Genetic and clonal diversity for *Myrica cerifera* along a spatiotemporal island chronosequence

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Changes in genetic diversity and clonal structure were investigated along a spatiotemporal island chronosequence for the shrub *Myrica cerifera*. On our study site, Hog Island, Virginia, USA, island movement creates a sequence of dune ridges and intervening swales along an east–west axis of the island that produces an age-structured geomorphology. This substrate-mediated age structure, called the chronosequence, superimposes itself upon cohorts of *M. cerifera* that colonize behind nascent dune ridges as they are formed. This chronosequence allowed comparisons of levels of genetic diversity and clonal structure among different aged cohorts of *M. cerifera*. We observed little change in allelic diversity along the chronosequence and no evidence for

heterosis, although there was moderate change in genotypic diversity. The spatial distribution of individuals within 10 plots established along three transects intersecting the island chronosequence identified a nonrandom spatial distribution of individuals in all cohorts, with increasing aggregation of above-ground stems into multistemmed clusters in the older sites. This aggregation of individuals did not correspond to a significant increase in clonal growth with cohort age, nor was there significant spatial genetic autocorrelation within any of the plots.

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Introduction

The distribution of genetic diversity among plant populations has been extensively investigated, with particular emphasis on its spatial distribution (Hamrick and Godt, 1996). A few studies have examined the temporal distribution of genetic diversity, typically comparing cohorts within populations, and sometimes different aged populations (Linhart *et al.*, 1981; Fore *et al.*, 1992; Giles and Goudet, 1997; Mahy *et al.*, 1999). Results from these studies vary, but some general conclusions are that younger cohorts often have lower genetic variation than older cohorts, and in particular younger cohorts exhibit less individual heterozygosity than older cohorts. Mechanisms that account for such patterns are rarely directly tested. It is often concluded that young cohorts or populations contain a subset of the genetic diversity observed in the larger, surrounding global populations, and that over time gene flow augments the limited genetic base (Dumolin-Lapegue *et al.*, 1997; Giles and Goudet, 1997). Temporal changes in genetic diversity within a cohort where recruitment has ceased cannot be explained by gene flow, whereupon overdominant selection is often invoked.

The importance and prevalence of overdominant selection in natural plant populations has been a subject of debate (Mitton and Grant, 1984; Savolainen and

Hedrick, 1995; Schierup, 1998). Although the observation of increased heterozygosity with age is common in natural populations of many woody plants (Bush and Smouse, 1992), the mechanisms responsible for increased heterozygosity are often unknown. Recent studies have suggested that selection against inbred individuals results in increased heterozygosity (Dudash and Carr, 1998). However, regardless of the mechanism and specific locus of selection, documenting the occurrence and prevalence of heterosis, and its magnitude in natural populations, remains relevant because of its potential importance in maintaining genetic diversity within populations.

Many factors other than heterosis affect levels of genetic diversity in natural populations. One such factor is clonal growth (Widen *et al.*, 1994). While many plant species regenerate clonally, rarely is this taken into account when addressing levels and distribution of genetic diversity. Clonal structure may reduce the number of genetically distinct individuals within a population and hence decrease the genetic effective population size, increasing genetic drift. Clonal structure may also result in positive spatial genetic autocorrelation, which may be falsely attributed to limited propagule dispersal distance or kin-structured colonization (Ingvarsson, 1998; Kalisz *et al.*, 2001). Alternatively, if clonal growth and overdominant selection operate in concert, an excess of heterozygosity may be observed together with increased allelic diversity within populations and decreased differentiation among them. While clonal and asexual populations may have similar levels of genetic diversity relative to exclusively sexually reproducing species (Ellstrand and Roose, 1987; Parker

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and Hamrick, 1992), the effect of increasing clonal structure on genetic diversity is not widely known. Since clonal plants begin as a single unit and then replicate to make a genet composed of multiple, genetically identical ramets, it is reasonable to expect temporal genetic changes within a clonally reproducing population, particularly with regard to genotypic diversity. Genetic studies of clonal plants have investigated diversity at a single stage of population development; thus how increased clonal growth affects populations is unknown.

