CHANGING GENETIC STRUCTURE OF A SAVANNA BUR OAK POPULATION

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ABSTRACT

Genetic relatedness among adult bur oaks and among saplings in a remnant savanna in Illinois was compared using data from four microsatellite loci for evidence of changes in genetic structure. Relatedness among all adults in the stand was close to zero (0.005) while that among all saplings was substantially higher (0.075). When the stand was divided into spatial groups of trees, relatedness of adults within those spatial groups was always slightly negative (indicating lower relatedness than average) while spatial groups of saplings had significantly higher relatedness coefficients of about 0.10. Comparisons of individual microsatellite genotypes indicated that no more than 19% of adult trees are likely to be the offspring of trees that are currently alive in the stand, but 94% of the saplings have a parent in the stand. Possible sib relationships among adults were tested by spatial group, inferring hypothetical maternal genotypes that would maximize the number of half-sibs. All results suggested that unlike the saplings, the adults in the stand do not represent clusters of offspring from a few seed parents and are largely unrelated. Although saplings will thin and new seedlings may establish as the adults in the stand die, it is unlikely that these processes will be sufficient to prevent a change in the genetic structure of the stand in the future. Secondary growth stands, where trees are more densely spaced than in native savanna, may already reflect these changes.

Key words: *Quercus macrocarpa,* relatedness coefficient, microsatellites, genetic structure, seedling recruitment

INTRODUCTION

The population structure of many North American tree species changed profoundly following European settlement. Temperate savannas were dramatically altered by fragmentation and fire suppression as settlers cleared land for lumber and agriculture. One species native to Illinois savannas, the bur oak (*Quercus macrocarpa*), is now found in both presettlement remnant savannas and in dense, second growth stands. Presettlement trees, now well over a hundred years old, are still found in forest preserves, parks, pastures and residential areas. Many of these mature trees may be reaching the end of their reproductive life, but still offer the opportunity to collect information on presettlement genetic structure, and to compare current recruitment patterns from those that occurred prior to human disturbance. Here we apply DNA microsatellites to characterize the genetic structure of a remnant bur oak savanna across two generations, adult trees and established saplings. Although previous studies have compared structure across life stages in trees (LINHART *et al.* 1981; YAZDANI *et al.* 1985; FORÉ *et al.* 1992; BERG & HAMRICK 1995; ALVAREZ-BUYLLA *et al.* 1996; EPPERSON & ALVAREZ-BUYLLA 1997), ours is the first to employ microsatellites to examine relatedness of individuals in different generations. Microsatellite loci, comprised of short, tandem DNA repeats, exhibit codominant, Mendelian inheritance, have extremely high levels of variability, and are likely to be selectively neutral. They offer a much higher level of resolution for inferring relatedness than allozymes (BRUFORD & WAYNE 1993; ASHLEY & DOW 1994). The study described here focuses on the mature trees in the stand, and compares these findings to those previously reported for the saplings (DOW & ASHLEY 1996).

Like many temperate deciduous trees, bur oaks are wind pollinated, monoecious and nut-bearing. Because of the similarity of oaks to other temperate trees, our results may have general implications for regeneration of temperate trees. Information on recruitment of savanna oaks is of particular conservation interest because many savanna oaks, notably in California, are failing to regenerate (*e.g.* WHITE 1966; GRIFFIN 1976; MOMEN *et al.* 1994; HOLMES 1995). Regeneration of bur oaks in the Midwest may also be of concern. In Green County in southern Wisconsin, at a site about 80 km from ours, the decline of oak species has been

documented and previously abundant bur oaks occur now only in woodlands derived from presettlement savanna (SHARPE *et al.* 1987).

Our study site consisted of a relatively isolated stand of mature bur oaks and saplings. We have previously developed and applied microsatellite genotyping (DOW *et al.* 1995) to all the adults bur oaks, many saplings, and a large sample of acorns to study seedling establishment (DOW & ASHLEY 1996), pollination patterns (DOW & ASHLEY 1998a), and male reproductive success (DOW & ASHLEY 1998b). In our study of sapling establishment, we found that only four adult trees were seed parents to 80% of the saplings, and recruitment of about half of the current sapling generation had occurred as clusters of half-siblings around a seed parent (DOW & ASHLEY 1996). As a result, join-count spatial autocorrelation analysis revealed more clustering of microsatellite alleles in the sapling generation (eight of 40 alleles) than in the adult generation (one of 36 alleles). This suggested that the genetic structure of the stand may be in transition due to changes in recruitment patterns, and that a regenerated forest may come to consist of spatial clusters of first degree relatives. This led us to conduct the analysis described here, which examines in greater detail that genetic structure of the adults in the stand and compares it to the sapling generation. First, we calculate and compare relatedness coefficients (QUELLER & GOODNIGHT 1989) within and among groups of trees. Second, we identify all possible parent/offspring pairs among the adults in the stand by looking for pairs of adult trees that matched one microsatellite allele at each of four loci. Finally, we looked for siblings (half or full) among the adults in the stand. This final analysis is complicated by the fact that identification of siblings using genetic data is more difficult than assigning parentage. Siblings may by chance share neither allele at a locus, having inherited alternate alleles from each parent. Half-sibs will share, on average, at least 25% of their alleles and full sibs, on average 50%, but a sibling relationship cannot be excluded even between individuals that share zero alleles, although the probability of this happening decreases as the number of loci scored increases. We therefore used an approach to look for half-sibs among the adults by dividing the stand into spatial groups, then inferring hypothetical maternal genotypes that would maximize the number of trees that could be half-sibs in each cluster. This approach takes into account the fact that while half-sibs must always share half their alleles with the maternal parent, they may not share any alleles with each other. We also examined adults in clusters of three, because the maximum number of alleles at any locus for three half-sibs is five (*i.e.*, at any locus two of three half sibs must share one allele). Taken together these analyses provided unbiased estimates of relatedness in adults and saplings based on allele sharing and quantified the maximum number of first degree relatives among adults based on composite microsatellite genotypes.

