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CLONAL POPULATION STRUCTURE AND GENETIC VARIATION IN SAND-SHINNERY OAK, *QUERCUS HAVARDII* (FAGACEAE)[1]

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We investigated clonal population structure and genetic variation in *Quercus havardii* (sand-shinnery oak), a deciduous rhizomatous shrub that dominates vegetation by forming uninterrupted expanses of ground cover over sandy deposits on the plains of western Texas, western Oklahoma, and eastern New Mexico. Isozyme electrophoresis (15 loci coding 11 enzymes) was used to recognize and map clones arrayed in a 2000-m transect (50-m sample intervals) and a 200 × 190 m grid (10-m sample intervals). Ninety-four clones were discovered, 38 in the transect and 56 in the grid, resulting in an estimated density of ~15 clones per hectare. Clones varied greatly in size (~100–7000 m^2), shape, and degree of fragmentation. The larger clones possessed massive interiors free of intergrowth by other clones, while the smaller clones varied in degree of intergrowth. The population maintained substantial levels of genetic variation (P = 60%, A = 2.5, H_{exp} = 0.289) comparable to values obtained for other *Quercus* spp. and for other long-lived perennials. The population was outcrossing as evidenced by conformance of most loci to Hardy-Weinberg expected genotype proportions, although exceptions indicated a limited degree of population substructuring. These data indicate that despite apparent reproduction primarily through vegetative means, *Q. havardii* possesses conventional attributes of a sexual population.

Key words: allozyme; clone; Fagaceae; genetic variation; isozyme; population structure; *Quercus*.

Among the most unusual of biotic communities in North America is the sand-shinnery oak community occurring on sandy deposits in western Texas, eastern New Mexico, and southwestern Oklahoma (Fig. 1). The overwhelming dominant in this community is the plains-endemic *Quercus havardii* Rydb. (sand-shinnery oak), a clonal deciduous shrub of low stature, with sparsely branched aerial shoots rarely exceeding 1 m in height. It forms large patches through horizontal growth effected by subterranean rhizomes, which may achieve a diameter of >9 cm (Muller, 1951; Wiedman, 1960; Scifres, 1972; Pettit, 1979; Dhillion et al., 1994). In the eastern portion of the species' range these patches remain more or less discrete, but in the more western sand-shinnery oak community, the patches coalesce to form a continuous ground cover with stem densities ranging from 30 to 70 stems/m^2 (Dhillion et al., 1994; Zhang, 1996). The resulting aspect of the community is a vast homogeneous ''sea'' of uniformly spaced *Q. havardii* stems similar in height and other attributes. This vegetation type is estimated to have originally covered an area of 2.3 million hectares, but present abundance has been substantially reduced through eradication, as *Q. havardii* is considered a pest by ranchers (Dhillion et al., 1994).

Demographic features and clonal biology of *Q. havardii* have been sparingly studied and remain poorly understood. Individual ramets generally live <15 yr (Wiedman, 1960; Pettit, 1979), but genets may be of considerable age and were previously estimated to expand to sizes up to ~15 m (Muller, 1951; Wiedman, 1960). Herein we address clonal population structure and genetic variation in *Q. havardii*. The goal of this study was to use molecular markers (allozymes) to discriminate clones and thereby facilitate perception of a population comprising discrete genets of sexual origin. From this new perspective, features of the genets such as size, shape, and intergrowth could be evaluated, and levels and patterns of genetic variation could be addressed in a manner parallel to that available for nonclonal species.

MATERIALS AND METHODS

Study site and sampling strategy—This study was conducted at a permanent research site near Plains in Yoakum County, Texas (Fig. 1; Dhillion et al., 1994). The habitat consists of sand dunes dominated by *Quercus havardii*, which comprises >90% of the ground cover, interrupted in spots by disturbed sites and other plant species. Associated plant species included *Artemisia filifolia, Yucca campestris, Schizachyrium scoparium, Andropogon hallii, Aristida purpurea, Eriogonum annuum*, and *Euphorbia fendleri* (Dhillion et al., 1994; names of taxa based on Correll and Johnston, 1979).

As there was previously little more than anecdotal information on clone size for *Quercus havardii* (Muller, 1951), the most appropriate distance between sampling points was undetermined as this study commenced. To establish a first approximation of clone size, 40 samples were taken at 50-m intervals along a transect and electrophoresed (see below) to determine whether clones extended >50 m. As most samples in this transect possessed distinct genotypes and were therefore different genets (see Results), a finer sampling interval of 10 m was chosen. A grid measuring 200 × 190 m was established at the study site from which samples were obtained at 10-m intervals.

Electrophoresis of enzymes—Leaves were obtained for electrophoretic analysis from April through July 1993 and placed in sealable bags

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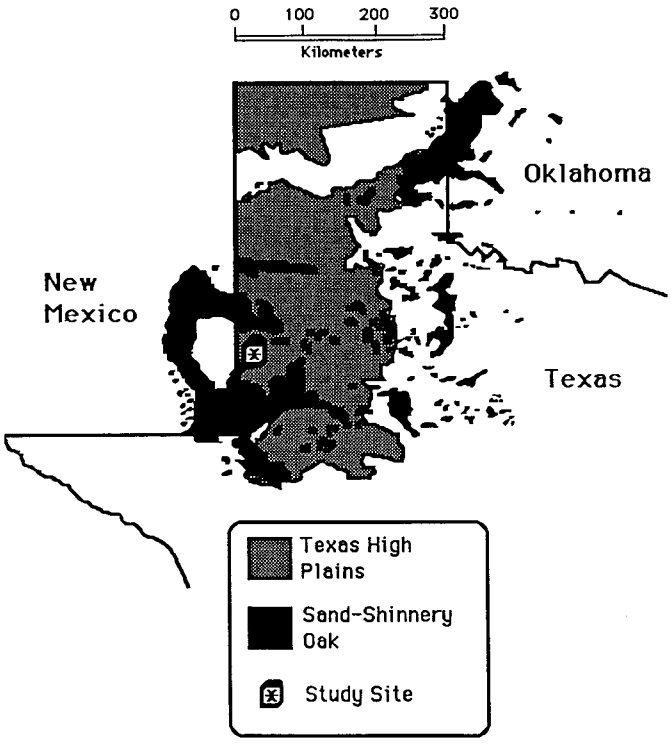