In this study, we examine populations of the shrub *Myrica cerifera* that inhabit a barrier island which shows a spatially distributed age structure referred to as a chronosequence. Thus, areas that were colonized at different times remain separated from one another and serve as a snapshot of a population through time. This spatiotemporal chronosequence allows us to address how changes in clonal structure along the chronosequence affect allelic and genotypic diversity. Also, we describe changes in heterozygosity with age and how that process interacts with changes in clonal structure to alter patterns of genetic diversity at both spatial and temporal scales.

Materials and methods

Study species

Myrica cerifera L. (Myricaceae) is an evergreen dioecious, perennial shrub that reaches 6 m in height; it is common in coastal or disturbed inland sites in the Southeastern US (Radford *et al.*, 1968). Fruits of *M. cerifera* are single-seeded drupe, small, ≈ 4 mm in diameter, and are covered by a resinous waxy substance from which the species takes its common name, wax myrtle. Fruits mature in early fall and are eaten by migratory bird species, particularly *Dendroica coronata* (D. Erickson, personal observation). Seeds do not dehisce, and those that remain attached to the plant begin to lose their ability to germinate after 9 months (unpublished data). *Myrica cerifera* has a symbiotic association with a nitrogen-fixing bacterium (*Frankia* sp.), which is suggested to facilitate colonization of nutrient-poor sandy soils (Young *et al.*, 1992).

Study site

Our study site was Hog Island of the Virginia Coast Reserve, which is part of the Long Term Ecological Research (LTER) network, and located approximately 7 miles off the coast of Virginia, USA (Figure 1a). Hog Island has an unusual geomorphology defined by a distinct east–west chronosequence of dunes (Hayden *et al.*, 1991). Island movement results in a succession of dune ridges built over time, which are punctuated by high saline swales that separate vegetation growing on the dunes (Figure 1b). In general, studies of vegetation on Hog Island have observed that elevation controls the distribution of most plants. Many plant species, including *M. cerifera*, cannot grow in high saline depressions, yet require a low enough elevation for their roots to reach an underground lens of freshwater (Young *et al.*, 1994; Hayden *et al.*, 1995).

The combination of the geomorphology of Hog Island and the restriction of plants to mid-elevation sites results

Figure 1 (a) Location of the Hog Island field site relative to the continental US, with a larger schematic of Hog Island showing the position of the mature stands of shrubs with each of the three transects. (b) A diagram of the island chronosequence showing the topology that regulates the distribution of the shrubs and the location of the field plots. Other images of Hog Island and the distribution of vegetation can be viewed at the VCR LTER website: www.atlantic.evsc.virginia.edu.

in the age structure of the chronosequence superimposing itself upon the population structure of *M. cerifera*. Thus we find *M. cerifera* to be highly age structured along the east–west chronosequence of Hog Island (Shao *et al.*, 1998). On Hog Island, *M. cerifera* is the dominant woody species, and other common members of maritime forests are largely absent (Shao *et al.*, 1998). Thus, the potential effect of other species upon the maintenance of age structure of *M. cerifera* on Hog Island is eliminated. Additionally, *M. cerifera* is largely unaffected by even severe storms such that environmental disturbance has not significantly altered the age structure of the shrubs on Hog Island (Young *et al.*, 1995a,b). Likewise, Crawford and Young (1998) found that *M. cerifera* did not replace itself in canopy gaps, a property that reinforces the substrate-mediated age structure upon the plant population.

There are four major zones of *M. cerifera* vegetation: (1) a colonizing zone (CS) of shrubs <10 years old where seedling recruitment continues; (2) a leading edge (LE) of thicket expansion where the 15 to 25-year-old canopy is largely closed and recruitment is very limited; (3) a mid-Island site (MI) composed of 40 to 60-year-old-plants where the canopy is closed and no recruitment occurs; (4) a bay side (BS) site with plants of >60 years old, which is established on the most westward and oldest above-ground habitat on the island.

