

# Population Structure and Genetic Diversity among Eelgrass (*Zostera marina*) Beds and Depths in San Francisco Bay

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## Abstract

The seagrass *Zostera marina* is widely distributed in coastal regions throughout much of the northern hemisphere, forms the foundation of an important ecological habitat, and is suffering population declines. Studies in the Atlantic and Pacific oceans indicate that the degree of population genetic differentiation is location dependent. San Francisco Bay, California, USA, is a high-current, high-wind environment where rafting of seed-bearing shoots has the potential to enhance genetic connectivity among *Z. marina* populations. We tested *Z. marina* from six locations, including one annual population, within the bay to assess population differentiation and to compare levels of within-population genetic diversity. Using 7 microsatellite loci, we found significant differentiation among all populations. The annual population had significantly higher clonal diversity than the others but showed no detectible differences in heterozygosity or allelic richness. There appears to be sufficient input of genetic variation through sexual reproduction or immigration into the perennial populations to prevent significant declines in the number and frequency of alleles. In additional depth comparisons, we found differentiation among deep and shallow portions in 1 of 3 beds evaluated. Genetic drift, sweepstakes recruitment, dispersal limitation, and possibly natural selection may have combined to produce genetic differentiation over a spatial scale of 3–30 km in *Z. marina*. This implies that the scale of genetic differentiation may be smaller than expected for seagrasses in other locations too. We suggest that populations in close proximity may not be interchangeable for use as restoration material.

**Key words:** gene flow, marine population genetics, natural selection, restoration genetics, San Francisco Bay, seagrass

Marine species that depend on currents for dispersal are predicted to show a positive relationship between dispersal potential and population connectivity, with greater connectivity leading to reduced population genetic differentiation (Bohonak 1999; Grosberg and Cunningham 2001). Although this relationship is often upheld, there are numerous exceptions in diverse taxa (e.g., Taylor and Hellberg 2003; Veliz et al. 2006). Within a single species, one might expect the pattern to be more consistent, with populations in areas with strong currents showing less genetic structure than populations living in low-current environments. However, this relationship may be disrupted by physical (Pineda et al. 2007) or biological (Morgan 1995) barriers to dispersal or if

selective forces prevent immigrants from successfully establishing in their new location (e.g., Koehn et al. 1976).

Eelgrass (*Zostera marina*) is a member of the group of marine angiosperms collectively called seagrasses that are suffering population declines globally (Short and Wyllie-Echeverria 1996; Orth et al. 2006; Waycott et al. 2009). Seagrasses reproduce sexually and asexually, the latter through rhizome extension (Tomlinson 1982) that often results in clusters of genetically identical meadow-forming shoots or ramets (Procaccini et al. 2007; Rozenfeld et al. 2007). Seagrass beds provide a number of valuable ecosystem services (reviewed in Kenworthy et al. 2006), including provision of foraging and nursery habitat for

several endangered marine animals (Hughes et al. 2009). Along the Pacific Coast of North America, *Z. marina* provides spawning habitat for commercially important Pacific herring (*Clupea harengus pallasii*), supports outmigrating juvenile salmon (*Oncorhynchus* spp.), and is an important food source for migrating and resident waterfowl (Phillips 1984; Wyllie-Echeverria and Ackerman 2003). Although seagrasses commonly grow in single-species stands (den Hartog and Phillips 2001; Moore and Short 2006), there is accumulating evidence of the importance of within-species genetic diversity on multiple ecological levels (reviewed in Procaccini et al. 2007; Hughes et al. 2008). Studies using microsatellite analysis have demonstrated that clonal richness within in situ stands of *Z. marina* enhances resistance to abiotic (e.g., temperature stress; Ehlers et al. 2008) and biotic (e.g., goose grazing; Hughes and Stachowicz 2004, 2009) stress. Therefore, measuring and comparing multiple facets of genetic diversity in eelgrass populations are a vital part of increasing our understanding of the communities for which seagrasses form the foundation.

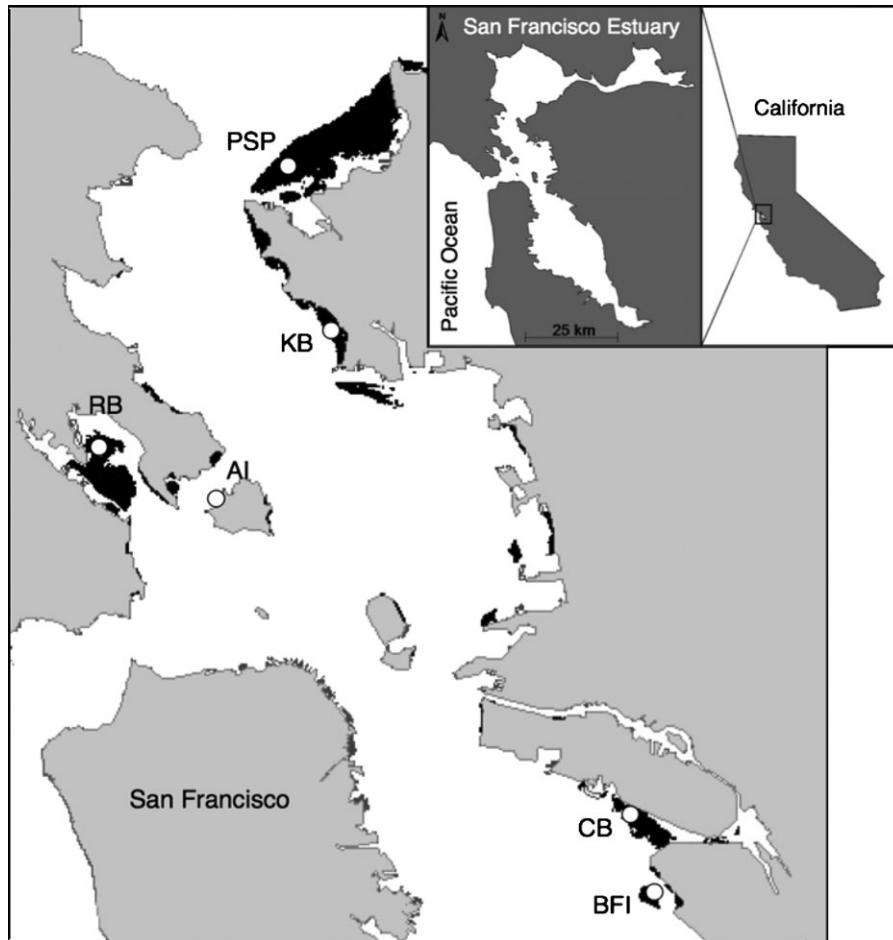
*Zostera marina* seed germination and new shoot growth in the Northeast Pacific commonly begins in early spring, followed by development of flower-bearing shoots in late spring and summer (Setchell 1929; Phillips, Grant, et al. 1983; Phillips, McMillan, et al. 1983). The negatively buoyant pollen and seeds (Cox et al. 1992; Ruckelshaus 1996) typically disperse only a few meters even when subject to strong currents (Orth et al. 1994; Ruckelshaus 1996). No regional studies directly document long-distance dispersal, but genetic isolation by distance (IBD) studies have suggested 150 km as an upper limit to the natural dispersal distance of *Z. marina* (Olsen et al. 2004; Muñiz-Salazar et al. 2005). Field studies show dispersal most likely occurs by seed-bearing shoots breaking from the rhizome and rafting up to 35 km (Harwell and Orth 2002) or even over 150 km (Kallstrom et al. 2008). Thus, there is potential for the annual exchange of genes within this rafting distance. If this occurs in the absence of localized natural selection, we would expect little or no genetic structure among populations that frequently exchange migrants by rafting (Reusch 2002).