Fig. 1. The distribution of sand-shinnery oak and the location of our field site in Yoakum County, Texas. The sand-shinnery oak community per se is predominantly in the western continuous portions of the species' range.

in an insulated container, and kept refrigerated until processing. Leaf tissue was homogenized within 3 d after harvest (longer delays resulted in significant loss of enzyme activity) in pH 7.5 ''microbuffer'' of Werth (1985) containing 5% polyvinylpyrrolidone (molecular mass 40 000 units) and 1% 2-mercaptoethanol. Homogenates were either immediately loaded into the starch gels (12% Sigma starch) or stored frozen at −85°C for later electrophoresis. Electrophoresis and staining of enzymes followed standard procedures (Soltis et al., 1983; Werth, 1985; Wendel and Weeden, 1989), but employed the ''zymecicle'' methodology of Werth (1990). Leucine aminopeptidase (LAP) and esterase (EST) were resolved on the lithium hydroxide system (Werth, 1985); phosphoglucose isomerase (PGI), alcohol dehydrogenase (ADH), and triosephosphate isomerase (TPI) on buffer system 6 of Soltis et al. (1983); phosphoglucomutase (PGM) and 6-phosphogluconic dehydrogenase (6PGD) on the tris-citrate pH 8.0 buffer system of Selander et al. (1971); and aldolase (ALD), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), 6PGD, and shikimate dehydrogenase (SKDH) on the morpholine-citrate buffer system (Werth, 1991).

Band interpretation and data analysis—Isozyme band patterns were interpreted using standard principles (Wendel and Weeden, 1989; Murphy et al., 1990). Alleles were designated by numbers representing the migration of the allozymes they encoded, with the lowest number assigned to the most anodal allozyme.

Genetic individuals (genets) were discriminated on the basis of their multilocus isozyme genotypes, and confidence levels for clone assignment estimated using the method of Parks and Werth (1993). Quantitative treatment of the data was carried out using BIOSYS-1 (Swofford and Selander, 1981), Statview Student for the Macintosh (1991), and NTSYS (Rohlf, 1988).

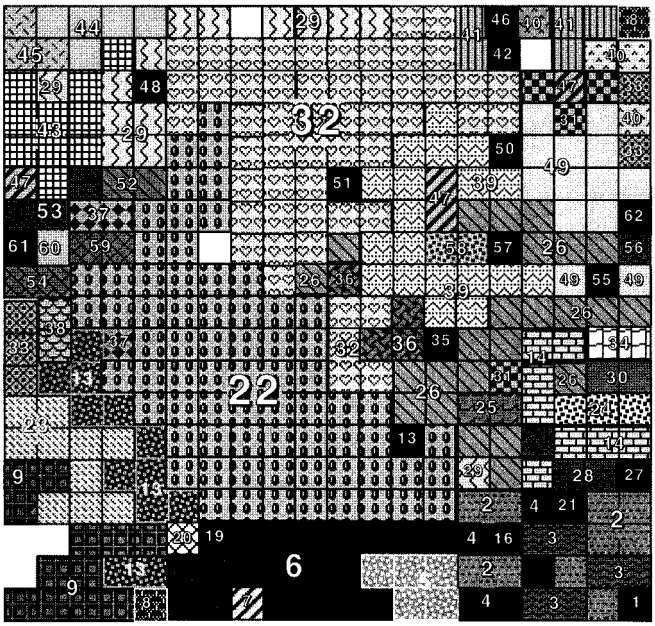

Fig. 2. Map depicting the identity of genotypes in our sampling grid (200 × 190 m). Each 10 × 10 m square was represented by a sample point in its center. Each pattern represents a different multilocus genotype, i.e., putative genet; numbers correspond to genet numbers in Appendix. Blank squares are regions devoid of *Q. havardii* ramets.

RESULTS

General—Fifteen loci coding 11 enzymes were consistently scorable and could be used for discrimination of genets. Of these 15 loci, 86.7% (13 loci-- *Adh, Est, Idh, Lap, Mdh-1, Mdh-2, Pgi-2, Pgm-2, 6Pgd-1, 6Pgd-2, Skdh, Tpi-1*, and *Tpi-2*) were polymorphic in the study area. The two nonvariable loci were *Ald* and *Pgi-1*. Three of the loci, *Idh, Mdh-2,* and *6Pgd-2,* were not variable within the grid subpopulation, and one, *Tpi-2*, was not variable within the transect subpopulation.

