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# Relationships between bed age, bed size, and genetic structure in Chesapeake Bay (Virginia, USA) eelgrass (*Zostera marina* L.)

Jennifer M. Rhode[†,*] & J. Emmett Duffy
*School of Marine Science, College of William and Mary, Virginia Institute of Marine Science, P.O. Box 1346, Gloucester Point, VA 23062, USA; [†]Current address: Department of Biological and Environmental Science, Georgia College and State University, CBX 081, Milledgeville, GA 31061, USA; ([*]Author for correspondence: fax: (478)445-5290; e-mail: jennifer.rhode@gcsu.edu)*

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**Abstract**

Genetic structure and diversity can reveal the demographic and selective forces to which populations have been exposed, elucidate genetic connections among populations, and inform conservation strategies. Beds of the clonal marine angiosperm *Zostera marina* L. (eelgrass) in Chesapeake Bay (Virginia, USA) display significant morphological and genetic variation; abundance has fluctuated widely in recent decades, and eelgrass conservation is a major concern, raising questions about how genetic diversity is distributed and structured within this metapopulation. This study examined the influence of bed age (<65 years versus <6 years) and size (>100 ha versus <10 ha) on morphological and genetic (allozyme) structure and diversity within Chesapeake Bay eelgrass beds. Although both morphology and genetic diversity varied significantly among individual beds ($F_{ST} = 0.198$), neither varied consistently with bed age or size. The Chesapeake eelgrass beds studied were significantly inbred (mean $F_{IS} = 0.680$ over all beds), with inbreeding in old, small beds significantly lower than in other bed types. Genetic and geographic distances within and among beds were uncorrelated, providing no clear evidence of isolation by distance at the scale of 10's of km. These results suggest that local environmental conditions have a greater influence on plant morphology than do bed age or size. They support the hypotheses that eelgrass beds are established by multiple founder genotypes but experience little gene flow thereafter, and that beds are maintained with little loss of genetic diversity for up to 65 years. Since phenotypic and genotypic variation is partitioned among beds of multiple ages and sizes, eelgrass conservation efforts should maximize preservation of diversity by minimizing losses of all beds.

## Introduction

The diversity and distribution of genotypes can provide information about a population's history, including disturbances, demography, local adaptation, and selective events (Van Dijk 1987, McCauley et al. 1995, Harada and Iwasa 1996, Linhart and Grant 1996). Genetic diversity in vegetatively-reproducing organisms can be measured at two levels: the number of different clones (i.e., multilocus genotypes or genets), and the total allelic diversity. Genetic diversity is generally measured using neutral markers (Endler 1986), but it can also be reflected in morphological and physiological traits. Levels and distribution of genetic diversity can influence populations profoundly, and these measures can be used to inform conservation efforts. For instance, genetically diverse populations are better able to adapt to environmental changes, while those with lower diversity tend to be more vulnerable to extinction (Beardmore 1983).

A population's genetic diversity is influenced by demography, especially its age and size (Wright 1978, Oostermeijer et al. 1994; Weidema et al. 1996). If migration and genetic drift are the primary processes affecting populations, then young populations, which have experienced little immigration and are more susceptible to drift, will often have the lowest genetic diversity. If selection is an important force acting on populations, then diversity of older populations might be reduced as unfit individuals are lost (Beardmore 1983); this process might be expedited in clonal species, where the best-adapted genotypes increase in relative abundance through vegetative propagation as well as sexual reproduction. Finally, larger populations also might be expected to show relatively high genetic diversity simply due to greater numbers of individuals. This relationship can be complicated in clonal organisms, however, by discrepancies between census of population size and number of genotypes, which can vary widely among populations. Because population age and size are often correlated, it can be difficult to differentiate between their effects, especially in populations of clonal plants (Eriksson 1993).