Data collection

In 1995, three transects intersecting *M. cerifera* along the chronosequence on Hog Island were established. Two transects intersect all four of the age zones of *M. cerifera*, while a third at the more narrow south end of the Island intersected only the CS and MI sites. Plots were square with a length of 15–20 m as needed to include ~45 above-ground stems in the sample. Every stem was mapped, tagged and was sampled for leaf tissue for allozyme analyses. Tissue was kept cold during transport to the University of Georgia (1–3 days), where enzymes were extracted. Enzymes were extracted from leaf tissue in a phosphate-PVC buffer (Mitton *et al.*, 1979), and the extract was absorbed onto filter paper wicks and stored at –70°C until analysis. Samples were subjected to electrophoresis for 3–5 h in 10% starch gels, stained and scored for polymorphism.

Six polymorphic allozyme markers were used in the analysis. These enzymes were phosphoglucose mutase 1 and 2 (PGM1 and PGM2), Shikimic dehydrogenase (SKDH), 6-phosphoglucose dehydrogenase (6-PGD), fluorescent esterase (FE) and phosphoglucose isomerase (PGI). PGM 1 and 2, 6-P and SKDH were resolved on buffer system 11 (Soltis *et al.*, 1983), while FE and PGI were resolved on a Poulik buffer (Mitton *et al.*, 1979). Recipes for the enzyme stains are found in Soltis *et al.* (1983).

Statistical analyses

Several parameters commonly used to measure genetic diversity were calculated for each of the 10 sites. These were the proportion of polymorphic loci (*P*) among the six loci used, observed (H_o) and expected (H_e) heterozygosity and total number of alleles. H_e was calculated where $H_e = 1 - \sum p_i^2$, where p_i is the frequency of the *i*th allele at that locus. To examine changes in genotypic diversity caused by clonal growth, we calculated the genotypic diversity statistic *D* for each of our study plots (Ellstrand and Roose, 1987). Where $D = 1 - \sum \{(n_i(n_i - 1)) / [N(N - 1)]\}$, n_i is the number of

individuals of multilocus genotype *i* and *N* is the population size. The *D* value ranges from 0 to 1, where 0 reflects all individuals containing the same multilocus genotype, and 1 is where each individual has a unique multilocus genotype.

Spatial dispersion of individuals

As a result of the apparent change in the spatial distribution of individuals with age, we used a measure of spatial dispersion within each of the 10 plots. We employed a statistic $L(t)$, which is a modification of Ripley's $K(t)$, to test the spatial pattern of individuals, or stems, within a site relative to a random distribution at successively greater distance intervals (Ripley, 1977; Peterson and Squiers, 1995). The experimenter chooses the distance interval (*t*) tested, and then spatial dispersion is tested for all increments of *t* up to half the size of the plot. We calculated dispersion via $L(t) = t - [K(t)/\pi]^{1/2}$, and then plot $L(t)$ vs *t* to examine the spatial dispersion of stems at all distance classes *t*; the parameter *L* is favored over *K* since the shape of *K* with increasing *t* is exponential, whereas *L* fluctuates around zero for all *t* when dispersion is random; $L(t)$ is negative when clumped and positive when even or hyperdispersed. We chose the increment of *t* to be 0.5 m; thus *L* is calculated for 0.5 m and then at each successive 1/2 m interval until the largest distance interval, which is specified as 1/2 the size of the smallest side of the plot. Each graph of $L(t)$ vs *t* has a 95% confidence envelope, where the upper 95% represents hyperdispersion and the lower 95% represents a clumped distribution, and $L(t)$ values between the upper and lower confidence intervals (CIs) correspond to random dispersion.

