0.001$ ) and consistent with the estimation of  $\theta$  given by our analysis of genetic differentiation using GENEPOP. A Mantel (1967) test of isolation-by-distance showed no significant relationship between genetic and geographic distance ( $P = 0.469$ ), with a correlation coefficient of  $r = 0.00232$ .

The Bayesian clustering approach implemented in *structure* yielded estimated ln probabilities for  $1 \leq K \leq 14$  ranging from –11367.5 to –23319.1, with the highest posterior probability given for  $K=1$  ( $\Pr(X|K) = 1$ ). Additionally, assignment of individuals to putative clusters was highly symmetrical,

Table 2

Number of alleles, expected ( $H_e$ ) and observed ( $H_o$ ) heterozygosities for five microsatellite loci in 14 stands of bur oak

Site name	$N$	QpZAG9	QpZAG110	MSQ13	MSQ4	MSQ16	Private alleles	$H_e$	$H_o$
Burnham Prairie	62	16	9	17	13	16	2	0.870	0.914
Burton Woods	25	9	8	10	10	10	0	0.848	0.968
Cranberry Slough	115	16	9	11	13	15	0	0.838	0.858
Crystal Lake Park	27	13	10	10	14	9	0	0.853	0.911
Gerth's Grove	30	13	11	13	15	11	2	0.893	0.973
Goose Lake Prairie	26	12	8	10	7	14	1	0.841	0.892
Lone Grove	25	11	10	10	13	13	2	0.872	0.936
Mary Beebe Center	20	9	8	9	12	11	0	0.859	0.970
Middlefork Savanna	30	13	9	14	14	11	1	0.878	0.933
Morton Arboretum	20	8	9	9	13	12	1	0.886	0.869
Royal Grove	20	12	10	11	11	10	1	0.871	0.890
Sibley Grove	25	8	8	10	10	11	0	0.845	0.936
Turtle Pond	30	11	11	10	11	12	0	0.875	0.900
Urbana Park	25	13	10	9	12	13	0	0.869	0.888
Mean	34.30	11.70	9.29	10.93	12.00	12.00	0.714	0.864	0.917

Table 3

Weir and Cockerham's (1984) estimator of genetic differentiation,  $\theta$ , at each locus

Locus	$\theta$
QpZAG9	0.0202
QpZAG110	0.0183
MSQ13	0.0466
MSQ4	0.0370
MSQ16	0.0132
Global $\theta$	0.0271

Table 5

Analysis of molecular variance, AMOVA

Source of variation	d.f.	S.S.	Variance	% Total	$P$	Fixation index, $F_{ST}$
Among populations	13	77.987	0.0588	2.69	<0.001	0.0269
Within populations	946	2010.587	2.125	97.31		
Total	959	2088.574	2.184			

d.f., degrees of freedom; S.S., sum of squares;  $P$ , significance of fixation index;  $F_{ST}$ , fixation index averaged across loci and populations.

indicating little or no genetic structure. Therefore, no underlying genetic structure was detected despite the small, but significant,  $\theta$  values found between several of the stands.

#### 4. Discussion

Genetic variation in *Q. macrocarpa* was high, as evidenced by the high heterozygosity and allelic diversity found across all stands (Table 2). The five microsatellite loci used were highly

polymorphic and provided good resolution for estimating levels of gene flow and population structure in the study stands. Using standard population genetic approaches, the global fixation index,  $F_{ST} = 0.0269$ , indicated little population differentiation overall. Given the low overall levels of  $F_{ST}$ , it is somewhat surprising that all but 11 of the 91 pairwise comparisons of  $\theta$  among stands showed small but significant differences after Bonferroni correction (Table 4). Inspection of the pairwise comparisons indicates no obvious spatial patterning. For

Table 4

Comparison of Wright's  $F_{ST}$  between all pairs of study sites (upper diagonal) and geographic distance in kilometers between all pairs of study sites (lower diagonal)