MATERIALS AND METHODS

The study site was a stand of 62 mature bur oaks (*Quercus macrocarpa* Michx.) and 16 red oaks (*Quercus rubra* L.) located on an abandoned farm in McHenry County near Harvard, Illinois. The adults and saplings in the stand were mapped (Fig. 1), trunk diameter at breast height (DBH) measured, leaf samples collected and DNA extraction and analysis performed as described previously (DOW *et al.* 1995). Nearest neighbor distances were determined for adult trees and saplings. All adults and 100 (of 140 total) randomly chosen saplings were genotyped at four microsatellite loci as previously described (DOW *et al.* 1995; DOW & ASHLEY 1996). Null alleles, which did not produce a visible PCR product, were found at two loci, MSQ3 and MSQ4. All individuals that were apparently homozygous for either of these two loci were assumed to be heterozygous for the null allele. This method prevents the exclusion of any true match, but will overestimate the number of matches (DOW & ASHLEY 1996).

For several of the genetic analyses, the trees in the stand were divided into five groups (Fig. 1) following natural breaks in their distribution. Each group included at least one cluster of adult trees with crowns that were adjacent or overlapping. More distant trees nearby were also included, as seed dispersal can exceed 150 m (DOW & ASHLEY 1996). The dimensions of the five groups (north-south × east-west) were 80 m × 90 m., 47.5 m × 60 m., 85 m × 55 m., 107.5 m × 75 m and 65 m × 122.5 m for Groups 1 through 5, respectively. The distance covered by each group is similar to the largest sibling cluster found in the sapling study (around Tree 17, Fig. 1), which had a north-south distance of 80.4 m (DOW & ASHLEY 1996). Saplings were only found in Groups 1 and 2.

Relatedness

In order to quantify and compare the relatedness of adults and saplings based on shared alleles, we analyzed genotype data for both groups using *Relatedness* 4.2c, which uses the algorithm described by QUELLER and GOODNIGHT (1989). The relatedness coefficient, R, calculated by this program is an unbiased estimate of the proportion of shared alleles within a group compared to the proportion of shared alleles in the general population. A positive R value indicates greater