Spatial genetic structure

To examine the distribution of genotypes within each of the 10 plots, we estimated a coefficient of coancestry, r_{ij} . The parameter r_{ij} measures the multilocus genetic correlation among pairs of individuals across a range of distance intervals (developed by Dr J Nason from Cockerham, 1969). For a given distance interval, all pairs of individuals that occur within it will be analyzed for coancestry. The r_{ij} value for that distance interval is then presented as a mean value across all pairs analyzed. The coefficient of coancestry r_{ij} is calculated as

$$r_{ij} = \left\{ \sum_{ij} [(p_i - p^*) / (p_j - p^*)] / kp^*(1 - p^*) \right\}$$

where p^* is the frequency of allele *p* in the population, p_i and p_j are the frequencies of allele *p* in individuals *i* and *j*, respectively, and *k* is the number of pairs for which r_{ij} is calculated. The six allozyme loci were used in conjunction with the two-dimensional map coordinates of each individual within a plot to generate r_{ij} values for distances of 0.5 and up to a 0.5 m interval. The maximum distance interval depends upon the largest distance among any pair of individuals within a plot. A 95% CI for r_{ij} was generated by simulation through randomly assigning map positions to extant genotypes and then recalculating r_{ij} . This randomization procedure was repeated 19 times at each distance interval to generate

Table 1 Estimates of genetic diversity within each of the 10 study plots (the number of above-ground stems in each plot is shown in parentheses)

Transect	Plot (N)	A_e	H_o	H_e	F_{IS}	<i>D</i>
1	CS (45)	1.10	0.094	0.084	–0.044	0.69
1	LE (48)	1.22	0.158	0.160	0.124	0.92
1	MI (38)	1.27	0.114	0.190	0.296	0.82
1	BS (47)	1.26	0.194	0.196	0.002	0.90
2	CS (44)	1.12	0.106	0.094	–0.074	0.72
2	MI (44)	1.12	0.102	0.097	–0.020	0.60
3	CS (47)	1.16	0.110	0.125	0.636*	0.73
3	LE (42)	1.20	0.123	0.144	0.128	0.76
3	MI (48)	1.10	0.069	0.080	0.075	0.60
3	BS (44)	1.27	0.063	0.150	0.614*	0.76
Mean	(446 total)	1.18	0.117	0.140	0.173	0.75

A_e : effective number of alleles; H_o : observed heterozygosity; H_e : expected heterozygosity; F_{IS} : inbreeding coefficient; *D*: multilocus diversity statistic. Mean values are calculated when all 446 individuals are pooled; **P* < 0.05.

Table 2 Estimates of allelic diversity and structure for each locus across all 10 plots

Locus	H_T	H_S	F_{ST}	F_{IS}	χ^2
Pgm1	0.321	0.309	0.036	0.133	39.55 (18)***
Pgm2	0.086	0.082	0.055	0.067	45.86 (9)***
SKDH	0.071	0.066	0.075	-0.049	62.91 (9)***
6-Pgd	0.101	0.098	0.029	0.009	24.09 (9)**
FE	0.224	0.215	0.038	0.317	26.31 (9)**
PGI	0.024	0.023	0.021	0.008	17.80 (9)*
Mean	0.138	0.132	0.042	0.074	

H_T and H_S are total and population level heterozygosities respectively. F_{IS} and F_{ST} are departures from Hardy-Weinberg equilibrium because of deviation within and among the 10 plots. χ^2 tests for differences in allele frequency among the 10 sites. Mean values are averages among all loci. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

a 95% confidence envelope around a mean of no spatial genetic structure. Only 19 simulations were performed because of limited access to computers where the analysis was run and because of our observation that when several simulations were repeated 99 times it made no difference in the outcome of the experiment.