	MB	BW	CL	GG	LG	MS	MA	RG	SG	UP	TP	BP	GL	CS
MB	0	0.0222*	0.0195*	0.0279*	0.0395*	0.0127*	0.0193*	0.0238*	0.0135	0.0259*	0.0304*	0.0333*	0.0666*	0.0338*
BW	60.82	0	0.0266*	0.0211*	0.0295*	0.0119	0.0182*	0.0272*	0.0219*	0.0359*	0.0249*	0.0214*	0.0455*	0.0275*
CL	252.45	192.18	0	0.0214*	0.0519*	0.0082	0.0241*	0.0083	0.0183*	0.0213*	0.0187*	0.0251*	0.0493*	0.0236*
GG	186.30	126.19	66.18	0	0.0188	0.0092	0.0081	0.0264*	0.0176*	0.0254*	0.0296*	0.0245*	0.0359*	0.0250*
LG	61.67	16.02	191.63	125.48	0	0.0267	0.0032	0.0470*	0.0354*	0.0469*	0.0462*	0.0412*	0.0506*	0.0380*
MS	55.87	61.13	238.92	175.45	74.13	0	0.0114	0.0137*	0.0123	0.0188*	0.0125*	0.0218*	0.0364*	0.0125*
MA	73.65	26.82	189.78	125.63	42.56	50.03	0	0.0324*	0.0205*	0.0320*	0.0376*	0.0328*	0.0472*	0.0332*
RG	248.93	188.15	21.76	65.82	189.41	230.77	182.78	0	0.0295*	0.0154*	0.0189*	0.0168*	0.0525*	0.0299*
SG	200.01	140.24	53.14	14.87	138.84	190.24	140.37	56.18	0	0.0293*	0.0341*	0.0293*	0.0481*	0.0267*
UP	252.75	192.44	1.29	66.52	191.99	238.94	189.86	20.60	53.63	0	0.0191*	0.0198*	0.0455*	0.0431*
TP	190.05	129.85	62.40	4.06	129.33	178.57	128.83	61.75	12.43	62.71	0	0.0160*	0.0339*	0.0330*
BP	116.29	73.05	178.84	122.69	87.74	73.57	47.20	166.90	137.17	178.59	124.75	0	0.0282*	0.0271*
GL	114.71	54.21	137.97	72.05	55.44	106.19	56.39	134.37	86.27	138.23	75.66	73.01	0	0.0307*
CS	92.40	44.78	179.87	118.06	59.51	59.88	19.69	171.03	132.93	179.83	120.86	28.29	55.32	0

Asterisks (\*) denote statistical significance after a Bonferroni correction.

example,  $\theta$  was not significantly different between the Mary Beebe Center and Sibley Grove, 200.01 km apart, yet was significantly different between the two closest stands, Crystal Lake and Urbana Park, 1.29 km apart (Table 4). Thus, although most stands were slightly differentiated, this did not appear to be the result of geographic structuring. Hedrick (1999) cautions that the statistical resolution provided by highly variable markers such as microsatellites may yield measurements of differentiation ( $F_{ST}$  values) that are statistically significant but not biologically relevant. This may be the case in our study as  $F_{ST}$  was the only analysis that provided evidence for significant population structure.

All other analyses conducted suggested that *Q. macrocarpa* represents a single population over large spatial scales, and historical levels of gene flow have been very high despite the highly fragmented distribution of stands found in the prairie/savanna region. For example, the Bayesian clustering approach implemented by *structure* (Pritchard et al., 2000), which searches for underlying genetic structure among the stands, found the highest posterior probability for a single genetic cluster. The Bayesian approach assigns individuals to genetic clusters based solely on their multilocus genotype without including information regarding population of origin. In addition to the Bayesian results, a Mantel test of isolation-by-distance showed no correlation between genetic and geographic distance among the stands ( $r = 0.0023$ ;  $P = 0.469$ ), while an AMOVA revealed that only 2.69% of genetic variation was partitioned among the stands. These results indicate very little genetic structure among the stands and that geographic restrictions to gene flow have not occurred as a result of long-term fragmented distributions of savanna oaks. Our sample included trees that were either established prior to European settlement, or were at most one to two generations post-settlement. Thus we can suggest that prior to European settlement, the bur oaks of this region comprised a single population, although they likely occupied less than 5% of the landscape (McBride and Bowles, 2001). Indeed, they may still represent a single regional population connected by pollen flow.