Eelgrass (*Zostera marina*) is an interesting subject for studies of population genetic structure because of its great economic value (including its role as habitat for species of commercial interest) (Costanza et al. 1997), broad distribution (den Hartog 1970; McRoy and Helfferich 1977), clonal and sexual reproductive strategies (Orth et al. 1994; Ruckelshaus 1994; Ewanchuk and Williams 1996; Harwell 2000; Rhode 2002; Rhode and Duffy 2004), dispersal *via* both shoots and seeds (Harwell and Orth 2002a), and morphological diversity (Rhode 2002). In addition, seagrasses worldwide have declined precipitously in recent decades; they are now targets for conservation and restoration in many countries (McRoy 1996), including the United States, and restoration efforts are especially intense in Chesapeake Bay (Virginia, USA). Initial allozyme studies of eelgrass population genetics in North America and Europe concluded that eelgrass populations contained very little genetic diversity (Gagnon et al. 1980; McMillan 1982; Heij and Nienhuis 1992). Observations of rapid vegetative growth, low flowering rates (Phillips et al. 1983), and limited seed (Orth et al. 1994) and pollen (Ruckelshaus 1996) dispersal supported conclusions from the initial genetic data. Researchers concluded that most *Zostera marina* reproduction was clonal (McMillan 1982) and that eelgrass responded to environmental variation primarily *via* phenotypic plasticity. More recent surveys using additional allozyme loci or DNA-based molecular markers (RFLPs, microsatellites) have found more genetic and phenotypic substructuring both within and among patches of eelgrass (Fain et al. 1992; Laushman 1993; Erikkson 1993; Alberte et al. 1994; Ruckelshaus 1996; Williams and Orth 1998; Reusch et al. 1999a). Such studies also supported earlier hypotheses that clones, though sometimes very small, tended to be large (range: 2–5000 ramets per clone; Reusch et al. 1999b), and that clonal propagation, not sexual reproduction, was the primary force structuring eelgrass demography (Reusch et al. 1999c).

Chesapeake Bay eelgrass populations have experienced substantial areal declines in recent decades (Orth and Moore 1983), which might have important implications for genetic structure and connectivity among beds. Several historical events have strongly impacted these eelgrass populations and might be apparent in the beds' population genetic structure. First, Chesapeake eelgrass probably went through a demographic bottleneck in the 1930s, when an outbreak of the pathogenic slime mold *Labyrinthula* sp. apparently caused the regional demise of eelgrass (Rasmussen 1977; Short et al. 1987). Later, in the 1960s and 1970s, freshwater input from Tropical Storm Agnes, combined with anthropogenic eutrophication and high sediment input, further decimated many Chesapeake Bay eelgrass populations (Orth and Moore 1983); some of these have yet to recover fully (Orth et al. 1994; Orth et al. 2001). Because the size and persistence of Chesapeake Bay eelgrass beds has been monitored for over 65 years in unusual detail, the influence of known population parameters (age, size) on genetic structure of Chesapeake Bay eelgrass can be examined.

In this study, we used a metapopulation of eelgrass (*Z. marina*) of known history to test the influence of bed size and age on patterns of genetic diversity. This study was motivated by observations of significant interpopulation differences in eelgrass morphology within Chesapeake Bay and by the need to create conservation and restoration strategies for these beds. The survey included beds

of: (1) similar sizes (areal coverage of eelgrass) but different ages (persistence of eelgrass in an area), and (2) similar ages but different sizes. Allozyme electrophoresis was used to estimate the magnitude and spatial arrangement of genetic diversity within and among beds. First, relationships among genetic diversity, bed age, and bed size were examined. Next, relationships between genetic differentiation and geographic distance among eelgrass beds were explored. Finally, these data were used to make both inferences about demographic forces that structure these populations and recommendations for eelgrass conservation and restoration.

## Methods

### *Field sampling*

This genetic survey included 12 disjunct *Zostera marina* beds (Figure 1). Aerial photographs and ground monitoring records (US Environmental Protection Agency Chesapeake Bay Program; Orth et al. 1998 and earlier reports; R. J. Orth pers. comm.) were used to identify historically persistent beds, designated old (greater than 65 years old), and recently founded eelgrass beds, designated young (less than 7 years old). Four recently founded and four historically persistent


*Figure 1.* Map of Chesapeake Bay (Virginia, USA) indicating locations of beds surveyed for this study.

patches of less than 10 ha areal coverage (small) were included in this survey. Though smaller beds are present throughout Chesapeake Bay, they were not used in this study because very small beds: (1) might be transient, and are therefore, unlikely to be targets of conservation concern, and (2) are difficult to select randomly because beds less than a few meters, in diameter do not appear in aerial photographs. Four old, large (greater than 100 ha areal coverage; an order of magnitude larger than small beds) patches were also surveyed. Areal coverage was assumed to be related to the number of individuals within a population, though the relationship is probably not linear (Harwell 2000). Thus, the total number of beds surveyed included four old, large beds; four old, small beds; and four young, small beds.