In the initial transect, 38 of the 40 ramets sampled at 50-m intervals exhibited distinct genotypes and could thus be inferred to be different genets (Appendix). Two pairs of adjacent points, numbers 2/3 and 32/33 had identical genotypes, indicating the presence of single clones spanning greater than 50 m. In the two-dimensional grid, 56 distinct genotypes were observed among the 380 ramets sampled (Appendix; Fig. 2). The genotypes were numbered as encountered. In some cases the same genotype was inadvertently given two different numbers, in which case the higher number was omitted, resulting in ordinal values up to 62. Because the different sampling intervals and vastly different linear extent (2000 m vs. 200 m) of the grid and transect might influence their genetic makeup if genotypes were unevenly distributed across the population, most population genetic parameters were computed separately for these two subsamples. Allele frequencies for all loci were calculated separately for the grid and transect and then averaged to obtain population means (Table 1). For the grid subpopulation, in which closely spaced ramets resulted in repeated sampling of genets, allele frequencies were estimated both by tallying all genets (unadjusted) and by using the ''round-

TABLE 1. Estimates of allele frequencies for 15 enzyme loci examined for the grid and transect subpopulations of *Quercus havardii*. Allele frequencies in grid were estimated using all individuals (standard estimate) and by the ''round-robin'' method of Parks and Werth (1993).

Locus	Allele	Grid		Transect	Population mean
		Standard	Round robin		
Adh	1	0.264	0.269	0.263	0.264
	2	0.709	0.704	0.737	0.723
	3	0.027	0.028	0.000	0.014
Ald	1	1.000	—	1.000	1.000
Est	1	0.580	0.570	0.473	0.527
	2	0.273	0.279	0.108	0.191
	3	0.148	0.151	0.405	0.277
	4	0.000	0.000	0.014	0.007
Idh	1	1.000	—	0.961	0.981
	2	0.000	—	0.039	0.020
Lap	1	0.063	0.029	0.176	0.120
	2	0.616	0.637	0.378	0.497
	3	0.232	0.245	0.311	0.272
	4	0.089	0.088	0.135	0.112
Mdh-1	1	0.929	0.927	0.882	0.906
	2	0.071	0.073	0.118	0.095
Mdh-2	1	1.000	—	0.987	0.994
	2	0.000	—	0.013	0.007
Pgi-1	1	1.000	—	1.000	1.000
Pgi-2	1	0.732	0.741	0.658	0.695
	2	0.268	0.259	0.342	0.305
Pgm-2	1	0.417	0.417	0.355	0.386
	2	0.120	0.115	0.171	0.146
	3	0.028	0.031	0.105	0.067
	4	0.435	0.438	0.368	0.402
6Pgd-1	1	0.880	0.877	0.263	0.572
	2	0.102	0.104	0.697	0.400
	3	0.000	0.000	0.039	0.020
	4	0.019	0.019	0.000	0.010
6Pgd-2	1	1.000	—	0.974	0.987
	2	0.000	—	0.026	0.013
Skdh	1	0.798	—	0.711	0.755
	2	0.202	—	0.013	0.108
	3	0.000	—	0.276	0.138
Tpi-1	1	0.759	0.759	0.658	0.709
	2	0.241	0.241	0.342	0.292
Tpi-2	1	0.946	0.945	1.000	0.943
	2	0.054	0.055	0.000	0.027

robin'' method, a subsampling routine that can avoid overestimation of frequencies for rare alleles, as described in Parks and Werth (1993). Both techniques resulted in nearly identical frequencies (Table 1), indicating that sufficient genetic variation was present to provide robust genet discrimination.

Genotype probabilities—Following Parks and Werth (1993), genotype probability P_{gen}, i.e., the probability that a zygote formed by combining randomly chosen gametes will have a particular genotype, was calculated for each genotype in the grid as the product across loci of Hardy-Weinberg expected frequencies, based on population mean allele frequencies. The values obtained for P_{gen} (Appendix), representing the chance that two consecutive ramet samples not belonging to the same genet could independently possess the same genotype, were <0.01 for all putative genets. Therefore, contiguous ramets of identical genotype could be confidently assigned to the same genet. Additionally, for each genet the probability of second encounter P_{SE}, i.e., the chance of observing its genotype one or more additional times among the 56 genets sampled, was computed as $1 - (1 - P_{gen})^{55}$. Values for P_{SE} were also small, <0.01 for most genotypes, although for seven genotypes $P_{SE} > 0.05$. Therefore, most noncontiguous ramets of identical genotype probably belonged to the same genet.

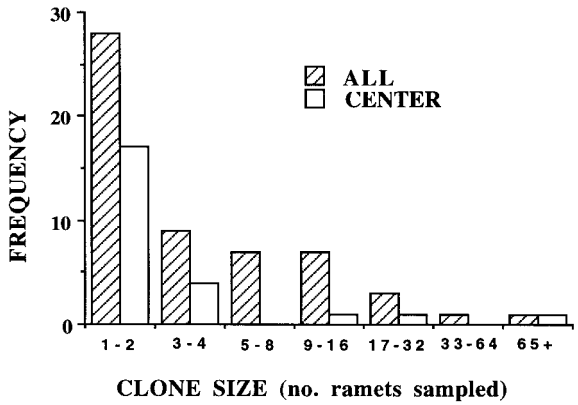

Fig. 3. Clone size (number of ramets sampled) frequency distribution. The barred columns include all clones, whereas the open columns include only clones that do not come into contact with the edge of the sampling grid.

Clone size—Features of size and shape of *Q. havardii* clones in the grid were evaluated by construction of a map of genets (Fig. 2) and a histogram depicting the distribution in clone size (Fig. 3). Among the 56 clones of the grid, the majority were small (37 with only 1–4 sample points), and a few were large (five with 17 or more sample points). To rule out edge effects as a possible cause of this distribution, the clone size histogram was also plotted using only those clones that did not touch the edge of the grid, resulting in a similar distribution of clone sizes (Fig. 3). The 12 largest clones occupied >70% of the grid area, 40% being attributable to the three largest clones, clones 22, 26, and 32. The largest clone, number 22, was sampled at 70 points and thus occupied an estimated area of 7000 m^2 (at 100 m^2 per sample point).