Until recently, gene flow in trees has usually been deduced by using indirect genetic methods that infer historical patterns of gene flow based on current population genetic structure (e.g. Mitton, 1994), as we have done here. Rarely have local studies of pollen movement using paternity assignments been available to “ground-truth” the estimates of gene flow based on indirect methods. In the case of Illinois bur oaks, however, pollination patterns have been characterized at the level of single stands (Dow and Ashley, 1996, 1998a,b; Craft and Ashley, submitted for publication). These studies show that the majority of pollen comes from external sources (at distances of  $>150$  m), and that pollen movement within the stand is nearly random, predicting that little genetic structure should exist at the landscape level, and populations over fairly large areas would be reproductively connected via regional “pollen clouds”. These local pollination studies measure current pollination patterns, while the indirect methods used here infer historical patterns of gene flow. Because many of the trees used in our study were of pre-settlement age,

we can conclude that the lack of genetic differentiation among our study stands is due to historical inter-stand pollen flow on a landscape scale, and that these patterns of pollen flow are ongoing today. Thus we have linked local and landscape patterns as well as contemporary and historical patterns of gene flow for this species.

The evidence for long distance pollen movement in the genus *Quercus* is now quite substantial (Streff et al., 1999; Chung et al., 2005; Dodd and Afzal-Rafi, 2004; Muir et al., 2004). It therefore appears unlikely that habitat fragmentation in this genus will result in reproductive isolation. It is thus somewhat surprising that Sork et al. (2002) conclude that increasing isolation and limited pollen flow are threatening species such as *Quercus lobata*. However, their study employed a model called TWOGENER rather than direct measures of pollination patterns using paternity analysis. TWOGENER has been reported to underestimate pollen dispersal distances and numbers of pollen donors (Dutech et al., 2005; Smouse and Sork, 2004; Craft and Ashley, submitted for publication). As Koenig and Ashley (2003) point out, the studies using TWOGENER to date involve relatively small sample sizes and have not yet been conducted on a larger scale or over several seasons. Additionally, TWOGENER has not yet been “ground-truthed” by direct paternity analysis of many parent-offspring pairs.

Our results, together with a growing number of studies indicating high levels of pollen movement in temperate trees (Burczyk and Chybicki, 2004; Foré et al., 1992; Schuster and Mitton, 2000), suggest that among the many issues facing conservation biologists, *reproductive isolation* of fragmented stands may not be of great concern. Spatially isolated stands of wind-pollinated trees will likely not be reproductively isolated or suffer losses of genetic diversity. However, the possibility of pollen limitation in isolated stands has not been ruled out. Two separate issues are involved, genetic diversity of pollen and abundance of pollen. The pollen received by trees in isolated stands may be genetically diverse and represent many pollen donors, but the overall amount of pollen received may be less than that received by trees in larger or denser stands. This could explain the findings of Knapp et al. (2001) that acorn production was correlated with the number of nearby flowering cohorts for *Quercus douglasii*. More studies comparing seed set with density or isolation of trees, combined perhaps with pollen supplementation studies, would shed light on relationships between stand size, density, isolation and pollen limitation.

Our results suggest some species of wind-pollinated trees may be resilient to the negative genetic consequences of habitat fragmentation. Concerns regarding the dire genetic consequences that will result from forest fragmentation rely on the assumption that forest fragments delineate populations. If, in fact, pollen or seed dispersal curves do not conform to the boundaries of forest fragments, these concerns may be exaggerated. However, oak savannas are a globally threatened ecosystem and occur on just 0.02% of their pre-settlement extent in the Midwestern United States (Nuzzo, 1986). Thus, we certainly do not suggest diminishing current conservation efforts.

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