Using GIS (Geographic Information System) technology and aerial photographs from the Virginia Institute of Marine Science's Submerged Aquatic Vegetation mapping laboratory (http://www.vims.edu/bio/sav/), 100 random, non-clustered GPS (Global Positioning System) sampling points were generated for each eelgrass bed. To maintain a balanced statistical design, the same number of sampling points was used for each bed, regardless of bed size (as in Williams and Davis 1996). Sampling points were at least 2 m apart (as in Ruckelshaus 1994) to minimize the probability of sampling a single genet more than once (but see Reusch et al. 1999a).

In the field, each point was located using a combination of GPS tracking and ground-based triangulation. Eelgrass shoot density was measured by counting individual shoots within a $10 \times 10$ cm quadrat and extrapolating this to shoots per $m^2$. At each point a single *Z. marina* shoot was collected and stored in cool water to preserve protein integrity until laboratory extractions.

All samples were collected within a five-week period in spring 1998. The restricted time frame was chosen to minimize the chance of confounding temporal effects on population genetic structure. Spring sampling was also advantageous because collections were done at the point of maximal population stability, before a new generation of seeds recruited and before eelgrass's predictable summer defoliation (Orth and Moore 1986).

*Morphometric and genetic analyses*

In the laboratory, number of blades and the length and width of the longest blade were recorded for each shoot. Blade area was then used as a proxy for plant fitness since size is both a good predictor of reproductive value (Caswell 2000) and an important component of fitness for plants that reproduce asexually, *via* vegetative propagation. A preliminary survey of randomly collected plants from 4 of the 12 sites sampled herein showed that, for *Z. marina*, blade area is closely correlated with biomass (linear regression; $r^2 = 0.662$, $df = 1/159$, $F = 311.9$, $P = 0.0001$), and there is also a correlation between number of blades and biomass (linear regression; $r^2 = 0.245$, $1/159$ $df$, $F = 51.4$, $P = 0.0001$). Since plants were collected before the time of seed set, it was not possible to make a more direct measure of fitness.

The methods of Williams and Orth (1998) were used to extract proteins from each shoot's primary (youngest) blade. Briefly, blades were rubbed with Kimwipes and rinsed in distilled water to remove any epiphytes. A mixture of eelgrass and extraction buffer was ground with a mortar and pestle, and the extract was divided into four aliquots, which were distributed among cell well plates. Quadruplicate protein extracts were stored at −80 °C until electrophoresis. Sample division allowed replicates to be run at multiple times or on different buffer systems without subjecting an individual sample to potentially destructive freeze-thaw cycles.

Subsets of the samples were screened with 34 allozyme buffer/stain systems (Soltis et al. 1983, Richardson et al. 1986; Murphy et al. 1996; Williams and Davis 1996; Williams and Orth 1998) to identify systems that produced consistently scorable bands for these samples. Of the 34 systems, seven yielded visible and reliably scorable bands for all test samples. All seven systems were used to test extracts from all 1200 shoots. Systems used were as follows: tris-citrate buffer for ADH; morpholine-citrate buffer for GPI-1, GPI-2, IDH, MDH-1, MDH-3, and ME. Five of these systems were also used in Williams and Orth (1998): GPI-1, GPI-2, IDH, MDH-1, and MDH-3. Gels for all stain systems were run under current and time conditions identical to those reported in Williams and Orth (1998). After gels had run, they were sliced and stained according to the methods

of Williams and Orth (1998) and Murphy et al. (1996). All gel slices were scored and photographed; an autoimage analyzer archived pictures to allow electronic comparison of gel banding patterns.