Despite the potential for population connectivity via rafting, population genetic structure has been found, somewhat inconsistently, at scales of less than 150 km. Examples occur in island populations off the Baja California and California coasts (Coyer et al. 2008), along the Western Atlantic coast (Campanella et al. 2010), in the lower Chesapeake Bay (Williams and Orth 1998) and in Northern Puget Sound and the San Juan Archipelago, Washington, USA (Ruckelshaus 1998; Wyllie-Echeverria et al. 2010). Becheler et al. (2010) recently uncovered genetic differentiation at every scale they measured, from hundreds of kilometers down to a few meters, which they attributed to temporal differences in the genetic makeup of recruits that opportunistically repopulate openings in the eelgrass canopy created by disturbance.

In addition to distance between populations, the velocity of currents seems an obvious factor to consider in the potential for gene flow; populations in high current regimes should be more connected than similarly spaced populations in low currents. However, microsatellite-based genetic comparisons of extant *Z. marina* populations from both the Wadden (reported only descriptively as having “strong tidal currents”) and Baltic Seas (“atidal”) indicated no such relationship in either environment among populations 30–54 km apart (Reusch 2002). Other studies in Baja California (Muñiz-Salazar et al. 2006) and the San Juan Islands (Wyllie-Echeverria et al. 2010) have found similarly equivocal relationships between genetic differentiation, distance, and water currents measured over speeds from 15 to 100 cm s<sup>-1</sup>.

Taken together, these studies suggest that the spatial scale of genetic connectivity between discrete *Z. marina* stands is idiosyncratic, ranging from just a few kilometers to 150 km. However, the existence of genetically differentiated “populations” in marine organisms does not always mean that the clusters in question necessarily function as independent units. Temporally or spatially chaotic patterns of recruitment can create a mosaic of patches that are genetically differentiated over both large and small areas (Hedgecock 1994; Becheler et al. 2010, but see Flowers et al. 2002). Thus, population structure may be dependent on regional and local environmental conditions and on the nature of physical and temporal isolation between sites. To offer more insight, we designed a study to determine if genetic diversity was influenced by 1) geographic location within a bay, 2) position of plants along a depth gradient, and 3) a perennial or annual life-history strategy.

The San Francisco Bay was the site of our investigation. Besides being the largest estuary on the Pacific Coast of North America, with well-characterized flow rates (Conomos 1979), it provides an excellent model system for restoration in a heavily impacted urban estuary. *Zostera marina* is discontinuously distributed from San Pablo Bay (northern San Francisco Bay) into the northern region of the South Bay with beds ranging in size from 609 ha (1504 ac) to 0.8 ha (0.2 ac) (Wyllie-Echeverria 1988; Merkel and Associates 2004). Maximum distance along a north–south transect between beds is approximately 33 km, whereas minimum distance between adjacent beds is as short as 2 km (Figure 1). Maximum tidal currents in the Bay typically range from 35 cm sec<sup>-1</sup> in shoal areas to over 150 cm sec<sup>-1</sup> in deeper channels (high-frequency radar monitoring: <http://norcalcurrents.org/COCMP/Home.html>), capable of moving a typical parcel of water ~10 km over the course of a tidal cycle due to both tidal- and wind-generated currents (Conomos 1979). Thus, *Z. marina* beds at extreme distributional points in the bay are well within potential rafting distances of 35–150 km (Kallstrom et al. 2008), and seed-bearing reproductive shoots could theoretically travel between most sites in the bay. Consequently, in the absence of localized selection or other barriers to dispersal and recruitment, considerable gene flow and a corresponding lack of genetic differentiation between sites is the null hypothesis. However, in a previous study of *Z. marina*



**Figure 1.** *Zostera marina* sampling locations (white circles), and 2004 eelgrass distribution (black patches), courtesy of Merkel and Associates and NOAA Restoration Center, in the San Francisco Bay, California, USA. East bay populations: PSP, Point San Pablo (N37.97295, W122.420889), KB, Keller Beach (N37.921933, W122.388589), CB, Crown Beach (N37.758883, W122.2688), and BFI, Bay Farm Island (N37.731233, W122.263683). West bay populations: RB, Richardson Bay (N37.8798, W122.487956) and AI, Angel Island (N37.864117, W122.263683). Site abbreviations as in text.

sampled from 8 locations in San Francisco Bay, Talbot et al. (2004) found all but 2 pairwise comparisons were genetically differentiated. The 8 localities generally contained low levels of heterozygosity, allelic richness (AR), and clonal diversity. The study was hampered by limited power to fully detect clonality, and the authors recommended further study with larger sample sizes and, if possible, more variable markers. We have added new sites and increased sampling to test for population structure and assess genetic variation in *Z. marina* in San Francisco Bay.

Moreover, because *Z. marina* adult ramets exhibit a shade-adapted response to turbidity within the Bay (Zimmerman et al. 1991), we hypothesized that position along a depth gradient could result in spatial genetic patterning in response to selection. As wind waves and tidal currents increase sediment resuspension and turbidity, lower light levels along the depth gradient may negatively influence seedling establishment in deeper waters and favor

asexual growth through rhizome extension as the primary mechanism of stand persistence. If so, we would expect reduced clonal diversity among plants in deeper water.

We can also document that *Z. marina* growing in at least one site in the Bay is an annual population (Fonseca et al. 2003; Talbot et al. 2004). *Zostera marina* populations that replace themselves each year by sexual reproduction may be expected to maintain greater clonal diversity than asexually expanding perennial populations (e.g., Reusch 2002). In addition, clones in perennial populations not subject to frequent extirpation probably grow to larger sizes over many years (Hämmerli and Reusch 2003b), which should increase the probability of self-pollination rather than outcrossing with another genet, with the effect being an increase in inbreeding. Therefore, we expected to find the highest clonal richness and heterozygosity in the annual population.

Although our study design contributes to a basic understanding of the forces that shape population structure

in *Z. marina*, another intent of our research was to direct efforts to restore eelgrass in San Francisco Bay. We therefore focused our effort on characterizing genetic and clonal diversity patterns at locations identified as potential donor sites in order to support decision making as projects advance from experimental and small-scale restoration projects to a Bay-wide restoration program.