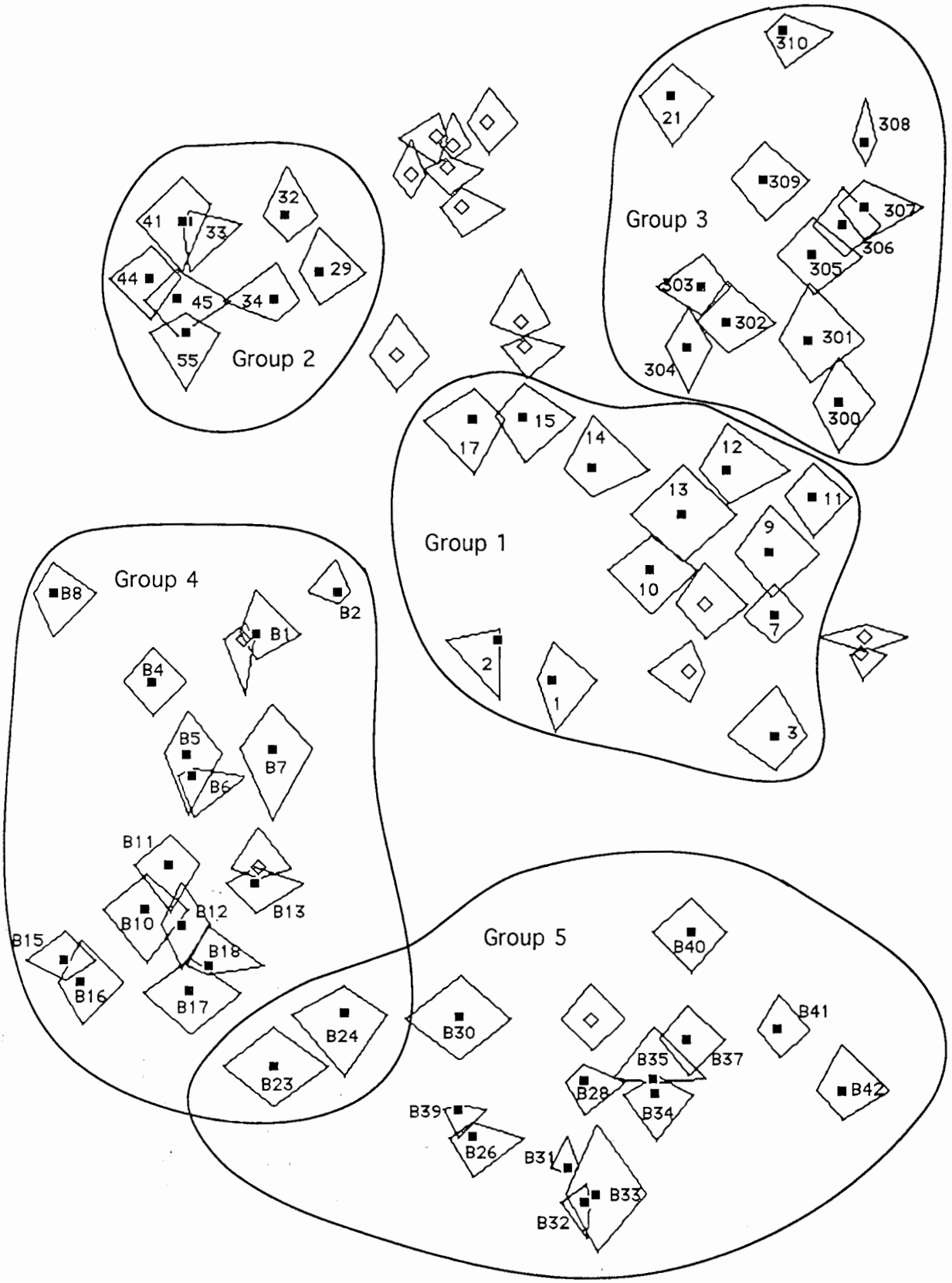

Figure 1. Map of the study site indicating tree numbers and groups used in analysis. Closed squares are bur oaks; open diamonds are red oaks. North is at the top of the figure. Dimensions of each group are given in the text.

Table 1. Relatedness values of groups of saplings and adults. *R* = relatedness; SE = jackknife estimate of standard error, *N* = sample size (numbers of known half-sibs in small sapling groups are in parentheses). 80% confidence intervals were calculated as ± (1.282) (SE).

Half-sibs	*R*	SE	*N*	80% conf. int.
Offspring of Tree 2	0.188	0.053	17	0.120–0.256
Offspring of Tree 3	0.249	0.038	19	0.200–0.298
Offspring of Tree 17	0.168	0.037	10	0.121–0.215
Offspring of Tree 35	0.281	0.099	26	0.154–0.408
Saplings by Group (as shown in Figure 1)				
Group 1	0.089	0.052	59	0.022–0.156
Group 2	0.109	0.030	41	0.071–0.147
Adults by Group (as shown in Figure 1)				
Group 1	–0.005	0.019	12	–0.030–0.019
Group 2	–0.001	0.031	9	–0.040–0.039
Group 3	–0.007	0.023	12	–0.040–0.022
Group 4	–0.002	0.013	17	–0.020–0.015
Group 5	–0.005	0.020	13	–0.030–0.021
All adults	0.005	0.017	63	–0.020–0.027
All saplings	0.075	0.044	100	0.019–0.131
Adults to saplings by Group				
Group 1	0.046	0.024	12, 59	0.015–0.077
Group 2	–0.007	0.020	9, 41	0.030–0.019

relatedness within the group than is present in the population as a whole. Clusters of previously identified half-sib saplings (*i.e.*, individuals that matched the same adult tree) were used to evaluate the performance of the program on our data. Relatedness among the adults was analyzed over the spatial groups described above and among saplings in Groups 1 and 2 (there were no saplings in the other groups). Relatedness was also calculated over all saplings and all adults, as well as between saplings and adults in Groups 1 and 2. Standard errors for each relatedness calculation were estimated by jackknifing (QUELLER & GOODNIGHT 1989). Relatedness values for adults and saplings on the same spatial scale were compared with a Mann-Whitney U test.

Parent/offspring pairs among adults

If one individual is a parent of another individual, the two trees must have one allele in common at every locus. All possible haplotypes (*i.e.* every combination of one allele at each locus) of each adult were entered into a database, and identical haplotypes were grouped together (DOW & ASHLEY 1996). If haplotypes of two individuals matched at all loci, a possible parent/offspring dyad was identified. Such dyads were not necessarily parent and offspring, as such a match might also occur by chance. The probability of a match occurring by chance rather than relatedness (probability of random match or PRM) was calculated for each dyad based on allele frequencies in the stand (DOW & ASHLEY 1996). A PRM of 0.10, for example, indicates that the matching alleles were relatively rare in the population and that the probability that the match was due to chance rather than relatedness was less than 10%. A low PRM is not conclusive evidence of a parent/offspring relationship, since other relatedness, such as between siblings, may also result in shared alleles at all loci, although the probability is lower.