*Data analyses*

Measurements of shoot density, blades per shoot, shoot length, and shoot width were subjected to Principal Components Analysis (PCA) to detect relationships among these parameters and to generate a composite morphological variable for each plant (SAS 1999). Data for all allozyme loci were collapsed to generate a composite genotype for each plant. Composite genotype data were entered into Arlequin (Schneider et al. 2000), which generated indices of genetic diversity for beds and, when appropriate, for individuals. The calculated indices included $P$, the percent of loci (of 7) that revealed polymorphisms (i.e., frequency of the most common allele $<99\%$); A, the mean number of alleles over all seven loci; $G$, number of multilocus genotypes within a bed; and $H$, observed heterozygosity (Endler 1986). Observed and expected heterozygosities were also compared to determine whether populations were in Hardy–Weinberg equilibrium. Finally, Wright's (1978) $F$ statistics were calculated in Arlequin (Schneider et al. 2000). $F_{ST}$ measured the amount of genetic subdivision among all beds. $F_{IS}$ estimated the degree of inbreeding within the population, although in a clonal organism this value can be biased by multiple samplings of individual clones. We attempted to minimize the incidence of resampling intact clones by collecting samples at least 2 m from one another. To differentiate between true inbreeding and multiple clone sampling, we also recalculated $F$ statistics using only one individual per genotype in each population, with the expectation that $F_{IS}$ would not change if it was due to inbreeding rather than replicate sampling of individual clones. $F_{IS}$ values based on this reduced data set changed little (0.624 versus 0.680).

Nested ANOVA (Zar 1998; SAS 1999) was used to examine the influence of age, size, and age/size combinations on each genetic diversity measure. In these ANOVAs, site (i.e., $k = 4$ individual beds per bed type) was nested within bed type ($k = 3$: old and large, old and small, young and small), with 100 replicate plants per individual bed. Bed type was treated as a fixed factor. Because data did not meet ANOVA assumptions for some genetic variables, resampling analyses were used to test for differences among bed types. For a given variable, the values for the 12 beds were resampled (with replacement) 10,000 times using an Excel add-in (Blank et al. 1999), and, with each iteration, a mean value per bed type was calculated. The difference between the largest and smallest mean was then calculated, and the observed value was compared to the distribution calculated from the bootstrapped replicates. The number of bootstrapped replicates whose value was greater than this observed difference was divided by the total number of bootstrapped replicates to obtain a $P$-value.

Nested ANOVA (Zar 1998, SAS 1999) was also used to examine the relationship between each plant's genotype and morphotype, to see if the former was predictive of the latter. Analyses were conducted only for genotypes found in more than one of the 12 sites. In these ANOVAs, the composite genotype was nested within site, to account for environmentally-induced phenotypic variation, and the composite morphotype (based on PC1, which explained 96% of the variation in the data) was the dependent variable.

An ArcView (2001) macro (K. Farnsworth 2001 pers. comm.) was used to calculate geographic distances between all sampling points. Data for Nei's (1972) genetic distances were generated by Arlequin. We used Mantel tests (Schneider et al. 2000) to correlate genetic and geographic distances both within and among beds.

## Results

Eelgrass beds differed substantially in blade morphology (blade area; Figure 2a), blades per shoot (Figure 2b) and shoot densities (Figure 2c, $P < 0.0001$ for all), but no morphological measure differed significantly among the three bed types (old large, old small, young small). Thus, there was no consistent effect of bed age or size on eelgrass morphology.

Overall genetic diversity of the eelgrass beds surveyed was high. In samples from nearly 1200 eelgrass individuals, a total of 109 composite (7-locus) genotypes were found. Sixty-nine of these genotypes were unique to a single bed (52% of