## Materials and Methods

### Sample Collection

Six study locations were chosen to encompass the geographic extent of *Z. marina* in San Francisco Bay and to represent a range of meadow conditions and attributes, for example, life-history strategy and flowering rates, current and wind strength and direction, depth, and sediment texture (Figure 1). The east bay populations were Point San Pablo (PSP, number of ramets collected  $N_r = 44$ ), Keller Beach (KB,  $N_r = 45$ ), Crown Beach (CB,  $N_r = 45$ ), and Bay Farm Island (BFI,  $N_r = 46$ ). West bay populations were from Richardson Bay (RB,  $N_r = 44$ ) and Angel Island (AI,  $N_r = 37$ ). The PSP and BFI beds occur on offshore, sandy shoals. The KB, CB, and AI beds are along sandy beaches, AI being the most exposed adjacent to the deepest strait within the bay. RB is a deeper bed in fine sediments in a relatively protected bay. Finally, CB plants exhibit an annual life history in the shallower portion of the bed's distribution (Kiriakopolos S, thesis in preparation). All of the sampling locations have supported *Z. marina* for the last several decades, and none are known to have been recipients of previous restoration work (Wyllie-Echeverria and Rutten 1989). A total of 261 samples of vegetative shoots were collected in July and August 2006 at intervals of at least, and generally greater than, 10 m, to try to avoid multiple samples from the same clonal plant (Hämmerli and Reusch 2003a), using a haphazard walk or swim with SCUBA. The sampling within each bed covered areas of approximately 400–800 m by 200–400 m, depending on the geometry of the bed perimeter. Approximately 6 cm of tissue was collected, returned to the lab, and stored at  $-80^\circ\text{C}$  until used for DNA extraction.

Depth-specific samples were collected in July 2006 from 3 locations: PSP, CB, and BFI, using a  $45 \times 4$  m grid scheme. A 45-m transect was established at the deepest portion of each meadow parallel to the shore. This became the deep transect. A similar shallow transect was established within the shallowest region of each bed, 200–400 m inshore from the deep transect. Deep and shallow depths (relative to NAVD88), corrected to local tidal datum using Vdatum (Hess et al. 2005), were  $-0.70$  and  $-0.31$  m for PSP;  $-1.04$  and  $-0.24$  m for CB; and  $-0.72$  and  $-0.53$  m for BFI. Starting at one end of the transect, the nearest vegetative shoot 2 m toward shore from the transect was collected. We then moved 1 m along the transect and collected the nearest vegetative shoot 2 m off the transect away from shore. We continued sampling from points alternating 2 m to either side of the transect at 1 m intervals,

such that the space between samples was  $\sim 2\text{--}4$  m. Thus, we collected 45 ramets at each depth. Shoots were floated in mesh bags in flow-through seawater tables until tissue was collected and frozen as above.

### DNA Extraction and Microsatellite Analysis

Approximately 100 mg of tissue from each sample was homogenized in a 1.5 ml microcentrifuge tube using a micropestle under liquid nitrogen. DNA was extracted using the cetyltrimethylammonium bromide method (Doyle JJ and Doyle JL 1987), resuspended in 60  $\mu\text{l}$  tris-ethylenediaminetetraacetic acid (10 mM Tris, 1 mM EDTA), and final DNA concentration was quantified using a Nanodrop ND-1000 spectrophotometer. Working aliquots of 5 ng  $\mu\text{l}^{-1}$  DNA were stored at  $4^\circ\text{C}$  and used for subsequent PCR. Previous DNA sequencing of the 5.8S rRNA/ITS-1 and ITS-2 genes gave results consistent with *Z. marina* being the only *Zostera* species present in San Francisco Bay (Talbot et al. 2004).

Eight microsatellite loci, Zosmar CT3, CT12, CT17, CT19, CT20, GA3, GA4, and GA5, were amplified by PCR using previously described primers designed from plants in the Baltic Sea (Reusch et al. 1999; Reusch 2000). Preliminary data showed that these were the most variable for our region among the microsatellite loci available for the species at the inception of the project. A GTGTCTT "tail" was added to the 5' end of all reverse primers to avoid artifacts caused by inconsistent addition of adenine nucleotides to PCR products (Brownstein et al. 1996). Reaction conditions were 10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.1% Triton X-100, 0.1% bovine serum albumin, 200  $\mu\text{M}$  each dNTP, 1.5 mM  $\text{MgCl}_2$  (2.0 mM  $\text{MgCl}_2$  for CT19), 0.8 units Taq DNA polymerase (New England Biolabs), and 5 ng template, in a final volume of 25  $\mu\text{l}$ . A negative control was included for each set of reactions. Thermocycling conditions were  $94^\circ\text{C}$  denature step for 3 min followed by 33–35 cycles of  $94^\circ$  for 20 s,  $57^\circ$  for 30 s,  $72^\circ$  for 30 s, and a final extension of  $72^\circ$  for 12 min. PCR products were verified by agarose gel electrophoresis, diluted 0- to 100-fold, and pooled with up to 2 other loci compatible for simultaneous fragment analysis in 9  $\mu\text{l}$  of denatured formamide (HiDi; Applied Biotechnologies, Inc.) and an internal size standard GS500-ROX or GS600-LIZ (ABI) on an ABI 3130 Avant automated sequencer. Fragment sizes were determined using GeneMapper v3.7 software (ABI). PCR and sizing were repeated on at least 10% of randomly selected samples to calculate an error rate in genotyping. We tested for the presence of null alleles using MICRO-CHECKER (Van Oosterhout et al. 2004).

### Statistical Analysis

#### Genetic Diversity

Identical genotypes may be observed within an eelgrass population due to sampling of multiple shoots (ramets) from a single clone (genet) or by the chance recombination of identical alleles during sexual reproduction. Clonal

diversity describes the proportion of sampled individuals with unique genotypes and was calculated within each sample as  $R = (G - 1)/(N - 1)$ , where  $G$  = the number of genets and  $N$  = the total number of ramets sampled following Dorken and Eckert (2001). Thus,  $R = 0$  in a monoclonal stand and  $R = 1$  if every sample has a unique genotype, regardless of sample size. We assessed the statistical power of the marker set to discriminate among clones using the probability of identity values  $P_{IDunbiased}$  and  $P_{IDsib}$  (Waits et al. 2001) calculated in GIMLET (Valiere 2002) for each population and depth sample.

The genet is the unit to be considered when determining heterozygosity and inbreeding coefficients. If 2 or more ramets had identical genotypes, we decided whether to define them as clonal replicates and remove them from the analysis set by using the statistic  $P_{SEX}$ , the probability that 2 or more identical genotypes arose due to sexual reproduction, as calculated in GenClone 2.0 (Arnaud-Haond and Belkhir 2007). If a reencounter of an identical genotype occurred with a probability  $P_{SEX} < 0.05$  within the ramet's population of origin and the overall sample, that sample was excluded from further analysis. We then used the trimmed data set in GenClone to construct saturation curves of genetic distance. Mutations and scoring errors can cause the frequency curves to be bimodal, with a small peak at low genetic distances. If that peak is present, it can indicate the need to define multilocus lineages to avoid overestimating the number of clones in a sample (Arnaud-Haond et al. 2007). We also created a second more conservative data set in which all but one exemplar of each multilocus genotype (MLG) was excluded from each population, regardless of  $P_{SEX}$ . This data set was applied to population structure analyses to mitigate the possibility that the statistical power of  $P_{SEX}$  was overly weak.