Half-sib relationships among adults

In a previous study of sapling groups, we found that at each locus, there were two alleles that occurred at high frequencies (DOW & ASHLEY 1996). These high frequency alleles matched the genotype of a nearby tree, which was consistent with the interpretation that the saplings represented a cluster of half-sibs around their seed parent. If the adults in the stand were also arranged in clusters of half-sibs, we would expect that

Table 2. Example of inferred maternal genotypes of Group 1 (see Figure 1, trees 1–17). For each of 4 loci (MSQx), alleles are given in the left column and the individuals with those alleles are listed to the right. The composite genotype of the inferred seed parent is shown at the bottom along with trees matching at least one allele per locus. Individuals matching the seed parent at all four loci are inferred half-siblings. This example shows one of several possible genotypes which were examined. No other genotype was found to be consistent with more than three half-sibs in the group. * = inferred maternal allele, H = homozygous individuals.

MSQ3					
203	9				
207	13				
209	11				
211	14				
213	2				
217	1	13			
219*	7	10	12	14	15
221*	3	7	9	17	
223	10				
225	11				
227	12	17			
NULL 1	1	2	3	15	
MSQ4					
202	17				
203*	12	2	10		
204*	3	9	15		
205	7				
206	3	15			
207	1	12			
209	13	7			
211	13				
215	11				
217	1				
219	14	10	2		
225	17				
NULL 9	9	14	11		

MSQ13					
228	3				
232*	9H	12	14H	15	
234*	2	7	10H	11	
236	13H				
238	1	2	12	17	
240	11	17			
242	1	3	7	15	
MSQ16					
182	10				
183	14				
185	2	3H	12	13H	17
187	1	12			
189*	7	10	15		
191	14				
193	11H	2			
199*	1	7	9H		
207	15				
209	17				

Inferred seed parent										
MSQ3 219/221		3	7	9	10		12	14	15	17
MSQ4 203/204	2	3		9	10		12		15	
MSQ13 232/234	2		7	9	10	11	12	14	15	
MSQ16 189/199	1		7	9	10				15	

spatial groups of adult trees would have some alleles that occurred at high frequency. Genotype data were used to infer a hypothetical genotype of a maternal tree of a half-sib cluster.

All the alleles in each of the five groups were listed (an example is given in Table 2), then beginning with the most common alleles at each locus, we inferred a maternal genotype that would maximize the number of trees that could be included in the half-sib group. This analysis does not prove that these trees are indeed half-sibs, but provides a maximum possible number of half-sibs in each group, and, perhaps more importantly, the number of trees per group that cannot be half-sibs.

Microsatellite genotypes were also used to test whether specific groups of three trees could be half-siblings. Because two half-siblings do not necessarily have to share any alleles, there is no way to exclude two trees as being half-siblings. However, when the number increases to three, two of three half-sibs must share one allele at any locus because only two alleles could have come from the common maternal tree. In other words, if at any locus three trees have six different alleles, those three trees could not possibly be half-sibs. Clusters of three trees growing close together that were not identified as possible half-sibs in the analysis

Table 3. Trees with matching haplotypes (designated by Tree 1 and Tree 2), their respective diameters at breast height (DBH), the difference in DBH, the distance between the two trunks and the probability of random match (PRM).

Tree 1	DBH 1 (cm)	Tree 2	DBH 2 (cm)	DBH2-DBH1	Distance (m)	PRM
34	72	B16	88	16	152	0.05
34	72	B17	96	24	149	0.07
B23	87	B33	112	25	74	0.08
B15	74	13	101	27	163	0.10
307	89	B17	95	6	222	0.10
B12	82	308	85	3	223	0.10
306	81	12	94	13	59	0.11
34	72	B23	87	16	164	0.12
B34	86	B8	108	22	167	0.15
B40	90	55	97	7	168	0.15
B10	81	B17	95	14	20	0.20
306	81	45	82	1	144	0.22
33	79	B1	84	5	89	0.26
B35	76	306	81	5	188	0.27
306	81	307	89	8	6	0.27
B35	76	307	89	13	193	0.27
B18	79	B17	95	16	7	0.30
7	66	307	89	23	90	0.30
45	82	307	89	7	149	0.30
300	86	305	87	1	33	0.34
B32	41	B10	81	40	112	0.34

of the large groups were tested for half-sib relationships.

RESULTS

As reported previously (DOW & ASHLEY 1996), the mean nearest neighbor distance among adult trees in the stand was 12.4 m (SD = 6.7). The wide spacing and open-grown architecture (horizontal branching) of these trees suggest that the stand is a savanna remnant. The average trunk diameter of adult trees was 88.7 cm (SD = 15.1), and the range was 40.7 cm to 125.7 cm. The saplings in this stand had a mean nearest neighbor distance of 1.7 m (SD = 3.0 m.). The relatively large standard deviation reflects a wide range of nearest neighbor distances, from 20 cm to 24 m. Ninety-five of 140 saplings (68%) were tall enough for trunk diameter to be measured. For these trees, mean diameter was 3.3 cm (SD = 1.7) and the range was 0.7 cm to 8.3 cm.

Relatedness

The *Relatedness* 4.2c program (QUELLER & GOODNIGHT 1989) performed well on the groups of previously identified half-sibs, particularly for the larger sample sizes (Table 1). The expected value for half-sibs is 0.25, and in three of the four groups, this number was included in the 80% confidence interval. The offspring of Tree 17 had a low relatedness value (0.168), possibly because we had data for only 10 individuals and four loci. The offspring of Tree 35 had a relatedness of 0.281, probably because this tree was homozygous for one locus, and therefore all offspring shared that allele. Relatedness calculated over all adults was 0.005, and over all saplings was 0.075, indicating that the saplings are much more closely related to each other than are the adults in the stand.