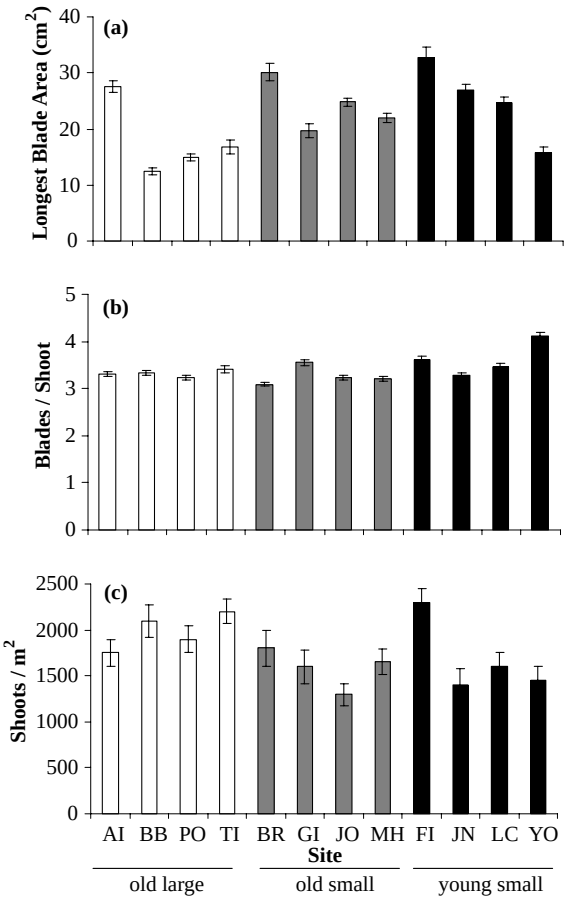


*Figure 2.* Morphological differentiation among 12 eelgrass beds by bed age and size. a) Mean (± 1 SE) longest blade area, b) number of blades per shoot, and c) shoot density. n = 100 per bed. Bed types are shown below site labels. Blade area, blades per shoot, and shoot density all differed among the 12 beds (1-way ANOVAs, $P = 0.0001$ for each), but not among the three age-size classes ($P > 0.05$ for all variables).

unique genotypes were heterozygous), while 40 genotypes were shared among more than one bed (45% of shared genotypes were heterozygous). Most of the seven polymorphic loci tested were polymorphic within all beds (range: 67–100% of beds), and mean allelic diversity ($A$) at a locus ranged from 1.50–2.00 over all beds and loci (Table 1). Genotype diversity (G) ranged from 0.12 to 0.40 (mean = 0.20) (Table 1). Although there was substantial variation among individual beds in $P$, $A$, and especially $G$, none of these genetic diversity measures varied significantly with bed age or size (Table 2), although $P$ tended to be lower in old, small beds (resampling analyses; $P = 0.0567$). The proportion of allozyme

*Table 1.* Measurements of genetic diversity for 12 Chesapeake Bay eelgrass beds: $P$, percent loci polymorphic; $A$, average allelic diversity; and $G$, proportion distinct genotypes. Values were based on composite genotypes (7 allozyme loci); $n = 100$ plants per bed. Resampling analysis ($n = 10{,}000$) showed no differences in $P$ ($P = 1.000$), $A$ ($P = 0.727$), or $G$ ($P = 0.906$) among bed types

| Bed type | Site | $P$ | $A$ | $G$ |
|---|---|---|---|---|
| Old, large | AI | 100 | 2.000 | 0.300 |
| Old, large | BB | 100 | 2.000 | 0.220 |
| Old, large | PO | 100 | 2.000 | 0.120 |
| Old, large | TI | 57.1 | 1.714 | 0.150 |
| Old, small | BR | 100 | 2.000 | 0.190 |
| Old, small | GI | 100 | 2.000 | 0.170 |
| Old, small | JO | 71.4 | 1.714 | 0.120 |
| Old, small | MH | 100 | 2.000 | 0.230 |
| Young, small | FI | 100 | 2.000 | 0.288 |
| Young, small | JN | 85.7 | 2.143 | 0.120 |
| Young, small | LC | 85.7 | 2.000 | 0.230 |
| Young, small | YO | 85.7 | 1.867 | 0.130 |