We tested for linkage disequilibrium (nonrandom associations of alleles from different loci) in GENETPOP (Raymond and Rousset 1995) on the web ([http://genepop.curtin.edu.au/genepop\\_op2.html](http://genepop.curtin.edu.au/genepop_op2.html)) with 1000 dememorization steps, 100 batches, and 1000 iterations per batch. Expected and observed heterozygosities were calculated in Arlequin 3.1 (Excoffier et al. 2005). AR rarefied to the smallest sized sample of genets ( $N_g$ ), and inbreeding coefficient  $F_{IS}$  of Weir and Cockerham (1984) were calculated in FSTAT 2.9.3 (Goudet 1995), testing for significance using 500 randomizations among individuals within samples. We tested for evidence of a genetic bottleneck in BOTTLENECK (Cornuet and Luikart 1996). The test takes advantage of the fact that during a genetic bottleneck, rare alleles will be eliminated from a population quickly, but levels of heterozygosity will decline more slowly. Therefore, heterozygosity in excess of expectations calculated from AR is interpreted as evidence for a recent genetic bottleneck. We followed the recommendations of Piry et al. (1999) in using a two-phase model (TPM) that stipulates most microsatellite mutations to occur in a stepwise manner but allows a small amount of deviation from that model in the form of infinite allele mutations. Parameters were set to 95% single-step mutations and

variance = 12. Strictly infinite allele and stepwise mutation models were also investigated with no notable differences from the TPM reported here. Statistical significance over 1000 replicates was assessed by a one-tailed Wilcoxon test for heterozygosity excess.

### Population Structure

Pairwise  $F_{ST}$  and analysis of molecular variance (AMOVA) (Excoffier et al. 1992) were performed in Arlequin 3.1 using assumptions for  $F_{ST}$ -like (allele frequency-based) models. Permuting genotypes between populations 16 000 times gave the null distribution of pairwise  $F_{ST}$ .  $P$  values were calculated as the proportion of permutations leading to an  $F_{ST}$  value greater than or equal to the observed  $F_{ST}$ .

We also examined population structure using Bayesian methods in STRUCTURE (Pritchard et al. 2000) using no a priori information regarding the geographic origin of individuals. Instead, STRUCTURE calculates a likelihood score when the data are forced into a given number of genetic clusters,  $K$ . We tested 10 iterations at each  $K = 1-8$ , with 50 000 burn-in steps followed by 500 000 Markov chain Monte Carlo steps. We then used the method described by Evanno et al. (2005) to infer the most likely value of  $K$ . We used CLUMPP (Jakobsson and Rosenberg 2007) to combine the outputs of the 10 iterations from STRUCTURE at each  $K$  value using the "FullSearch" algorithm for  $K \leq 3$ , or 10 000 random permutations of the computationally faster "Greedy" algorithm for  $K > 3$ . The output from CLUMPP was used as input into DISTRUCT (Rosenberg 2004) for the creation of bar plots showing the probability of each individual's membership in each cluster  $K$ .

We assessed the relationship between genetic and geographic distances in all pairwise comparisons between populations using the IBDWS program (Jensen et al. 2005). Slatkin's (1993) genetic distance  $M\text{-hat} = F_{ST}/1 - F_{ST}$  was calculated between all pairs of populations and regressed against the shortest distance over water between populations. Nonparametric Mantel tests for nonrandom associations between genetic and geographic distances were performed and tested for significance using 30 000 permutations of the data. The false discovery rate was assessed for multiple tests using the bootstrap method in QValue (Storey et al. 2004).

## Results

### Microsatellite Data Properties

We used 8 variable microsatellite loci to score 262 ramets from 6 *Z. marina* meadows in the San Francisco Bay, and 267 ramets collected along transects placed in the shallow and deep portions of 3 of the meadows. Locus CT17 was dropped from analyses due to multiple samples not yielding PCR products and others showing the presence of 3 alleles, which was taken to be indicative of the presence of null alleles and a duplicated marker. The remaining 7 loci were

**Table 1** Genetic diversity

Site	$N_r^a$	$N_g^b$	MLG <sup>c</sup>	$P_{ID}^d$	$P_{IDsib}^e$	$R^f$	AR <sup>g</sup>	$H_O^h$	$H_E^i$	$F_{IS}^j$
Population samples										
All populations	261	257	207	0.0018	0.0585	0.78	4.29	0.32	0.37	0.119**
PSP	44	43	31	0.0121	0.1466	0.70	2.74	0.26	0.25	-0.017
KB	45	45	38	0.0043	0.0830	0.84	3.30	0.34	0.34	0.004
CB	45	45	45	0.0006	0.0446	1.00	2.96	0.34	0.39	0.125*
BFI	46	46	32	0.0102	0.1308	0.74	2.87	0.28	0.26	-0.048
RB	44	43	38	0.0015	0.0512	0.88	2.93	0.38	0.39	0.025
AI	37	35	27	0.0028	0.0721	0.79	3.00	0.34	0.36	0.034
Mean	43.5	42.8	35.2	0.0053	0.0881	0.82	2.97	0.32	0.33	0.00
Depth samples										
All depths	267	242	182	0.0031	0.0814	0.90	3.57	0.30	0.31	0.039*
PSP shallow	45	43	27	0.0122	0.1483	0.60	2.53	0.23	0.24	0.066
PSP deep	45	38	28	0.0091	0.1334	0.61	2.87	0.27	0.25	-0.051
PSP combined	90	81	49	0.0115	0.1393	0.55	2.95	0.25	0.25	0.008
CB shallow	43	43	42	0.0005	0.0452	0.98	3.06	0.38	0.39	0.035
CB deep	44	32	24	0.0009	0.0514	0.56	3.00	0.36	0.38	0.069
CB combined	87	75	66	0.0007	0.0472	0.76	3.26	0.37	0.38	0.049
BFI shallow	45	43	29	0.0117	0.1444	0.64	2.89	0.27	0.24	-0.122
BFI deep	45	43	32	0.0085	0.1229	0.70	2.97	0.31	0.27	-0.143
BFI combined	90	86	56	0.0104	0.1298	0.62	3.23	0.34	0.30	-0.126*
Shallow mean	44.3	43.0	32.7	0.0082	0.1136	0.74	2.8	0.29	0.29	-0.01
Deep mean	44.7	37.7	28.0	0.0062	0.1033	0.63	2.9	0.31	0.30	-0.04
All depths mean	44.5	40.3	30.3	0.0072	0.1076	0.68	2.9	0.30	0.30	-0.024

<sup>a</sup> Number of ramets sampled.

<sup>b</sup> Number of probable genets in sample.

<sup>c</sup> Number of multilocus genotypes in sample.

<sup>d</sup> Probability of identity among MLG.

<sup>e</sup> Probability of identity among MLG with high sibling mating.

<sup>f</sup> Clonal diversity.

<sup>g</sup> AR rarefied to smallest sample size of  $N_g = 35$  for population samples or  $N_g = 32$  for depth samples.

<sup>h</sup> Observed heterozygosity.

<sup>i</sup> Expected heterozygosity under HWE.

<sup>j</sup> Population inbreeding coefficient.

\* $P < 0.05$ , \*\* $P < 0.01$ , sequential Bonferroni correction applied.

scored with an error rate of approximately 1.5% over all loci. Alleles from CT20 and CT3 were associated with each other more frequently than expected by chance (linkage disequilibrium) within AI and BFI ( $P < 0.0001$ ), but not at the other populations. This result led to a signal of linkage between the same 2 loci across the entire pooled population data set ( $P < 0.0001$ ). CT20 and CT3 were also linked when tested over the entire depth data set but only in PSP shallow when tested within each depth sample. In both cases, this is less than the number of false-positive results (6.3) expected for the 126 pairwise comparisons (7 loci over 6 populations). Because the markers were not linked in all sampling and show an informative difference between PSP shallow and deep, we retained all markers. However, we also compared results with and without CT20, with no differences.