Results

Diversity

No significant differences in genetic diversity were observed among the different aged sites or among the three transects (Table 1). We found significant differences ($P < 0.05$) in allele frequencies among the 10 sample sites at each locus (Table 2). However, the statistically significant differences in allele frequency among sites did not produce high F_{ST} values. Rather, F_{ST} values for all six loci were low, with a mean of 0.042. Low F_{ST} values are consistent with the life history of the species, since outcrossing, wind-pollinated plants typically have low F_{ST} values (Hamrick *et al.* 1993; Hamrick and Godt 1996). The significant differences in allele frequencies observed are at least partially explained by the high sensitivity with which significance is inferred when using large sample sizes. There was also no evidence for heterosis in these populations (Table 3). There was an increase in both observed and expected heterozygosity among the youngest (CS) and oldest (BS) sites (Table 3), but the increase was not monotonic. There was also an increase in the difference between observed and expected heterozygosity (Table 4) with age such that F_{IS} was less in the CS (0.010) than in the BS sites (0.301). However, increases in both observed and expected heterozygosity with age were not significant (Table 3). Additionally, variance in allele frequency among sites was greater among individual sites (Table 2) than that observed among different age classes (Table 4). There was also no difference in the relative frequency of males and females along the chronosequence (Table 3).

Spatial and clonal structure

Along the chronosequence, we observed an increase in the grouping of individuals into multistemmed clusters. At the youngest sites on the island, no multistemmed

Table 3 Genetic diversity statistics for each age class pooled for all sites within that age class

Site	N	%P	AP	H_o (SE)	H_e (SE)	D	A	M/F
CS	135	100	2	0.104 (0.026)	0.103 (0.043)	0.73	12	0.98
LE	90	83.3	2	0.142 (0.038)	0.153 (0.053)	0.82	11	1.02
MI	130	100	2	0.093 (0.026)	0.119 (0.043)	0.70	12	0.92
BS	91	100	2	0.130 (0.037)	0.186 (0.054)	0.85	12	1.11
Mean	446	96.8	2	0.117 (0.016)	0.140 (0.024)	0.75	12	0.97

N: number of individuals for each age class; %P: percent polymorphic loci; H_o : observed heterozygosity with standard errors in parentheses; H_e : expected heterozygosity with standard errors in parentheses; D: index of multilocus genotypic diversity; A: number of alleles present within a cohort. The relative number of males to females is presented as the quotient of the number of males divided by the number of females.

individuals were apparent, but in the oldest sections, most individuals were within multistemmed aggregates (Figure 2). We tested the significance of the observed change in spatial patterning along the chronosequence with Ripley's L . For all plots, the test statistic fell below the lower 95% confidence bound (Figure 2), reflecting significant aggregation of stems. A random distribution was observed in only two sites (T1-MI and T3-LE), and only at a few of the intervals tested ($t = 5$ and 6 for T1-MI, and 7 and above for T3-LE). Thus, although there appears to be an increase in this spatial clustering with age, individuals at all age classes were significantly clumped.

The increase in multistemmed architecture along the chronosequence does not appear to be because of clonal growth. Few multistemmed clusters were composed of a single genotype. Clustering of unrelated individuals appears to be the consequence of a nonrandom distribution of individuals described above rather than of an increase in size and clonal spread individual genets. In the CS sites, individuals, particularly juveniles colonizing in 1997, were significantly clumped. Thus, the spatial aggregation observed in very early stages was maintained at all age classes and was independent of genotype. This lack of clonal structure is mirrored in the high D value (Table 1). The D statistic does not change consistently with age for any of the three transects (Table 1). Pooling similar aged sites also does not result in a consistent trend of increasing clonal structure (decreasing D value) with age (Table 3). Values of D are high given the level of allelic diversity observed, and suggest a general absence of clonal structure. Likewise, for all 10 plots the calculation of spatial genetic autocorrelation, r_{ij} , fell within the 95% confidence envelopes, indicating a lack of spatial genetic structure at all intervals tested (Figure 3). Estimates of F_{IS} that may indicate clonal growth and reproduction varied among sites. The mean of F_{IS} for all sites is low (Table 1), suggesting no clonal structure, with two of the 10 sites (T3-CS and T3-BS) exhibiting significantly positive F_{IS} values (Table 1). However, neither of these sites were observed to have a positive r_{ij} , nor did they have low D values.