When spatial groups of saplings and adults were examined (Fig. 1), the relatedness of saplings was an order of magnitude greater than the relatedness of the adults (Table 1) and this difference was statistically significant (Mann-Whitney U test, $p < 0.05$). All of the adult groups had relatedness values very near zero whereas the two groups of saplings had values close to 0.1. In comparisons of adults to saplings within spatial groups (Table 1), relatedness coefficients were relatively low, although the value was larger in Group 1 (0.046) than in Group 2 (–0.007). Low relatedness values between adults and saplings is likely a result of a disproportionate contribution of a few maternal trees which have outcrossed widely with trees outside the stand, as has been suggested previously (DOW & ASHLEY 1996). Group 1 included groups of half-sibs around Trees 2, 3 and 17, while Group 2 included only the large half sib group around Tree 35. Because many

Table 4. Genotypes of hypothetical seed parents of five clusters of mature trees and trees that match the genotype at each locus. Possible half-siblings are shown in bold. See Table 1 for further explanation..

Group 1 (1-17)										
Inferred seed parent										
MSQ3 219/221			3	7	**9**	**10**		12	14	**15**
MSQ4 203/204		2	3		**9**	**10**		12		**15**
MSQ13 232/234		2		7	**9**	**10**	11	12	14	**15**
MSQ16 189/199	1			7	**9**	**10**				**15**
Group 2 (29-55)										
Inferred seed parent										
MSQ4 203/221		**32**	**33**	**34**		**41**	44	45		
MSQ13 234/236		**32**	**33**	**34**		**41**	44	45		
MSQ3 213/219		**32**	**33**	**34**		**41**		45	55	
MSQ16 185/189	29	**32**	**33**	**34**	35	**41**	44		55	
Group 3 (21, 300-310)										
Inferred seed parent, 1 of 2										
MSQ13 232/234	**300**	**301**		304	**305**	306	307	308		
MSQ4 203/205	**300**	**301**	302		**305**	306	307	308	309	**310**
MSQ3 203/221	**300**	**301**		304	**305**					**310**
MSQ16 185/195	**300**	**301**		304	**305**				309	**310**
Inferred seed parent, 2 of 2										
MSQ13 232/234	**300**	301		304	**305**	**306**	**307**	308		310
MSQ4 203/205	**300**	301	302		**305**	**306**	**307**	308	309	310
MSQ3 219/221	**300**				**305**	**306**	**307**		309	
MSQ16 185/191	**300**			304	**305**	**306**	**307**			310

Group 4 (B1-B24)																	
Inferred seed parent																	
MSQ13 232/234	B1	B2	B4		**B6**	B7	B8	B10	B11	B12	**B13**		B16	**B17**	**B18**	**B23**	
	B1			B5	**B6**		B8	B10			**B13**	B15		**B17**	**B18**	**B23**	B24
MSQ4 204/221			B4	B5	**B6**			B10			**B13**	B15	B16	**B17**	**B18**	**B23**	
MSQ3 207/215	B1		B4		**B6**	B7	B8				**B13**	B15		**B17**	**B18**	**B23**	
MSQ16 185/189																	

Group 5 (B23-B42)															
Inferred seed parent, 1 of 2															
MSQ13 323/242	B23	**B24**	**B26**	**B28**	B30		B32	B33	B34	B35			**B41**		
MSQ4 204/205		**B24**	**B26**	**B28**	B30				B34			B40	**B41**	B42	
MSQ3 215/229	B23	**B24**	**B26**	**B28**		B31	B32	B33			B37	B39	**B41**	B42	
MSQ16 185/191		**B24**	**B26**	**B28**		B31	B32			B35		B39	B40	**B41**	B42
Inferred seed parent, 2 of 2															
MSQ13 323/242	B23	**B24**	B26	**B28**	B30		**B32**	B33	B34	B35			**B41**		
MSQ4 204/205		**B24**		**B28**	B30		**B32**			B35	B37		B40	**B41**	
MSQ3 215/229	B23	**B24**	B26	**B28**		B31	**B32**	B33			B37	B39		**B41**	B42
MSQ16 185/191		**B24**	B26	**B28**		B31	**B32**			B35		B39	B40	**B41**	B42

adults did not contribute alleles to the saplings in Group 2, relatedness was therefore lower in this group.

Parent/offspring pairs

Of 794 total haplotype combinations, 21 were shared by two or more trees (Table 3). No trees matched all alleles at every locus, indicating that there was no evidence of clonal reproduction, as in stump sprouting. Of the 21 shared haplotypes, 9 had PRMs higher than 0.25, suggesting that these genotypes had common alleles and may have matched by chance rather than because they are related. Therefore, only 12 of 62 living trees in the stand have a greater than 75% probability of matching by relatedness. Although tree diameter cannot be used to accurately estimate age in old oaks, these data are presented in Table 3 to give relative sizes of the trees with matching haplotypes.