## Population Samples

### Genetic Diversity

Estimates of genetic diversity are summarized in Table 1.  $P_{IDunbiased}$  (hereafter referred to as  $P_{ID}$ ) was 0.0018 overall

(range 0.0006–0.0121), indicating a reasonable power to identify unique clones using our markers and sample sizes (Waits et al. 2001). However,  $P_{IDsib}$  was 0.0585 overall (range 0.0446–0.1466). This more conservative statistic suggests that if there is a high degree of mating between siblings in our populations, our estimates of clonal diversity will be underestimates. Nevertheless, clonal diversity  $R$  was by far highest in the annual population at CB, in which every individual sampled had a unique diploid genotype ( $R = 1.0$ ). Clonal diversity among the perennial populations ranged between 0.88 at RB and 0.70 at PSP. AR, the average number of alleles per locus standardized to the smallest sample size, was approximately 3 and varied nonsignificantly over all populations ( $F = 0.148$ ,  $P = 0.98$ , 5 degrees of freedom). Only 4 single-copy private alleles were found, 2 in KB and 1 each in PSP and CB.

The 261 ramets collected ( $N_r$ ) contained 207 distinct MLG. Four ramets, representing 3 different MLG, were determined to have a high probability ( $P_{SEX} < 0.05$ ) of having arisen by clonal replication. These 4 clonal ramets were removed from all further analyses. Subsequent analyses were therefore performed on the remaining 257 genets ( $N_g$ ).

**Table 2** Pairwise  $F_{ST}$  values among population and depth (S, shallow, or D, deep) samples

	Population samples						Depth samples					
	PSP	KB	CB	BFI	RB	AI	PSP-S	PSP-D	CB-S	CB-D	BFI-S	BFI-D
PSP	—											
KB	<b>0.055</b>	—										
CB	<b>0.114</b>	<b>0.084</b>	—									
BFI	<b>0.060</b>	<b>0.100</b>	<b>0.086</b>	—								
RB	<b>0.112</b>	<b>0.086</b>	<b>0.088</b>	<b>0.180</b>	—							
AI	<b>0.176</b>	<b>0.092</b>	<b>0.156</b>	<b>0.217</b>	<b>0.102</b>	—						
PSP-S	−0.003	—	<b>0.118</b>	<b>0.055</b>	—	—	—					
PSP-D	0.000	—	<b>0.114</b>	<b>0.050</b>	—	—	−0.002	—				
CB-S	<b>0.110</b>	—	−0.003	<b>0.080</b>	—	—	<b>0.113</b>	<b>0.099</b>	—			
CB-D	<b>0.085</b>	—	−0.002	<b>0.071</b>	—	—	<b>0.091</b>	<b>0.088</b>	−0.003	—		
BFI-S	<b>0.054</b>	—	<b>0.093</b>	0.001	—	—	<b>0.054</b>	<b>0.039</b>	<b>0.080</b>	<b>0.074</b>	—	
BFI-D	<b>0.090</b>	—	<b>0.110</b>	0.011*	—	—	<b>0.086</b>	<b>0.070</b>	<b>0.092</b>	<b>0.085</b>	0.010*	—

Upper left quadrant: comparisons among population samples; lower right quadrant: comparisons among depth samples; lower left quadrant: comparisons of depth samples with population samples. Bold,  $P < 0.0000$ , \* $P < 0.05$ .

All other instances of MLGs being shared among 2 or more ramets were determined to have occurred through the action of sexual reproduction producing the identical MLG by chance ( $P_{SEX} > 0.05$ ). The curve of frequency versus genetic distance was unimodal, not bimodal, but with a curious propensity for genetic distances to be even numbers, an artifact of our microsatellite markers being dinucleotide repeats (Supplementary Figure S1a). Somatic mutations or scoring errors were therefore determined to not be an important source of bias in estimating clonality (Arnaud-Haond et al. 2007).

We found a significantly large  $F_{IS}$  over all population samples, a departure from Hardy–Weinberg equilibrium (HWE) that is consistent with genetically differentiated populations being erroneously analyzed as a single population.  $F_{IS}$  indicated a significant deficiency of heterozygotes within CB, consistent with the possibility of population substructure or inbreeding. Tests in STRUCTURE did not support the substructure hypothesis (data not shown). All other populations were in HWE with nonsignificant  $F_{IS}$  values. The ranges of  $H_O$  and  $H_E$  were 0.26–0.38 and 0.25–0.39, respectively. Heterozygosity within the annual population at CB was within the range of values found at other populations. None of the populations showed evidence of a recent genetic bottleneck (Wilcoxon  $P > 0.50$ ) with the possible exception of CB ( $P = 0.078$ ).

#### Population Structure

All pairwise comparisons of  $F_{ST}$  were highly significant (all  $P < 0.0000$ ) among the population samples, confirming that the 6 populations are all genetically differentiated from each other (Table 2). The same result (not shown) was obtained if locus CT20 was eliminated as a precaution against the effects of linkage disequilibrium. We repeated our genetic structure analyses using the smaller data set in which only one exemplar of each MLG was retained for each population, a commonly used but more conservative approach than using  $P_{SEX}$  to cull clonal samples.  $F_{ST}$  values

differed slightly, and not consistently upward or downward, compared with the  $P_{SEX}$ -based data set, but all  $P$  values remained significant at or below the  $P < 0.002$  level (Supplementary Table S1). Differentiation was not due to a single locus;  $F_{ST}$  was significant ( $P < 0.05$ ) for all 7 individual loci (Table 3). The results from STRUCTURE are shown in Figure 2. When 6 genetic clusters ( $K = 6$ ) were assumed, individuals were assigned to any of the 6 with roughly equal probability (Figure 2a), suggesting  $K = 6$  was not the best solution. STRUCTURE analysis reached a maximum log likelihood score at  $K = 2$  (Supplementary Figure S2a). The mode of  $\Delta K$  transformation (Evanno et al. 2005) also occurs at  $K = 2$ , with height = 15 (Supplementary Figure S2b), supporting the existence of either 1 or 2 clusters. The method cannot distinguish between 1 and 2 clusters, but given the highly differentiated  $F_{ST}$  values, we reject the possibility of all populations belonging to the same cluster and conclude our samples formed 2 genetic clusters. The  $\Delta K$  statistic identifies the highest hierarchical level of population structure (Evanno et al. 2005). Therefore, there is evidence that the 6 differentiated populations are organized into 2 groups.