*Table 2.* Results of ANOVAs testing the effects of (1) age and (2) size on measures of genetic variation

| | df (test/error) | MS | Error MS | P |
|---|---|---|---|---|
| *Age* | | | | |
| P | 1/10 | 0.0014 | 0.0768 | 0.8939 |
| A | 1/10 | 0.0033 | 0.1373 | 0.8805 |
| G | 1/10 | 0.0047 | 0.0064 | 0.4164 |
| H | 1/10 | 0.0032 | 0.0026 | 0.2955 |
| *Size* | | | | |
| P | 1/10 | 0.0291 | 0.0737 | 0.5451 |
| A | 1/10 | 0.0032 | 0.1373 | 0.8818 |
| G | 1/10 | 0.0060 | 0.0063 | 0.3531 |
| H | 1/10 | 0.0010 | 0.0029 | 0.5621 |

genotypes that were shared with at least one other site also did not vary consistently among bed types (Figure 3). Heterozygosity ranged from 0.21 to 0.87 among the 12 beds (Figure 4a), and all beds deviated from Hardy–Weinberg equilibrium (ANOVA; $P = 0.0002$) (Figure 4b) due to significant heterozygote deficiencies. $F_{IS}$ was variable among beds, ranging from 0 to 0.91 (mean = 0.68) (Figure 4c). Resampling tests showed that this inbreeding coefficient differed significantly among the three bed types ($P = 0.0321$), as old, small beds were less inbred on average than other bed types.

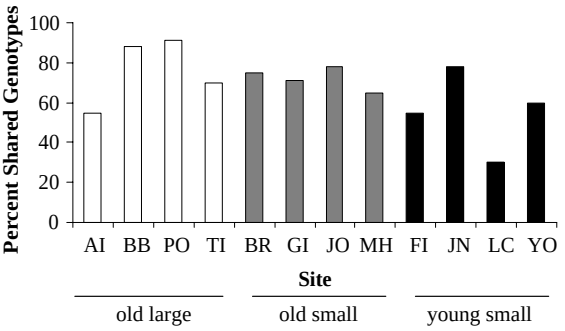


*Figure 3*. Percent of all composite genotypes within a site that are shared with at least one other site. Bed types are shown below site labels.

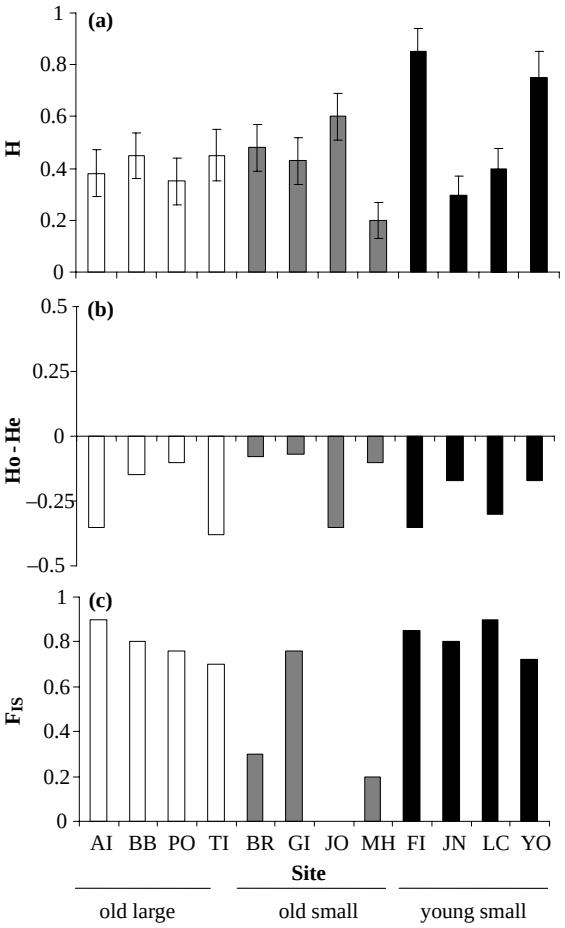


*Figure 4*. Measurements of heterozygosity and inbreeding for 12 Chesapeake Bay eelgrass beds. a) mean (±1 standard error) proportion heterozygous individuals (of n = 100) within bed (H), over 7 loci, b) deviation from expected heterozygosity for each bed, and c) $F_{IS}$, inbreeding coefficient, for each bed. Measurements of $H_O - H_E$ and $F_{IS}$ were based on composite genotypes (7 allozyme loci) of 100 plants per bed. $F_{IS}$ differed significantly among bed types (resampling analysis; n = 10,000; P = 0.032). Bed types are shown below site labels.