Figure 2 Scatter plots of all 10 study plots are shown with corresponding graphs of spatial structure, $L(t)$, of individuals within the plots. Graphs of spatial structure contain the test statistic, and a 95% confidence envelope defining the upper and lower boundary of random dispersion. Values of $L(t)$ above the 95% CI are hyperdispersed, and those below are clumped. Plots are labeled by letter, with a–d representing the BS, MI, LE and CS plots of Transect 1. Likewise, (e) and (f) are MI and CE for transect 2, and g–j are BS, MI, LE and CS for transect 3.

Table 4 Estimates for plots pooled within each age class

Locus	H_T	H_S	F_{ST}	F_{IS}	χ^2
Pgm1	0.321	0.319	0.005	0.163	8.3 (6)
Pgm2	0.086	0.085	0.009	0.110	7.8 (3)
SKDH	0.071	0.070	0.014	0.015	12.1 (3)*
6-Pgd	0.100	0.100	0.014	0.024	11.7 (3)*
FE	0.233	0.217	0.030*	0.322	19.9 (3)**
PGI	0.024	0.024	0.006	−0.018	4.5 (3)
Mean	0.138	0.136	0.010	0.103	

H_T and H_S are total and population level heterozygosities, respectively. F_{IS} and F_{ST} are departures from Hardy–Weinberg equilibrium because of deviation within and among the 10 plots. χ^2 tests for differences in allele frequency among the 10 sites. Mean values are averages among all loci. ** $P < 0.01$, * $P < 0.05$.

Discussion

Studies examining separate cohorts or age groups within populations for evidence of heterosis or increased genetic diversity offer mixed results. Some studies have found increased genetic diversity and heterozygosity, or de-

creased genetic structure with increasing cohort age (Smyth and Hamrick, 1984; Giles and Goudet, 1997). Others have found little temporal differentiation among cohorts (Mahy *et al.*, 1999; Schnabel and Hamrick, 1990; Linhart *et al.*, 1981). In this study, we have only weak evidence for increased diversity and decreased genetic structure with increasing cohort age. There was a slight but nonsignificant increase in most genetic diversity parameters with age (Table 3), which was mirrored by low, but significant, F_{ST} values among the four age classes (Table 4). Nor is their compelling evidence for heterosis in this population. In fact, we observed a decrease in observed heterozygosity relative to expected heterozygosity with increasing population age (Table 3). If this difference was significant, it would suggest increased inbreeding or increased spatial genetic structure in the older age classes. However, we did not observe a significant increase in clonal structure as measured by the multilocus genotypic index, D . Although we do not see a decrease in D with age, and hence the suggestion of little clonal structure, we did see an increase in the number of multistemmed clusters composed of a single genotype. The observed increase in F may actually be a result in part of increased clonal

Figure 3 Plots of r_{ij} , which measures spatial genetic autocorrelation, are shown for all 10 study plots. Each panel shows the test statistic and a 95% confidence envelope. The r_{ij} statistic is plotted over a series of distance intervals as described in the text. Values of r_{ij} that lie above the 95% CI imply increased relatedness among pairs of individuals at that distance class, while values below the 95% CI imply decreased relatedness relative to random. Values of r_{ij} that fall within the 95% CI suggest that individuals are no more or less related than expected at random.

structure. The observation of a slight, though non-significant, increase in genetic diversity along the chronosequence is difficult to explain since the lack of recruitment in older cohorts precludes the addition of genetic diversity because of gene flow. Levels of genetic diversity in the younger sites were relatively high, indicating that there is little evidence for a founder effect in these newly colonized sites.