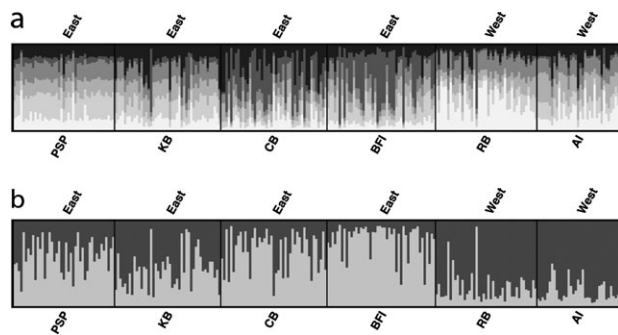
**Table 3** Locus-by-locus AMOVA with populations organized into east bay (populations PSP, KB, CB, and BFI) and west bay (RB and AI) groups

Locus	$F_{SC}^a$	$P$	$F_{ST}^b$	$P$	$F_{CT}^c$	$P$
CT12	0.064	0.000	0.047	0.000	−0.018	0.426
CT19	0.037	0.004	0.291	0.000	0.263	0.066
CT20	0.077	0.000	0.080	0.000	0.004	0.335
GA3	0.030	0.004	0.015	0.022	−0.016	1.000
GA4	0.067	0.000	0.058	0.000	−0.010	0.521
GA5	0.090	0.000	0.163	0.000	0.080	0.134
CT3	0.146	0.000	0.160	0.000	0.016	0.205

<sup>a</sup> Variance of genotypes permuted among populations within groups.

<sup>b</sup> Variance of genotypes permuted among populations and among groups.

<sup>c</sup> Variance of whole populations permuted among groups.



**Figure 2.** Bar plot of the probability of assigning each individual from the population samples to an inferred genetic cluster. Each individual is represented along the  $x$  axis. On the  $y$  axis is the probability of that individual belonging to each of the genetic clusters. (a) Plot assuming 6 genetic clusters, as suggested by pairwise  $F_{ST}$  analyses. (b) Plot assuming 2 genetic clusters, the solution supported by STRUCTURE.

Likelihood analysis in STRUCTURE with the number of clusters  $K = 2$  supports a division between the east and west sides of the bay, but that it is permeable to gene flow; that is, some individuals sampled from one side of the bay show a high probability of being genetically similar to populations on the opposite side of the bay (Figure 2b). An AMOVA with east and west sites in 2 groups provides marginally significant support for an east bay–west bay split ( $F_{CT} = 0.053$ ,  $P = 0.058$ ; Table 4). No other combinations of populations received close to significant support (results not shown). Mantel tests provide no evidence of nonrandom correlations between genetic similarity and geographic distance among all populations ( $r^2 = 0.032$ ,  $P = 0.781$ ) nor among a group of only the more linearly arranged east bay populations ( $r^2 = 0.100$ ,  $P = 0.545$ ).

## Depth Samples

### Genetic Diversity

The standardized clonal diversity  $R$  was marginally lower in the depth samples compared with the population samples (Table 1; one-tailed  $t$ -test,  $P = 0.046$ ).  $R$  ranged from 0.60 to 0.70 in both transects from BFI and PSP, slightly lower than what was found in the population samples from those locations. In the shallow transect of CB, located in the annual portion of the bed, only 2 samples had identical genotypes at all 7 loci ( $R = 0.98$ ), whereas the deep transect in the perennial portion had the lowest clonal diversity of

the study ( $R = 0.56$ ).  $P_{ID}$  was 0.0031 overall (range 0.0005–0.0122), whereas  $P_{ID_{sib}}$  was 0.0814 overall (range 0.0452–0.1483) (Table 1), again indicating reasonable statistical power to detect unique clones according to  $P_{ID}$  but less power according to the more conservative  $P_{ID_{sib}}$ . Our estimates of clonal diversity from the depth samples should therefore be taken as lower bounds.

One hundred and eighty-two unique MLG were found among the 267 ramets collected. Twenty-five ramets, representing 16 MLG, were determined to be clonal replicates ( $P_{SEX} < 0.05$ ). These samples were removed from the data set, leaving a total of 242 genets for further analysis. As with the population samples, the curve showing the frequency of genetic distance in pairwise comparisons of MLG was clearly unimodal (Supplementary Figure S1b), so we did not consider somatic mutations or scoring error to be a source of bias.

Genetic diversity ( $H_O$  and  $H_E$ ) calculated after the removal of clonal ramets was similar between the depth and population samples (Table 1), as was AR ( $t$ -tests paired according to locus,  $P > 0.05$ ). Observed heterozygosity departed from HWE when analyzed over all depth samples combined ( $F_{IS} = 0.039$ ,  $P = 0.029$ ), consistent with the previous result of differentiated populations. HWE was observed within each individual depth sample and when both depths at each location were combined into single samples at PSP and CB, suggesting that there is interbreeding among plants at the different depths.  $F_{IS}$  showed an excess of heterozygotes over expected at BFI, where the 2 depths were also genetically differentiated (see below). We found no statistical support for a bottleneck among any of the depth samples (Wilcoxon  $P = 0.59$  and 0.31 for CB shallow and deep, respectively;  $P > 0.94$  for PSP and BFI).

### Population Structure

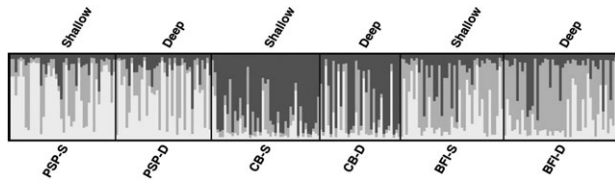
Within localities, we found marginal support for differentiation between shallow and deep samples at BFI ( $F_{ST} = 0.01$ ,  $P = 0.05$ ), but not at PSP ( $P = 0.56$ ) or CB ( $P = 0.66$ ). Confirming results from the population data, transects were differentiated by population location according to  $F_{ST}$  (Table 2). As with the population samples, depth samples were differentiated at all loci ( $F_{ST}$  range 0.015–0.171, all  $P < 0.01$ ). AMOVA of the depth samples did not show differentiation among samples grouped by population of origin (PSP, CB, or BFI,  $F_{CT} = 0.082$ ,  $P = 0.067$ ) nor grouped by depth (shallow or deep,  $F_{CT} = 0.028$ ,  $P = 0.91$ ). Using the smaller data set with all but one ramet

**Table 4** Results from AMOVA over all loci with population samples organized into east bay and west bay groups as in Table 3

Source of variation	df	Variance component	% Variation	$F_{ST}$	$P$
Between east and west bay	1	0.070	5.34	$F_{CT} = 0.053$	0.058
Among populations within groups	4	0.109	8.29	$F_{SC} = 0.088$	0.000
Among individuals within populations	251	0.020	1.55	$F_{IS} = 0.088$	0.221
Within individuals	257	1.117	84.82	$F_{IT} = 0.152$	0.000

df, degrees of freedom.





**Figure 3.** Bar plot of the probability of assigning depth samples to 1 of 3 genetic clusters, the solution supported by STRUCTURE. Axes as in Figure 2.

representing each MLG culled from the analyses did not alter our results (Supplementary Table S2) except to weaken support for differentiation between BFI deep and shallow ( $P = 0.57$ ) and between BFI deep and PSP shallow ( $P = 0.1$ ). Bayesian analysis in STRUCTURE identified 3 genetic clusters corresponding to the 3 collection locales rather than 2 depths (Figure 3), that is, the mode of  $\Delta K$  occurred at  $K = 3$  (with height = 29), corresponding to the 3 source populations, rather than at  $K = 2$ , for the 2 depths (Supplementary Figure S3b).