$F_{ST}$ over all beds was 0.1976, a high level of genetic substructuring (Wright 1978).

There was no relationship between genotype and phenotype (nested ANOVA; $df = 43/910$, $F = 0.90$, $P = 0.6630$), though there was a strong relationship between genotype and site (nested ANOVA; $df = 11/942$, $F = 10.45$, $P = 0.0001$). Mantel tests showed no relationship between Nei's (1972) genetic distance and geographic distance among beds ($r^2 = 0.0559$, $P = 0.0541$). Finally, there was no relationship between Nei's (1972) genetic distance and geographic distance within any bed (Mantel tests; for each bed, $P \geq 0.0650$).

## Discussion

We found strong genetic and morphological differentiation among the 12 eelgrass beds studied but no relationship between morphotype and genotype; most morphological variation could be attributed to site. These findings reinforce conclusions from transplant experiments (Rhode 2002) that morphological variation in Chesapeake Bay eelgrass is affected more by environmental than genetic factors. Phenotypic plasticity, a crucial adaptive strategy for many plant species (Sultan 2000; Agrawal 2001), evidently is responsible for much of the variation observed among these beds.

With respect to the study's primary question, we found no consistent difference in either morphology or genetic structure of eelgrass beds as a function of bed age. This is in contrast to a study of the seagrass *Posidonia oceanica*, which found that older beds contained more genetic diversity than their younger counterparts (Jover et al. 2003). There are several possible explanations for our results. First, the scale of age differences included in our study might have been too coarse. Studies by Harwell (2000) and Williams and Orth (1998) show that very young beds are especially variable in demographic characters; although they might have lower genetic diversity, these very young beds were excluded from our study due to practical considerations. Second, bed age estimates could be misleading. This study benefited from accurate data on bed persistence (presence of eelgrass at a particular spot), but beds are dynamic entities; though eelgrass coverage persists, individual genets might be relatively short-lived (Cerco

and Moore 2001). If demographic bottlenecks reduced the genetic diversity of Chesapeake Bay eelgrass, historically persistent (old) beds would be expected to have lower diversity than beds established after such population reductions and losses of diversity. Since old eelgrass populations were not significantly less diverse than their younger counterparts, these bottlenecks seem to have had little impact on genetic variance. This perhaps is not surprising, as both models and experiments suggest that population sizes must drop to very low levels before reducing genetic diversity significantly (Leberg 1992).

Because they contain more individuals, large beds were expected to have more genetic diversity and genetic substructure than small beds. Our findings did not support this expectation, although since sample size was standardized across bed classes, we may have reduced the likelihood of detecting such an effect. It is also possible that bed size is a function of clonal growth, with seed recruitment playing a less important role, so that larger beds contain more ramets but not more genets. Alternatively, the genetic diversity of small beds could be relatively high because they are founded by multiple clones (Oostermeijer et al. 1994), a hypothesis consistent with the observation that seeds are transported as sibling clusters attached to maternal reproductive shoots (Setchell 1929; Harwell and Orth 2002a).

The single strongest result of this genetic survey was the large degree of genetic differentiation among beds (high $F_{ST}$; Wright's scale of comparison; Wright 1978), a pattern also observed by Williams and Orth (1998). High population differentiation may appear inconsistent with the finding that most multilocus genotypes were shared among more than one bed (Figure 3). One possible explanation for these patterns is that the distribution of genotypes among Chesapeake Bay eelgrass beds reflects patterns of original colonization, as new beds were founded by floating, seed-bearing reproductive shoots. High $F_{ST}$ values would then reflect the random colonization and inbreeding that has occurred since beds were established, and suggest that post-colonization movements of genets has little effect on levels of population differentiation. Another possibility is that clonal identity was not, in fact, maintained over such large geographic distances, and that the apparently identical genotypes found in different beds are not, in fact, identical by descent. The relatively low resolution of allozyme markers (compared, for example, with microsatellites analysis or genome sequencing) could have lead to underestimates of clonal diversity and lumping genetically similar individuals into the category of a single clone.