Clone fragmentation—Most of the 56 clones of the grid were apparently continuous and unfragmented. However, 15 of the putative genets appeared to be fragmented, i.e., including one or more ramets isolated from other ramets of the same genotype by at least one sampling point. Notable examples of putatively fragmented clones included genotype 8 ($P_{SE} = 0.030$), whose two sampled ramets were ~240 m apart, genotype 33 ($P_{SE} = 0.069$), with five sampled ramets in two groups ~180 m apart, genotype 47 ($P_{SE} = 0.017$), with four sampled ramets in three groups spread over ~160 m, genotype 31 ($P_{SE} = 0.088$), with four sampled ramets in two groups ~70 m apart, genotype 29 ($P_{SE} = 0.008$), with 14 sampled ramets in two main groups ~100 m apart, and genotype 26 ($P_{SE} = 0.031$), with 25 sampled ramets in two groups ~50 m apart (Fig. 2). Of these 15 putatively fragmented clones, 13 had values for $P_{SE} < 0.05$ and therefore were unlikely to comprise different genets coincidentally possessing the same genotypes (but see below). Genotypes 31 and 33

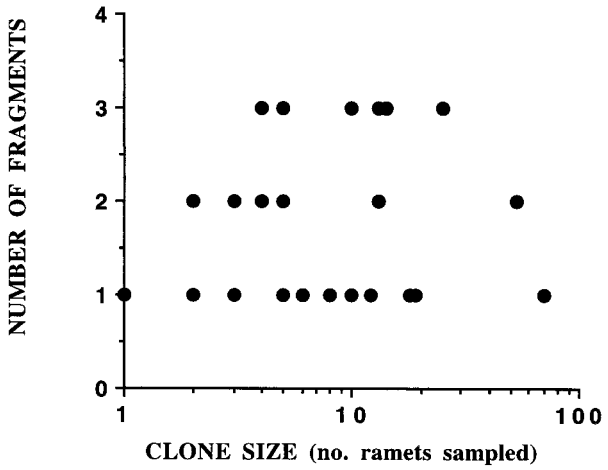

Fig. 4. Graph of the number of clone fragments as a function of clone size (number of ramets sampled) for all clones that did not come into contact with the edge of the sampling grid.

had values for $P_{SE} > 0.05$, such that there is a significant probability that the fragments with these genotypes could be different genets.

In evaluating the likelihood that each of these cases of separated ramet clusters represents clone fragmentation, it is necessary to consider that P_{SE} is the probability for second encounter of an independently formed ramet with one *particular* genotype. The collective probability that any *one or more* of the detected genotypes would be reencountered is much greater than P_{SE} because the number of ''trials'' (pairs of genets) is much greater. The probability of not reencountering a particular genotype is $(1 - P_{SE})$. The chance that none would be reencountered is the product of $(1 - P_{SE})$ for all genotypes in the grid subpopulation, which was computed as 0.329. Thus, although the chance of reencountering a particular genotype is low, the chance of reencountering one or more of the 56 genotypes is high, i.e., 0.671. Therefore, it is probable that at least one of the putatively fragmented clones may actually comprise a pair of different genets with identical genotypes, although it remains highly improbable that more than a few of the 15 cases of putative fragmentation are instead cases of distinct genet formation. It cannot be ascertained with certainty which groups of separated putative fragments with identical genotype actually comprise distinct genets. However, widely separated fragments, such as genotype 8, are especially suspect.

The number of fragments was unrelated to clone size (Fig. 4; Spearman rank correlation $N = 24$, $r_s = 0.094$, $p = 0.66$). This result apparently deviated from the random expectation, which is a positive relationship, since larger clones should have a higher probability of becoming fragmented.

Clone shape—Clones exhibited irregular and variable shapes that presumably reflect their growth history (Fig. 2). Some of the larger clones, such as clones 32, 22, and 6, consisted of massive central regions, free of invading rhizomes of surrounding clones, from which radiated one or more narrower extensions winding into or between other clones. Examples of this can be seen in the northward extension of clone 22 into clone 32 and in the northeastern extension of clone 43 that divides clone 29. However, much of the perimeter of these large clones seemed to form long, fairly straight boundaries with adjacent clones, characterized by little intergrowth as perceived at the scale of sampling employed (i.e., 10-m intervals). Other clones, such as clones 13 and 26, exhibited long narrow convoluted shapes, characterized by intergrowth with adjacent clones and tending to be fragmented. The irregular boundaries of clone 13 with clones 22 and 23 illustrate this. Perhaps the most distinctive shape was exhibited by clone 39, a large C-shaped clone sampled at 18 points, whose central portion was occupied by several other clones, including clones 26 and 47. This could have resulted from the initial growth of clone 39 in competition with its neighbors, or later invasion and displacement of the interior of a circular clone.