## Discussion

*Zostera marina* from the 6 sites we sampled within the San Francisco Bay are genetically differentiated according to multiple analyses based on 7 microsatellite loci. The results were consistent whether the data set was constructed by using  $P_{SEX}$  to identify which identical genotypes to cull or simply by removing all but one duplicate MLG from each population. Thus, the finding of strong population structure is not simply a statistical artifact of insufficient power to discern clones from truly independent genets. This result is unexpected given that the locations investigated are as close as 2.5 km apart and that the maximum distance between the sites is 33 km, that is, within the range of flowering shoot dispersal for this species (Harwell and Orth 2002). Furthermore, tidal currents and winds are capable of moving a parcel of water over 10 km in a single tidal cycle (Conomos 1979), suggesting potential for extensive gene flow between sites in San Francisco Bay. Previous work has shown *Z. marina* populations to be undifferentiated over distances comparable to those in our study in the Baltic and Wadden Seas (Reusch 2002). It is difficult to make a direct comparison of the influence of tidal currents in that study compared with ours, however, because Reusch (2002) reports the 2 environments only as “atidal” and “tidal,” respectively. On the other hand, populations in Portugal (Billingham et al. 2007), Washington state (Wyllie-Echeverria et al. 2010), and Baja California (Muñiz-Salazar et al. 2006) are differentiated over distances similar to our study in environments with lower maximal tidal currents (60–90 cm sec<sup>-1</sup>). Our results are unusual in that they show strong population differentiation despite flow rates reaching 150 cm sec<sup>-1</sup> or greater.

Bayesian analyses in STRUCTURE provided support for a separation between western (RB and AI) and eastern sites

but also show low rates of gene flow (note the few individuals in Figure 2b that show a high probability of being from a site other than the one from which they were sampled). Over time and in the absence of natural selection, even this low level of gene flow could theoretically homogenize populations under a model of neutral evolution dominated by mutation, gene flow, and genetic drift. Therefore, we conclude that additional factors have influenced the evolution of *Z. marina* beds within San Francisco Bay.

Possible explanations, which are not mutually exclusive, for differentiation include 1) recent independent founding of individual populations, each with a unique subset of alleles from a larger unidentified source population; 2) limited temporally heterogeneous genetic exchange between sites and subsequent genetic drift within each subpopulation (e.g., Becheler et al. 2010); and 3) ongoing localized natural selection maintaining genetic differences. Human disturbance over the past 150 years is thought to have led to one or more scenarios of local extirpation and recovery (e.g., Boyer and Cohen 2011; Tang X, Cohen CS, unpublished data). However, in this study, we did not find evidence of a severe genetic bottleneck, as one would expect from recent or repeated founding events. If dispersal is limited, differentiation due to genetic drift should be detected in multiple loci across the genome. Each of our 7 loci revealed spatial effects (significantly positive  $F_{SC}$  values), giving support to this hypothesis. However, differentiation by genetic drift requires populations to remain isolated for a sufficient amount of time for differences in allele frequencies to develop in each population. Given the evidence from STRUCTURE for low, but ongoing, levels of gene flow, a strictly neutral model of differentiation by genetic drift is less likely. Realized gene flow may therefore be more limited than one might expect based on water movement and wind patterns, with east–west movement especially curtailed (Figure 2). Chaotic differences in the genetic composition of the pool of recruits from year to year may explain the patterns of differentiation we observe, similar to Becheler et al. (2010). Unlike that example, we do not see strong differentiation at every scale, in that the depth transects are not differentiated at PSP or CB, and only weakly if at all at BFI. Seeds that disperse to distant locations are subjected to postsettlement selection, which probably varies among locations in intensity and direction. Supporting the hypothesis of local differentiation, we have unpublished data (Boyer KE, unpublished data) that seeds from several donor sites are not equally successful at germinating or persisting after introduction to novel laboratory or field environments, although another San Francisco Bay study did not find differences in early success among seeds from 3 sites in a greenhouse study (Tang X, Cohen CS, unpublished data). Localized adaptation has been reported in other *Z. marina* populations (Ruckelshaus 1995; Hämmerli and Reusch 2002). Differences in abiotic factors including sediment composition, wind exposure, tidal currents, light availability, and temperature or biotic factors such as herbivory or bioturbation could play a role in

diversifying selection among locales, as many of these factors vary among beds in our study. On the other hand, localized selection strong enough to cause differentiation at 7 microsatellite loci spread through the genome may produce a level of genetic load (Haldane 1957) that is perhaps unrealistic, and if so, this would support an explanation based on demography. The markers used in this study are not expected to directly reflect the influence of selection in this study or in prior research (e.g., Olsen et al. 2004). Reciprocal transplant experiments and additional genetic studies using a larger panel of markers at neutral and functional loci would be useful to address the importance of some of these factors.

*Zostera marina* phenology and growth is influenced by water depth (Dennison and Alberte 1982; Biber et al. 2009). For example, Provan et al. (2008) also found differences in reproductive strategies between plants living in shallow versus deeper water, but these differences may have been due to observed variation in salinity over the depth gradient rather than depth per se. We found no significant difference in clonal diversity between deep and shallow transects at PSP or BFI. However at CB, clonal diversity in the shallow region, where plants recruit from seed annually, was nearly double that of perennial plants growing in a deeper area. These genetic data support field observations that plants growing in the shallow area of CB are annuals. That we were unable to detect genetic differentiation between the annual and perennial portions of CB with our set of markers suggests that these life-history differences are not preventing gene flow across depths at CB. Further analysis using more sensitive genetic markers, designed from Eastern Pacific populations, is needed to detect possibly subtle differences along the depth gradient.

We did, however, detect weak genetic differentiation between shallow and deep zones at BFI. Also, when both depths were pooled, observed heterozygosity was elevated over expected heterozygosity, such as might occur with many accumulated somatic mutations or indirect selection favoring heterozygotes. Reasons for these observations are unclear, as differences in the depth gradient are relatively small (~20 cm), the sediment is consistently high in sand (Boyer K, unpublished data), and there are no other obvious gradients across the bed. By comparison, the sediment grain size at PSP and CB becomes increasingly less coarse with depth (Boyer K, unpublished data), but there was no corresponding genetic gradient with depth (Table 2). Measurements of flow rates, light attenuation, seedling versus clonal recruitment with depth, and additional genetic studies may be necessary to explain these results. Both of these marginally significant results may also be statistical rather than real, as they contradict each other. If the depths are in fact differentiated, the expectation is for heterozygote deficiency (Wahlund effect), not heterozygote excess.

Clonal diversity was, on average, lower in the depth samples compared with the population samples (Table 1). This was expected because the shorter distance between samples in the depth transects increased the probability of resampling from the same clone. Therefore, we infer that

*Z. marina* clones in San Francisco Bay commonly spread over the 2–4 m distance between samples along the depth gradient transects but are not usually greater than 10 m in width (the minimum distance between samples collected to determine population status). Although average AR in depth samples decreased when compared to population samples (AR = 3.57 and 4.29, respectively), the difference was not significant. We feel this trend, which we found using relatively low-resolution markers, cautions against collecting samples less than 10 m apart for restoration work. This finding is consistent with recommendations from other locations (Hämmerli and Reusch 2003a; Wyllie-Echeverria et al. 2010). Creating clone maps by sampling over a fine-scale grid at some of the sites in this study will be helpful to learn more about differences in clonal structure (e.g., Hämmerli and Reusch 2003a; Zipperle et al. 2009; Tang X, Cohen CS, unpublished data).