Analysis of the spatial structuring of individuals along the chronosequence with Ripley's L demonstrated a nonrandom spatial distribution for all age classes (Figure 2). This was not surprising since differences in micro-elevation regulate the distribution of newly established plants. The spatial patterning of *M. cerifera* on Hog Island suggests that clonal growth may increase with age. In contrast, the two measures used to infer clonal structure suggested that little clonal growth occurs in these populations of *M. cerifera*. The multilocus genotypic diversity statistic D did not decrease with age (Table 3), and genetic analyses of the multistemmed clusters showed that relatively few are composed of a single genotype. Rather, multistemmed clusters are typically composed of two or more genetically distinct individuals growing adjacent to one another. Although most clusters of stems are composed of genetically distinct individuals, there is an increase in the number of multistemmed clones relative to the CS sites. Therefore, the initial effects of seed dispersal and patchy establishment on the spatial distribution of individuals are maintained in the older aged sites. The limited clonal reproduction observed enhanced the initial spatial clustering observed in the colonizing sites, yet without significantly increasing spatial genetic structure.

Furnier *et al* (1987) investigated the clonal structure of whitebark pine, *Pinus albicaulis*, which exhibits a similar multistemmed architecture. They found that clusters of individuals were composed of genetically distinct individuals, but that individuals within a given cluster

are more closely related to one another than they are to individuals in separate clusters. The behavior of Clark's nutcracker, *Nucifraga columbiana*, likely generates this family structure through the caching of seeds from a single maternal plant, or kin-structured dispersal. This pattern of family structure results in a nonrandom distribution of individuals and alleles in space and hence some positive spatial genetic autocorrelation. Likewise, Hamrick *et al* (1993) found significant spatial genetic heterogeneity in a small tropical tree *Swartzia simplex* var. *ochracea*, which is also bird dispersed. They observed that *S. simplex* had the finest scale of genetic structure of the three species studied, and little decrease in genetic structure with age occurred, unlike the other, larger, tree species examined.

We found no evidence for the structuring of multilocus genotypes in space at any distance interval (Figure 3). Smouse and Peakall (1999) suggest that studies that fail to find spatial genetic structure may be flawed because of the limited resolution of methods such as Moran's I , which are based upon the spatial distribution of individual alleles. However, the measure r_{ij} is a true multilocus estimate of genetic correlation among spatially distributed genotypes (after Cockerham, 1969; see also Heywood, 1991 and Loiselle *et al*, 1995); yet we nonetheless failed to detect any spatial genetic structure. Our results suggest that individuals within a cluster are no more closely related to one another than to individuals in other clusters. The nonrandom spatial distribution of individuals contrasts with the random spatial distribution of alleles and suggests a complete absence of family-structured dispersal. The nonrandom aggregation of individuals into multistemmed clusters is similar for *M. cerifera* and *P. albicaulis*, but is likely because of different mechanisms, seed caching on the one hand and a pattern of patchy recruitment and establishment on the other. Kalisz *et al* (2001) used a similar multilocus estimator of spatial genetic structure in *Trillium grand-*

florum and observed differences in spatial genetic structure among different aged cohorts. They attributed the nonrandom distribution at later aged stages to the limited seed dispersal, but the lack of spatial genetic structure at younger aged cohorts was attributable to random pollen dispersal among individuals within the population. With *M. cerifera*, there does not appear to be limited seed dispersal, such that the nonrandom spatial distribution of individuals is uncoupled from the random distribution of genotypes and alleles. This is likely attributable to long-range seed dispersal by *D. coronata*, without associated kin-structured dispersal, as well as a random pollen gene pool.

The random spatial distribution of alleles and genotypes on Hog Island, in conjunction with the absence of pronounced genetic structure at any spatial scale as inferred through spatial genetic autocorrelation and F_{ST} , suggests that the entire island is a single large and nearly panmictic unit. The nonrandom spatial distribution of individuals appears to have no effect upon genetic diversity or structure in this population, and while the moderate increase in clonal structure may affect genotype frequencies, it has no overall effect upon levels of genetic diversity. The genetic structure of the population appears to be determined by a combination of gene flow and environmental disturbance, rather than by genetic drift or natural selection. The absence of significant spatial genetic structure at any scale suggests a primary role of gene flow in determining the genetic structure of population. The absence of heterosis with age may be attributed to the stochastic growing environment, where environmental disturbance rather than selection determines survival.

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