Levels and patterns of genetic variability—Genetic variability was quantified for the grid, transect, and combined samples by computing three indices: mean expected heterozygosity (H_{exp}), proportion of loci polymorphic (P), and mean number of alleles per locus (A) (Table 2). Values of all three indices ($H_{exp} = 0.289$, $P = 60\%$, and $A = 2.50$ for the combined sample) were higher than those reported for most other oaks that have been electrophoretically surveyed (Guttman and Weigt, 1989; Schnabel and Hamrick, 1990; Hamrick, Godt, and Sherman-Broyles, 1992; Berg and Hamrick, 1994) and were also high as compared to the mean values for flowering plants in general ($H_{exp} = 0.113$; $P = 34.2\%$; $A = 1.53$) (Hamrick and Godt, 1989). These values were also high when compared to subgroups defined by life-history characters shared with *Q. havardii*, e.g., other dicots ($H_{exp} = 0.096$; $P = 29.0\%$; $A = 1.44$), long-lived woody perennials ($H_{exp} = 0.149$; $P = 50.0\%$; $A = 1.79$), temperate plants ($H_{exp} = 0.109$; $P = 32.6\%$; $A = 1.51$), outcrossing wind-pollinated plants ($H_{exp} = 0.148$; $P = 49.7\%$; $A = 1.79$), and other clonal plants ($H_{exp} = 0.103$; $P = 29.4\%$; $A = 1.47$) (Hamrick and Godt, 1989).

Conformance of genotype proportions to expected values under Hardy-Weinberg equilibrium was tested using the chi-square test (with pooling where appropriate) for each locus separately in the grid and transect subpopulations, and deviations were quantified by computing the fixation index F (Table 3). Most loci were in Hardy-Weinberg equilibrium in both the grid and transect subpopulations and showed low positive values for F, ranging from 0.050 to 0.192, that were not statistically different from zero. Five instances, out of 22 valid tests, of statistical departure from Hardy-Weinberg occurred: *Adh* ($p = 0.005$), *Est* ($p = 0.046$), and *6Pgd-1* ($p < 0.001$) in the grid subpopulation and *Lap* ($p = 0.001$) and *Mdh-1* ($p = 0.023$) in the transect subpopulation. All five of these instances exhibited a deficiency in heterozygotes, as indicated by positive values for F (*Adh*, $F = 0.361$, *Est*, $F = 0.160$, and *6Pgd-1*, $F = 0.399$, for the grid and *Lap*, $F = 0.392$, *Mdh-1*, $F = 0.370$, for the transect). Heterozygote deficiencies at these few loci are unlikely to reflect intensive inbreeding, which should result in heterozygote deficiencies at all loci, but instead could reflect population substructuring, i.e., the Wahlund effect (Wah-

TABLE 2. Estimates of genetic variability in *Quercus havardii* (± 1 SE). Values are reported separately for the grid and transect samples, and for the combined sample of all genets treated as a single population sample.

Sample	Mean sample size per locus (N)	Percentage of loci polymorphic[a] (P)	Mean no. of alleles per locus (A)	Mean expected heterozygosity[b] (H_{exp})
Grid	54.3 ± 0.8	66.7 (66.7)	2.13 ± 0.3	0.249 ± 0.060
Transect	37.9 ± 0.1	60.0 (80.0)	2.33 ± 0.3	0.305 ± 0.069
Combined	92.2 ± 0.9	60.0 (86.7)	2.50 ± 0.3	0.289 ± 0.066

[a] The value for P is based on the 0.95 criteria, i.e., a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95. The value for P under the absolute criterion (i.e., a locus is polymorphic if more than one allele was detected) is indicated in parentheses.

[b] Unbiased estimate (see Nei, 1978).

lund, 1928). The possibility that the *Q. havardii* population might be genetically structured was evaluated in two ways, as follows.

Allele-frequency heterogeneity between the grid and transect population samples was addressed by computing F statistics (Wright, 1978) for all loci, and values of F_{ST} were statistically evaluated for deviation from 0 using contingency chi-square analysis (Table 4). The amount of divergence between the two samples, as estimated by F_{ST}, was generally low with a mean for all loci of $F_{ST} = 0.063$, and more than half of the loci having $F_{ST} < 0.02$. Six loci had $F_{ST} > 0.02$, ranging from 0.02 to 0.359, and were significantly greater than 0 as determined by contingency chi-square analysis. Two loci, *6Pgd-1* ($F_{ST} = 0.359$) and *Skdh* ($F_{ST} = 0.075$), had values of $F_{ST} > 0.05$, a value widely considered substantial (Hartl, 1980).

Genetic structure was also evaluated by testing whether genetic relatedness of neighboring clones was greater than expected by chance. Two correlation matrices were constructed comparing each pair of clones in the transect. In one matrix the physical distances between sample points were entered, and in the other pairwise values of Rogers' similarity, computed from the isozyme genotypes, were entered as an index of genetic relatedness. The Mantel Z test (carried out using NTSYS) showed no correlation between the two matrices ($r = 0.265$, $p \gg 0.05$). Clones from the grid were not similarly evaluated as there was no consistent method to assign distance between the genets. The results from analysis of Hardy-Weinberg proportions, F_{ST} computations, and distance/relatedness comparison combine to indicate that the population is structured (i.e., distribution of genotypes is not entirely random), but only weakly so.

DISCUSSION

Clonal populations often comprise an array of discrete patches that, depending on the growth strategy, may correspond to single genets (e.g., Daehler and Strong, 1994; Montalvo et al., 1997) or may each be composed of several genets (e.g., Parks and Werth, 1993; Berg and Hamrick, 1994). However, the *Quercus havardii* population addressed in this study comprises a single enormous patch, spread continuously over a large expanse of homogeneously suitable habitat. The use of isozymic markers has allowed for the first time a portion of this expanse to be viewed as genetically distinct individuals comprising a sexually interbreeding population. Despite rhizome growth that results in order-of-magnitude size variation among genets coupled with rarity of sexual recruitment (Muller, 1951; Wiedman, 1960; Pettit, 1979), this population appears to share features with populations of organisms with more determinate growth, i.e., large population size, an outcrossing breeding system in which mating approaches panmixia, and maintenance of a substantial level of genetic variation.