Overall, expected heterozygosity (0.25–0.39) and AR (2.74–3.30) within the San Francisco Bay populations are low relative to many populations in the northern hemisphere (Olsen et al. 2004; Muñoz-Salazar et al. 2005, 2006; Campanella et al. 2010; Wyllie-Echeverria et al. 2010, but see Coyer et al. 2008), but direct comparisons are difficult because different subsets of the available microsatellite markers were used in each study. The presence of many null alleles across all loci may cause a surfeit of apparent homozygotes, but our tests in MICRO-CHECKER, plus the fact that most of our alleles came in single repeat-unit increments, argue against this hypothesis. It seems, therefore, that *Z. marina* populations in San Francisco Bay on the whole are genetically isolated from other coastal populations that harbor greater diversity, but the Bay populations have persisted long enough in isolation to have reached HWE within each location except CB.

In the annual versus perennial comparisons, we expected results similar to Reusch's (2002) comparison of plants in the Wadden and Baltic Seas, that is, that annual plants at CB would exhibit the highest levels of clonal diversity and heterozygosity, but this was not entirely true. As expected, clonal diversity  $R$  was significantly greater within CB annuals—nearly, every sample from CB had a unique MLG—and AR showed a trend of being higher at CB in the pooled depth samples (Table 1). However, matings among close relatives are a possibility for our populations, and  $P_{ID\text{sib}}$  calculations suggest that, although  $R$  is near its maximum of 1 at CB, our estimates of clonal diversity should be taken as lower bounds in the other samples, where the application of markers with greater sensitivity could reveal higher levels of clonal diversity. The predictions for heterozygosity in clonal organisms are more complicated and may be influenced by relative levels of clonality (Balloux et al. 2003). Expected and observed heterozygosities at CB were similar to the perennial plants at several sites, where we observe a mix of clonal expansion and sexual reproduction (Table 1). Moreover, we observe relatively high flowering frequencies and seedling establishment in all 6 locations (Boyer K, unpublished data), suggesting that outcrossing, possibly among different interdigitating clonal plants, occurs

at rates sufficient to prevent the population-wide decrease in heterozygosity expected in populations that are highly inbred or rely solely on asexual reproduction. Thus, not all measures of genetic diversity will necessarily be higher in a purely sexually reproducing population compared to populations with mixed sexual and clonal reproductive modes. This is more similar to Reusch's (2002) comparison of annual versus mixed populations within the Wadden Sea, in which he found no differences in genetic diversity.

The heterozygote deficiency at CB is interesting and highlights the complex interaction between rates of sexual and asexual reproduction that determines local population structure. A deficiency of heterozygotes is not uncommon in marine population genetics (Addison and Hart 2005), but it is peculiar that we see it in only one of our samples. This deficiency could be the result of null alleles, selection, sampling across a subdivided population, that is, the "Wahlund effect," or patchy colonization from more than one genetically cohesive source. MICRO-CHECKER did not detect null alleles. If the population was genetically structured, then we would expect that the 2 depth transects, which were about 200 m apart, would have been differentiated, but they were not. In addition, tests in STRUCTURE did not support the existence of more than one genetic cluster at CB (results not shown). BFI is certainly close enough (~2.5 km) to disperse seeds to CB, but if it does, it is at a rate low enough to allow CB and BFI to remain genetically distinct. Reproduction between closely related plants, the lack of overlapping generations, and possibly a mild genetic bottleneck from the yearly die-off may also cause very fine-scale subdivision, increasing the difference between observed and expected heterozygosity compared with the other sites. Interestingly, a significant  $F_{IS}$  was not found in the shallow or deep samples collected for the depth comparison at CB. It is possible that the closer sampling scheme over a smaller portion of the site meant that we did not sample across subdivided portions of the bed in the depth study, thus avoiding the Wahlund effect. Sampling with higher resolution markers from a very fine-scale grid, to address localized inbreeding, will be required to define the size of the genetic neighborhood and provide a better understanding of the evolutionary consequences of annularity in this population.

Our population analysis results are qualitatively similar to a 2003 survey of San Francisco Bay *Z. marina* sites (Talbot et al. 2004). Four sites, PSP, CB, RB, and BFI, were examined in both studies. Genetic structure was found among most populations in 2003 except for 2 comparisons involving PSP and BFI, and PSP and another population close to KB. We found that the PSP-BFI and PSP-KB comparisons also had the smallest estimates of  $F_{ST}$  but were significantly differentiated. The difference in significance can be attributed to the larger sample sizes in the present study. Talbot et al. (2004) also found evidence for an east bay–west bay separation. Genetic diversity, too, was similar between the present study and Talbot et al. (2004). In both surveys, clonal diversity was highest at CB and lowest at PSP, whereas RB had the highest or was tied for the highest

and PSP and BFI had the lowest estimates of  $H_O$  and  $H_E$ . These comparisons suggest at a broad level that there have not been wide temporal fluctuations in the relative genetic diversity or population substructure at sites sampled in both studies, at least not over the 3 years (2003–2006) between the surveys. This period included a potentially strong selective event: a high-rainfall year (fall 2005 to winter 2006) that resulted in salinity levels near zero in some locations (<http://sfbay.wr.usgs.gov>). These data suggest that these sites support persistent populations with low realized genetic connectivity and little long-term dispersal success. Populations are possibly locally adapted, although firm conclusions cannot be drawn without definitive transplantation and rearing experiments. Nevertheless, this information is important to consider in a program to restore *Z. marina* at other sites in San Francisco Bay.

Numerous restoration efforts (e.g., Fonseca et al. 1998; Short et al. 2002; Kenworthy et al. 2006) have been initiated in response to the widespread decline of seagrasses worldwide (Orth et al. 2006; Waycott et al. 2009). During the process of selecting restoration donor sites, it is important to consider the extent to which site-specific populations are connected or locally adapted. Equally important is the extent to which natural populations may replenish each other through dispersal and subsequent gene flow (Palumbi 2003; Lee et al. 2007). In San Francisco Bay, extant populations of *Z. marina* growing in adjacent sites deviate from initial expectations by maintaining strong population genetic differentiation, despite strong winds and large tidal fluxes and despite detectable rates of gene flow. Explanations for the observed differentiation that are based solely on recent founding events or neutral genetic drift are not able to completely explain this phenomenon, suggesting that patchy recruitment and natural selection may be playing roles, as well. Placed in the context of restoration, this leads us to advise that, in the absence of the knowledge of specific localized selective pressures, and if the goal is not to preserve specific localized strains, restoration planners may decide to use material from multiple source populations in order to improve the probability of obtaining genotypes suitable for specific restoration sites. We have established baseline levels of genetic diversity and differentiation in San Francisco Bay eelgrass populations. Continued monitoring of the genetic composition of natural and restored populations, particularly using expanded suites of genetic markers, including candidate genes for important physiological parameters, would provide important data on the role of selection versus drift in promoting differentiation at local sites. Finally, our results support keeping the protection of extant *Z. marina* populations as a high priority, with the knowledge that, in the absence of more information, each location may be supporting a locally adapted genetically differentiated population.

## Supplementary Material

Supplementary material can be found at <http://www.jhered.oxfordjournals.org/>.

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