The distances among potential parent/offspring pairs are generally quite large. Fourteen of the 21 possible pairs with matching haplotypes (61%) were separated by distances of 90 m or more. Only two (B18 with B17 and 306 with 307) were closer than 15 m. This result contrasts sharply with the structure of the saplings in the stand, where 69% of saplings were found within 15 m of their maternal tree, and only 9% were found at distances greater than 90 m (DOW & ASHLEY 1996).

Half-sibs among adults

The genotypes for the inferred seed parents and their possible offspring are shown in Table 4. In order to be half-sibs, trees must have one of the maternal alleles at every locus. For Group 1, MSQ4 showed a fairly even distribution of alleles, which suggests that there were not a large number of half-sibs among these trees (Table 2). The maximum number of possible half-sibs in this group is three, Trees 9, 10 and 15. Trees 9 and 10 are 26 m apart, and Tree 15 is 61 m and 43 m from 9 and 10, respectively. Four groups of three neighboring trees within Group 1 were considered for possible half-sib relationships. These groups were 14, 15 and 17; 10, 12 and 13; 9, 11 and 12; 9, 12 and 13, and 7, 9 and 11. Of these small groups, only 7, 9 and 11 could be half-sibs, and only if 9 and 11 are heterozygous for a null allele at locus MSQ4.

The maximum number of half-sibs in Groups 2 was four, Trees 32, 33, 34 and 41. These trees are found close together (< 25 m) in the stand, particularly Trees 33 and 41, which have trunks about 1 m apart. The proximity of these proposed half-sibs is consistent with previous findings of sapling clusters around maternal trees (DOW & ASHLEY 1996). Interestingly, allele 213 of MSQ3 is fairly rare in the population as a whole, but is found in 4 of 9 trees in this cluster. Similarly, MSQ4-221 is rare, but is shared by 3 of 9 trees. When subgroups of three trees are considered, 41 and 33 could be half-sibs with 44. Three other groups (29, 32, 34; 34, 45, 55; 34, 44, 45) cannot be half-sibs. Trees 44, 45 and 55 can only be half-sibs if a null allele is inferred at locus MSQ3.

Group 3 has fairly even allele distributions. There were two possible seed parent genotypes that would include four trees in the half-sib group (Table 4). Of these two, the second shows more of a clustering pattern in the inferred half-sibs, since 305, 306 and 307 are separated by less than 10 m. Although the semi-circular spacing of 300, 301, 305, 306 and 307 suggests that these five trees may have been produced by one tree in the middle of them, at least one of these trees is not a half-sib to the others (*e.g.* 301). There is no inferred maternal genotype that can match all five. Although 302, 303 and 304 are less the 15 m from each other, at least one is not a half-sib to the other two. No other subgroups were considered in this group, as there were no other trees growing closely together.

Group 4 had a possible maximum of five half-sibs, Trees B6, B13, B17, B18 and B23. As mentioned previously, B17 and B18 shared half of their alleles, which may indicate a parent/offspring relationship. Eight subgroups of three trees were examined for possible half-sib relationships (4, 5, 6; 5, 6, 7; 10, 12, 18; 11, 12, 18; 10, 11, 12; 15, 16, 10; 15, 16, 17; 17, 23, 24). Of these two could represent half-sib groups (4, 5, 6 and 17, 23, 24), and one (10, 11, 12) could be half-sibs if a null allele is inferred.

There were two inferred maternal genotypes in Group 5 that included four possible half-sibs, although three of the four (B26, B28 and B41) were the same in both groups. The fourth tree was B24 for one genotype and B32 in the other. These trees were fairly scattered in the stand, being separated by 26 to 56 m. Unlike Group 3, no argument can be made in preference to one inferred genotype over the other based on proximity of trees. Trees 31, 32 and 33 could be half-sibs if a null allele is inferred, as could trees 23, 24 and 30. Three other subgroups (34, 35, 37; 28, 34, 35; 39, 26, 31) cannot be half-sibs.

Overall, there were potential half-sibs pairs in each group. Maternal genotypes could be inferred that would make it possible for between three and five of the trees in each group to be half-sibs. The best case can be made for the cluster of adults in Group 2 to represent a cluster of half-sibs that sprouted, without seed dispersal, from a maternal tree that is now dead. None of the other potential half-sib pairs show this type of clustering, which is the pattern that is currently in the sapling generation (DOW & ASHLEY 1996). It seems clear from both the parent/offspring analysis and the sib analysis that the genetic structure of the adults in the stand does not reflect a few seed parents with clusters of offspring.

DISCUSSION

Analysis of microsatellite genotypes and allele sharing among adults and saplings in a remnant savanna stand of bur oaks suggest that the genetic structure of these two groups is strikingly different. Adults were found to be largely unrelated, both by relatedness coefficients and by comparisons of individual genotypes. Levels of relatedness among adults, overall or in spatial groups, was close to zero. Genotype comparisons identified relatively few parent/offspring pairs among the adults; at most 21 adults (33%) could be the offspring of trees currently present in the stand, and this number decreases to 12 (19%) if matches above 25% PRM are excluded. We also failed to find large clusters of half-sibs among the adults in the stand, although there are potential half-sib groups scattered throughout the stand. In many instances, trees growing close together could not be half-sibs. In cases of potential parent/offspring and half-sib pairs among adults, the individuals are generally widely spaced, therefore one individual of the pair must have grown from a dispersed acorn.