In this respect, *Q. havardii* parallels other species for which vegetative propagation does not substitute for sexual reproduction, but rather effects horizontal spread of sexually reproduced individuals. Such populations have been shown capable of maintaining significant genetic variation (Thomas and Dale, 1975; Ellstrand and Roose, 1987; Wolf, Haufler, and Sheffield, 1988; Wolf, Sheffield, and Haufler, 1991; Aspinwall and Christian, 1992; Berg and Hamrick, 1994; Lokker et al., 1994; Sipes and Wolf, 1997; Montalvo et al., 1997) despite possibly lowered effective population sizes in clonally reproducing species

TABLE 3. Values for the inbreeding coefficient F computed for each polymorphic locus in the grid and transect samples. Statistical difference of F from 0 was evaluated using chi-square analysis, and is indicated as follows: ns = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Locus	Grid	Transect
Adh	0.361**	0.050 ns
Est	0.160*	0.099 ns
Idh	—	−0.041 ns
Lap	0.066 ns	0.392**
Mdh-1	0.192 ns	0.370*
Mdh-2	—	−0.013 ns
Pgi-2	0.180 ns	0.065 ns
Pgm-2	−0.102 ns	0.095 ns
6Pgd-1	0.399***	0.346 ns
6Pgd-2	—	−0.027 ns
Skdh	−0.134 ns	−0.132 ns
Tpi-1	−0.220 ns	−0.052 ns
Tpi-2	−0.057 ns	—

TABLE 4. Values of F_{ST} for all loci as an estimate of allele frequency heterogeneity between the grid and transect subsamples of the *Q. havardii* population. Statistical difference of F_{ST} from 0 was evaluated using contingency chi-square analysis and is indicated as follows: ns = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Locus	F_{ST}
Adh	0.001 ns
Est	0.043***
Idh	0.020*
Lap	0.030**
Mdh-1	0.006 ns
Mdh-2	0.007 ns
Pgi-2	0.007 ns
Pgm-2	0.006 ns
6Pgd-1	0.359***
6Pgd-2	0.013 ns
Skdh	0.075***
Tpi-1	0.012 ns
Tpi-2	0.028*
Mean	0.063***

relative to species that reproduce only sexually. It is unlikely that population size constrains maintenance of genetic diversity in this species. Assuming that the density of ~15 genets per hectare in the grid is representative for populations of this species, the number of individuals across the species range (2.3 million hectares), much of which was contiguous up until the early 20th century, can be estimated as exceeding 30 million. Furthermore, the apparently low degree of genetic structure indicates that neighborhood sizes are very large, as indicated for other *Quercus* species (Berg and Hamrick, 1994; Montalvo et al., 1997; but see Sork, Huang, and Wiener, 1993).

Attributes of clonal architecture are highly variable among and sometimes within plant species (Ellstrand and Roose, 1987). Clone size may range from <1 m^2 for some herbaceous species to >43 ha for a clone of *Populus tremuloides* Michaux (Kemperman and Barnes, 1976). Prior to the present study, the size range of *Quercus havardii* clones was unknown, although estimates of up to ~15 m had been made based on the sizes of discrete patches that occur in the eastern portion of the species range and of phenologically differentiated patches within the expansive communities (Muller, 1951). Here we have found that these previous estimates were conservative, as indicated by large clones such as number 22, which exceeded 150 m in maximum breadth and occupied ~7000 m^2.

Morphologies and growth strategies of clonal organisms vary across a broad spectrum from regularly shaped radiating circles of densely clumped ramets with minimal intergrowth (phalanx morphology) to irregularly shaped, meandering, and/or ramifying clones with more widespread ramets and a tendency for intergrowth and fragmentation (guerilla morphology) (Lovett-Doust, 1981; Silander, 1985). *Quercus havardii* clones, even the smaller ones, comprise numerous closely spaced ramets that occupy significant areas free of ramets from other clones (or other species) and tend not to be fragmented, thus possessing a fundamentally phalanx morphology. Phalanx morphology of *Q. havardii* is further suggested by anecdotal observation of its subterranean structure, usually revealed by exposure from wind erosion, rarely from purposeful excavations (J. C. Zak, Texas Tech University, personal communication). Such observations suggest a hierarchical system comprising groups of branches produced at regular intervals from a thick subterranean rhizome. However, to varying degrees individual clones also possess attributes associated with guerilla morphology. A meandering and branching form characterizes at least a portion of many of the clones, including the arms radiating from the larger ones, and some clones exhibit fragmentation. It remains uncertain, and a possible focus of future investigation, as to what kinds of spatial interactions occur between clones at a finer scale than addressed herein.

While the phenomenon of asexual proliferation through rhizome growth is readily observed in *Quercus havardii*, the nature of sexual reproduction and recruitment in this species is very poorly understood. Although large numbers of acorns are produced in ~3 out of every 10 yr (Muller, 1951; Pettit, 1979), *Q. havardii* seedlings rarely become established in the wild (Muller, 1951; Wiedman, 1960; Pettit, 1979), as virtually every available safe sight is occupied by existing genets, severely constraining the opportunity for seedling establishment. Moreover, disturbance by animals has been shown to facilitate establishment of herbaceous plant seedlings by opening sites in a deep layer of leaf-litter (Dhillion et al., 1994; Jeffery, 1998; Willig and McGinley, 1998). Perhaps disturbance may play a parallel role in recruitment of *Q. havardii* seedlings, but this remains to be observed directly.