Along with displaying strong genetic differentiation among beds, Chesapeake Bay eelgrass beds showed significant evidence of inbreeding. $F_{IS}$ was high over all beds, with significant heterozygote deficiencies, in striking contrast to European eelgrass beds (Reusch et al. 2000). Interestingly, old, small beds were the least inbred. Perhaps these beds receive more immigrants than other bed types. Alternatively, if beds are small because vegetative expansion of plants therein is weak, they could seem less inbred simply because a higher proportion of their reproduction is sexual.

Apparent heterozygote deficiencies in Chesapeake Bay *Z. marina* beds could be a consequence of multiply sampling the same clone, as eelgrass clones covering hundreds of square meters have been reported in some areas (Reusch et al. 1999b). Recalculating $F_{IS}$ using only one individual per clone changed its value little, so we concluded that this value was less likely to be a sampling artifact. Heterozygote deficiencies could also be attributable in part to Wahlund effects, an apparent reduction in genetic diversity that results from sampling multiple genetic populations and analyzing them as if they are a single population. Since dispersal of eelgrass pollen is somewhat limited (Ruckelshaus 1996), $F_{IS}$ is high, and selfing or close inbreeding is possible (Rhode and Duffy 2004), it is likely that beds (used here as units of population structure) are in fact mosaics of locally interbreeding groups, or neighborhoods, of plants. Finally, estimates of heterozygote deficiencies in Chesapeake Bay eelgrass beds could be accurate reflections of population structure. Extensive vegetative reproduction could create such population structure, as could non-random mating in the form of self-fertilization or inbreeding (including gamete exchange among clonemates). This is consistent with evidence that inbreeding occurs *in situ* with some regularity in eelgrass (Ruckelshaus 1996) and that Chesapeake eelgrass self-fertilizes with no apparent loss of fertilization success or seed set (Rhode and Duffy 2004); inbreeding without loss of reproductive effectiveness might be expected in

a population that inbred over enough generations to purge its deleterious alleles. Inbreeding in these beds might be further reinforced by pollen-dispersal distances of less than 15 m (deCock 1980; Cox et al. 1992; Ruckelshaus 1994, 1996), a range not broad enough to cover the unvegetated waters between beds (Williams and Orth 1998, Reusch et al. 1999b). This inbreeding, combined with extensive vegetative reproduction, could also help to explain the lack of relationship between geographic and genetic distance, even among beds less than 5 km apart. Though there is no isolation by distance at the scale of 10's of km, isolation by distance is probable over larger scales (for instance, between Chesapeake Bay and other eelgrass populations).

Genetic diversity is widely considered to buffer against changing environmental conditions and maintain the adaptive potential and resilience of populations of most species, including seagrasses (Ruckelshaus 1994; McRoy 1996; Williams and Orth 1998; Procaccini and Piazzi 2001; Williams 2001). Empirical studies have suggested that, in general, more genetically diverse populations have greater fitness (Oostermeijer et al. 1994). In fact, previous studies suggested that fitness of inbred eelgrass plants would be significantly lower than outbred plants (Reusch 2001) and showed an empirical correlation between population genetic diversity and bed growth for eelgrass specifically (Williams 2001; Hammerli and Reusch 2001). Because much genetic diversity is divided among high-diversity beds of eelgrass, the source from which transplanted material is taken for restoration efforts can greatly affect the genetic structure of the created population (Williams 2001). Data presented here suggest that the ideal size and diversity of restored beds could vary, and that small or young beds are not necessarily depleted in genetic resources. Instead of choosing source beds based on their size, age, or genetic diversity, our data suggest that, in the Chesapeake Bay, it is probably acceptable to choose beds according to convenience (i.e., proximity of donor bed to transplant site, bed depth, etc.), a strategy historically used by local restoration projects (Williams and Orth 1998; Orth et al. 1999). The longer-term effects of genetic homogeneity on these beds and of genetic homogeneity on sexual reproduction remain unknown, although correlative studies elsewhere suggest that low diversity may reduce eelgrass fitness (Williams 2001; Hammerli and Reusch 2001). We suggest that future studies monitor this eelgrass metapopulation over time to look for correlations between genetic diversity and performance or fitness parameters.

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