These findings for adults are in sharp contrast with the saplings in the stand. Relatedness among groups of saplings was significantly higher than among groups of adults. We previously reported that 94% of the saplings had at least one parent in the stand (DOW & ASHLEY 1996) and most of the saplings in the stand represent maternally related half-sibs from only four adult trees in the stand. For juveniles in the stand, at least half appear to have grown from acorns that were not dispersed but sprouted where they fell under or near the canopy of the maternal tree.

In any plant species with limited seed dispersal, genetic correlations among progeny clustered near their maternal parents might be expected (LIBBY *et al.* 1969; EPPERSON 1992). In species where progeny occur at much higher densities than parents, the correlations may disappear as the plants mature and are thinned out. For example, in an allozyme study of the tropical tree *Cecropia obtusifolia*, genetic variation of seedlings showed significant spatial autocorrelations, but juveniles and adults exhibited nearly random spatial distributions (EPPERSON & ALVAREZ-BUYLLA 1997). However, our study included only established saplings well past the filter of thinning from the seedling to juvenile stage. It is likely that a substantial proportion of our saplings will survive to become reproductive. Furthermore, second growth stands are often much denser; there is a second growth stand of bur oaks near our study site where the trees are more closely spaced (mean nearest neighbor distance 4.0 m compared to 12.4 m for the adults in the study site). Thinning of the saplings will not likely be sufficient for the genetic structure resulting from patterns of half-sib recruitment to disappear. Undoubtedly, new groups of saplings will become established as mature trees die and light gaps open over time. However, recruitment may continue to occur in clusters of maternally related half-sibs.

It is important to note that we are not presenting evidence for loss of genetic variability or genetic differentiation between the adult and sapling generation. In fact, there were more alleles present among saplings than among adults in the stand, and allele frequencies between the two generation are not significantly different (DOW & ASHLEY 1996). Rather, we are inferring that changes in fine-scale genetic structure across generations is likely due to differences in regeneration dynamics before and after settlement. Related studies on trees using allozymes to examine fine-scale genetic structure necessarily rely on statistical clustering of alleles through methods such as spatial autocorrelation, whereas the microsatellite data allowed us to actually test for first-order relatedness among individuals. Nevertheless, at least two allozyme studies have reported results analogous to ours, finding differing spatial genetic structure in populations of the same species established under different regeneration conditions. Differences in regeneration dynamics were suggested to account for spatial genetic structure found in a lowland stand of black spruce (*Picea mariana*) but not in at an upland site (BOYLE *et al.* 1990). The upland site was regenerated after fire. In a study of tamarack (*Larix laricina*), a stand that was regenerated on an abandoned field showed little population structure, but one regenerated on a clearcut site had significant spatial structure (KNOWLES *et al.* 1992). The authors hypothesize that the seed source for the old field site had come from off-site and was well mixed, whereas the clearcut stand regenerated from *in situ* surviving seedlings or seed.

Regeneration processes that favor recruitment of maternally related seedlings have operated in the last 25 years but apparently did not operate when the current adult generation was established. We can only speculate about factors that may be responsible for changing regeneration dynamics. It is possible that under the frequent fires that occurred prior to European settlement, the pattern of sapling establishment favored scattered individuals. Anthropogenic changes in land use tend to happen fairly suddenly, for example, fires are suppressed or livestock are introduced or removed. These relatively sudden changes may allow a few successful maternal trees to reproduce during seasons immediately following the change. The saplings in our study, for example, may represent a cohort of acorns that sprouted soon after the site was released from agriculture, about 25 years ago. In contrast, establishment of the adult oaks in the stand may have occurred

over many years and thinning of saplings due to frequent fires may have disrupted any detectable genetic structure grouping of related individuals. Alternatively, patterns of establishment of seeds may reflect behaviors of seed dispersers (caching, burial in favorable microhabitats, etc.) and communities of mammalian or avian seed dispersers may be altered from presettlement times. Changing herbivore communities may also play a role. In a long-term study of a deciduous forest complex in Ohio, extremely low survivorship of seedlings was found, and the overriding factor limiting survivorship was grazing by white-tailed deer (BOERNER & BRINKMAN 1996). Deer are also believed to suppress oak seedlings and prevent sapling growth in California oaks (WHITE 1966; GRIFFIN 1971). Finally, another factor implicated in the failure of California savanna oaks to regenerate may also be playing a role at our site, that is a change in the understory from native species to introduced grasses and herbs (*e.g.* (GORDON *et al.* 1989; MOMEN *et al.* 1994). No native grasses or forbes are currently present at the study site. The herbaceous layer is now almost a monoculture of brome grass (*Bromus inermis*) in well drained areas and reed canarygrass (*Phalaris arundinacea*) in low spots (DOW 1995), although we cannot know how long these introduced species have dominated. It would be interesting to compare the genetic architecture reported for sapling and adult bur oaks to nearby secondary growth stands of bur oak. These stands may already reflect the differences in genetic structure we are predicting will occur as the saplings at our study site mature and replace the current adult generation.

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REFENCES

ALVAREZ-BUYLLA, E. R., CHAOS, A., PINERO, D. & GARAY. A. A. 1996: Demographic genetics of a pioneer tropical tree species: patch dynamics, seed dispersal, and seed banks. *Evolution* **50**:1155–1166.