Processes and histories of recruitment of long-lived perennials such as forest trees and other woody plants are difficult to evaluate because of the large time scales involved. Vegetative proliferation compounds the problem by blurring the distinction between individuals. The recognition of *Q. havardii* genets through isozyme fingerprints achieved in the present study narrows the universe of possibilities and clarifies the nature of questions that can be addressed in future studies. The large density of distinct genets (15 genets/ha) comprising an interbreeding sexual population with conventional attributes implicates an important and continuing, if episodic, role for sexual recruitment. The establishment of the population must have involved a complex dynamic that occurred during antiquity, the timing and progression of which can only be speculated, as the ages of the clones and the biotic community they so thoroughly dominate are unknown. The highly variable sizes and shapes of the *Q. havardii* clones, as they collectively occupy nearly the totality of a vast surface, must have largely been influenced by their competitive interactions as they came to abut. However, it remains unknown whether the spatial features of the genets revealed are long-enduring, having reached a stasis, are continually changing but in dynamic equilibrium, or are subject to drastic chaotic changes following episodic occurrence of environmental extremes.

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APPENDIX. Distinct multilocus genotypes detected in the grid (N = 56) and transect (N = 40) subpopulations. Individual locus genotypes are provided as a pair of single-digit numerical allele designations for the 13 polymorphic loci (see Methods; monomorphic loci *Ald* and *Pgi-1*). Number of loci heterozygous and probabilities for occurrence (P_{gen}) and second encounter (P_{SE}) were computed for the grid only. Blank entries indicate missing data.

Clone no.	No. of ramets	Isozyme genotype													No. of loci heterozygous	P_{gen}	P_{SE}
		Adh	*Est*	*Idh*	*Lap*	*Mdh-1*	*Mdh-2*	*Pgi-2*	*Pgm-2*	*6Pgd-1*	*6Pgd-2*	*Skdh*	*Tpi-1*	*Tpi-2*			
Grid																	
G01	1	22	23	11	23	11	11	22	34	11	11	11	12	11	4	0.00000283	0.000157
G02	10	22	23	11	23	11	11	11	22	11	11	12	22	11	3	0.00000100	0.000057
G03	6	11	23	11	23	12	11	11	24	11	11	12	12	11	6	0.00000097	0.000053
G04	4	22	23	11	24	11	11	11	44	11	11	11	12	11	3	0.00006292	0.003519
G05	5	22	13	11	22	11	11	11	24	11	11	11	12	11	3	0.00025525	0.014193
G06	19	11	23	11	22	11	11	12	12	11	11	11	11	11	3	0.00001841	0.001031
G07	2	22	23	11	22	11	11	22	11	11	11	12	11	11	2	0.00002138	0.001198
G08	2	22	13	11	23	11	11	11	44	11	11	11	11	11	2	0.00054876	0.030272
G09	12	22	13	11	23	11	11	11	14	11	11	11	11	11	3	0.00105210	0.057244
G13	13	11	13	11	23	11	11	11	14	11	11	11	12	11	4	0.00009264	0.005174
G14	8	22	11	11	22	11	11	11	11	11	11	12	12	11	2	0.00042175	0.023347
G16	1	22	22	11	33	11	11	22	24	12	11	12	12	11	4	0.00000025	0.000013
G19	1	11	11	11	24	11	11	12	24	11	11	12	11	11	4	0.00001170	0.000654
G20	20	11	12	11	24	11	11	12	44	11	11	12	11	11	4	0.00001996	0.001118
G21	1	22	13	11	22	11	11	12	44	11	11	12	11	11	3	0.00027007	0.015012
G22	70	11	11	11	23	11	11	12	14	11	11	11	11	11	3	0.00020930	0.011652
G23	10	12	22	11	22	11	11	11	14	11	11	11	12	11	3	0.00028676	0.015932
G24	3	22	11	11	22	11	11	11	22	11	11	12	12	11	2	0.00003493	0.001954
G25	2	22	11	11	22	11	11	11	11	11	11		12	11	1	0.00130819	0.070685
G26	25	12	12	11	24	11	11	11	14	11	11	11	11	11	4	0.00055443	0.030580
G27	1	13	11	11	23	11	11	12	12	11	11	11	11	11	4	0.00001181	0.000661
G28	3	22	11	11	22	11	11	12	12	11	11	12	12	11	4	0.00017774	0.009905
G29	14	22	11	11	24	12	11	12	14	11	11		11	11	4	0.00013900	0.007754
G30	2	22	12	11	22	11	11	11	44	22	11	11	11	11	1	0.00001805	0.001011
G31	4	22	12	11	22	11	11	11	14	11	11	11	12	11	3	0.00163616	0.087621
G32	53	12	12	11	23	11	11	12	11	11	11	11	11	12	5	0.00005791	0.003239
G33	5	22	11	11	22	11	11	12	14	11	11	11	12	11	3	0.00127267	0.068832
G34	2	22		11	23	22	11	11	14	11	11	11	12	11	3	0.00002273	0.001271
G35	1	22	12	11	23	11	11	11	12	22	11	22	11	11	3	0.00000046	0.000027
G36	1	22	22	11	23	11	11	12	14	11	11	12	12	11	5	0.00010752	0.006004
G37	3	12	11	11	22	11	11	22	44	11	11	12	11	11	2	0.00007214	0.004031
G38	2	11		11	23	11	11	22	14	11	11	11	11	11	2	0.00011389	0.006359
G39	18	22	11	11	22	11	11	12	14	11	11	11	11	12	3	0.00022879	0.012730
G40	4	12	11	11	22	11	11	11	14	24	11	11	11	11	3	0.00001020	0.000571
G41	5	22		11	33	11	11	11	11	12	11	11	12	11	2	0.00008143	0.004549
G42	1	22		11	23	11	11	11	11	12	11	11	12	11	3	0.00014547	0.008115
G43	10	12	11	11	22	11	11	12	14	12	11	12	11	11	5	0.00017516	0.009763
G44	5	22	11	11	22	12	11	22	34	24	11	12	12	11	5	0.00000001	0.000000
G45	3	33	11	11	33	11	11	12	34	12	11	11	11	11	3	0.00000001	0.000000
G46	1	22	11	11	22	11	11	11	11	11	11	12	11	11	1	0.00066413	0.036519
G47	4	22	11	11	23	11	11	11	14	11	11	12	12	11	4	0.00031197	0.017321
G48	1	22	12	11	34	11	11	12	24	11	11	11	11	11	4	0.00037314	0.020682
G49	13	12		11	24	11	11	11	14	11	11	11	11	12	4	0.00003165	0.001771
G50	1	22	12	11	22	12	11	12	14	11	11	11	11	11	4	0.00028837	0.016021
G51	1	12	12	11	23	11	11	12	14	11	11	11	11	12	6	0.00012082	0.006743
G52	2	22	12	11	24	12	11	11	14	11	11	11	12	11	5	0.00015353	0.008562
G53	3	22	11	11	22	11	11	11	14	11	11	12	11	11	2	0.00411887	0.206366
G54	2	12		11	22	11	11	11	44	11	11	11	12	11	2	0.00004396	0.002460
G55	1	12	33	11	23	11	11	11	14	11	11	12	12	11	5	0.00146726	0.078938
G56	1	12		11	24	11	11	11	11	11	11	11	11	11	2	0.00083916	0.045925
G57	1	12		11	12	11	11	11					11	12	3	0.00079083	0.043337
G58	2	22		11	12	11	11	12	14	11	11	11	11	12	4	0.00013912	0.007761
G59	2	22		11	14	12	11	11	14	11	11	11	12	11	4	0.00002334	0.001308
G60	1			11	11	11	11	11					11	11	0	0.00094624	0.051633
G61	1	12		11	11	11	11	11	14	11	11	11	12	11	3	0.00004025	0.002251
G62	1	12	22	11	33	11	11	11	14	11	11	12	12	11	4	0.00002059	0.001151