ASHLEY, M. V. & DOW, B. D. 1994: The use of microsatellite analysis in population biology: Background, methods and potential applications. *In* : Molecular ecology and evolution: Approaches and applications. (eds. B. Schierwater, G. P. Wagner and R. DeSalle). pp. 185–201. Birkhauser Verlag, Basel, Switzerland.

BERG, E. E. & HAMRICK, J. L. 1995: Fine-scale genetic structure of a turkey oak forest. *Evolution* **49**:110–120.

BOERNER, R. E. J. & BRINKMAN, J. A. 1996: Ten years of tree seedling establishment and mortality in an Ohio deciduous forest complex. *Bulletin of the Torrey Botanical Club* **123**:309–317.

BOYLE, T., LIENGSIRI, C. & PIEWLUANG, C. 1990: Genetic structure of black spruce on two contrasting sites. *Heredity* **65**:393–399.

BRUFORD, M. W. & WAYNE, R. K. 1993: Microsatellites and their application to population genetic studies. *Current Opinion in Genetics and Development* **3**:939–943.

DOW, B. D. 1995: Characterization of mating system of bur oak (*Quercus macrocarpa*) using microsatellite DNA markers. Ph.D. dissertation, University of Illinois at Chicago.

DOW, B. D. & ASHLEY, M. V. 1996: Microsatellite analysis of seed dispersal and parentage of saplings in bur oak, *Quercus macrocarpa*. *Molecular Ecology* **5**:120–132.

DOW, B. D. & ASHLEY, M. V. 1998a: Paternity analysis using microsatellites reveals high levels of gene flow in bur oak. *Journal of Heredity* **89**: 62–70.

DOW, B. D. & ASHLEY, M. V. 1998b: Factors influencing male mating success in the bur oak, *Quercus macrocarpa*. *New Forests* **15**: 161–181.

DOW, B. D., ASHLEY, M. V. & HOWE, H. F. 1995. Characterization of highly variable (GA/CT)n microsatellites in the bur oak, *Quercus macrocarpa*. *Theoretical and Applied Genetics* **91**:137–141.

EPPERSON, B. K. 1992: Spatial structure of genetic variation within populations of forest trees. *New Forests* **6**: 257 –278.

EPPERSON, B. K. & ALVAREZ-BUYLLA, E. R. 1997: Limited seed dispersal and genetic structure in life stages of *Cecropia obtusifolia*. *Evolution* **51**: 275–282.

FORÉ, S. A., HICKEY, R. J. & VANKAT, J. L. 1992: Genetic structure after forest fragmentation: a landscape ecology perspective on *Acer saccharum*. *Canadian Journal of Botany* **70**:1659–1668.

GORDON, D. R., WELKER, J. M., MENKE, J. W., & RICE, K. J. 1989: Competition for soil water between annual plants and blue oak (*Quercus douglasii*) seedlings. *Oecologia* **79**:533–541.

GRIFFIN, J. R. 1971: Oak regeneration in the upper Carmel Valley, California. *Ecology* **52**:862–868.

GRIFFIN, J. R. 1976: Regeneration in *Quercus lobata* savannas, Santa Lucia Mountains, California. *American Midland Naturalist* **95**:422–435.

HOLMES, T. H. 1995: Woodland canopy structure and the light response of juvenile *Quercus lobata* (Fagaceae). *American Journal of Botany* **82**:1432–1442.

KNOWLES, P., PERRY, D. J. & FOSTER, H. A. 1992: Spatial genetic structure in two tamarack [*Larix laricina* (Du Roi) K. Koch] populations with differing establishment histories. *Evolution* **46**:572–576.

LIBBY, W. F., STETTLER, R. F. & SEITZ, F. W. 1969: Forest genetics and forest tree breeding. *Annual Review of Genetics* **3**:469–494.

LINHART, Y. B., MITTON, J. B., STURGEON, K. B., & DAVIS, M. L. 1981: Genetic variation in space and time in a population of ponderosa pine. *Heredity* **46**:407–426.

MOMEN, B., MENKE, J. W., WELKER, J. M., RICE, K. J. & CHAPIN III, F. S. 1994: Blue-oak regeneration and seedling water relations in four sites within a California oak savanna. *International Journal of Plant Science* **155**:744 –749.

QUELLER, D. C. & GOODNIGHT, K. F. 1989: Estimating

relatedness using genetic markers. *Evolution* **43**: 258–275.

SHARPE, D. M., GUNTENSPERGEN, G. R., DUNN, C. P., LEITNER, L. A. & STEARNS, F. 1987: Vegetation dynamics in a southern Wisconsin agricultural landscape. *In* : Landscape heterogeneity and disturbance. (ed. M.G. Turner). pp. 137–155. Springer-Verlag, New York.

WHITE, K. L. 1966: Structure and composition of foothill woodland in central coastal California. *Ecology* **47**: 229–237.

YAZDANI, R., MUONA, O., RUDIN, D. & SZMIDT, A. E. 1985: Genetic structure of a *Pinus sylvestris* L. seed-tree stand and naturally regenerated understory. *Forest Science* **31**:430–436.