APPENDIX. Continued.

Clone no.	No. of ramets	Isozyme genotype													No. of loci heterozygous	P_{gen}	P_{SE}
		Adh	*Est*	*Idh*	*Lap*	*Mdh-1*	*Mdh-2*	*Pgi-2*	*Pgm-2*	*6Pgd-1*	*6Pgd-2*	*Skdh*	*Tpi-1*	*Tpi-2*			
Transect																	
O01	2	22	11	11	22	11	11	12	34	22	11	12	12	11			
O03	1	22	33	11	22	11	11	12	24	12	11	11	22	11			
O04	1	22	13	11	22	11	11	11	24	22	11	11	11	11			
O05	1	22	33	11	34	11	11	12	44	11	11	11	12	11			
O06	1	22	11	11	12	11	11	12	11	22	11	11	12	11			
O07	1	12	13	11	22	11	11	22	34	12	11	11	12	11			
O08	1	12	23	11	24	12	11	22	44	22	11	13	22	11			
O09	1	22	13	11	22	22	11	11	14	22	11	13	22	11			
O10	1	22	11	11	34	11	11	22	24	12	11	11	22	11			
O11	1	22	33	11	23	11	11	11	33	22	11	11	12	11			
O12	1	22	11	11	33	11	11	11	24	22	11	13	12	11			
O13	1	22	13	11	24	12	11	12	24	12	11	13	12	11			
O14	1	22	11	11	33	11	11	11	22	11	11	13	12	11			
O15	1	22	11	11	33	11	11	12	24	22	11	13	11	11			
O16	1	22	33	11	44	11	11	12	24	23	11	13	12	11			
O17	1	12	12	11	33	11	11	11	44	12	11	11	11	11			
O18	1	12	13	11	23	11	11	22	24	11	11	13	12	11			
O19	1	12	12	11	33	11	11	11	24	12	11	11	11	11			
O20	1	22		11	13	11	11	12	24	11	11	33	12	11			
O21	1	12	13	11	12	22	11	11	14	22	11	13	12	11			
O22	1	12	13	12	14	11	11	12	14	12	12	13	11	11			
O23	1	12	11	11	12	12	11	12	13	22	11	33	11	11			
O24	1	22	13	11	11	11	11	22	11	11	11	11	11	11			
O25	1	12	23	11	11	11	11	11	33	12	11	11	12	11			
O26	1	22	11	11	01	11	11	12	11	22	11	11	12	11			
O27	1	12	11	11	12	11	11	11	34	22	11	13	11	11			
O28	1	11	13	12	14	11	11	12	14	22	12	13	11	11			
O29	1	22	13	12	13	11	11	11	11	22	11	11	12	11			
O30	1	12	34	11	33	12	11	11	14	33	11	13	11	11			
O31	1	12	13	11	22	11	11	11	14	22	11	11	12	11			
O32	2	12	33	11	22	12	11	11	11	22	11	13	11	11			
O34	1	22	22	11	14	11	11	11	14	22	11	13	11	11			
O35	1	11	13	11	33	11	11	12	11	22	11	13	11	11			
O36	1	12	33	11	34	11	12	12	12	22	11	11	11	11			
O37	1	22	11	11	33	11	11	11	11	22	11	13	12	11			
O38	1	11	23	11	22	11	11	12	11	12	11	11	11	11			
O39	1	22	13	11	22	11	11	12	14	22	11	11	12	11			
O40	1	22	23	11	22	11	11	11	14	12	11	11	11	11			