

The structure of population genetic diversity in *Vallisneria americana* in the Chesapeake Bay: implications for restoration

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Abstract Submersed aquatic macrophyte beds provide important ecosystem services, yet their distribution and extent has declined worldwide in aquatic ecosystems. Effective restoration of these habitats will require, among other factors, reintroduction of genetically diverse source material that can withstand short- and long-term environmental fluctuations in environmental conditions. We examined patterns of genetic diversity in *Vallisneria americana* because it is a cosmopolitan freshwater submersed aquatic macrophyte and is commonly used for restoring freshwater habitats. We sampled 26 naturally occurring populations of *V. americana* in the Chesapeake Bay estuary and its tributaries and found that the majority of populations have high genotypic diversity and are not highly inbred. Fourteen of the populations had high allelic and genotypic diversity and could serve as source sites for restoration material. However, substantial geographic structuring of genetic diversity suggests that caution should

be used in moving propagules to locations distant from their source. In particular, we suggest that propagules at least be limited within four primary geographic areas that correspond to freshwater tidal and non-tidal, oligohaline, and seasonally mesohaline areas of the Chesapeake Bay.

Keywords Submersed aquatic vegetation · SAV · Genotypic diversity · Gene flow

Introduction

Beds of submersed aquatic vegetation (SAV) provide habitat for fish and aquatic invertebrates (Rozas and Odum 1987, 1988; Wyda et al. 2002; Rozas and Minello 2006) and food resources for migratory waterfowl (Krull 1970; Korschgen and Green 1988). SAV also provides critical ecosystem services in that it improves water quality by stabilizing sediments (Sand-Jensen 1998; Madsen et al. 2001) and buffering nutrient levels (Brix and Schierup 1989; Takamura et al. 2003; Moore 2004). Unfortunately, the abundance, distribution, and diversity of SAV beds in coastal aquatic habitats have declined world-wide owing to extensive agricultural, industrial, and urban development in coastal zones (Cooper 1995; Short and Wyllie-Echeverria 1996; Orth et al. 2006; Procaccini et al. 2007). Such is the case in the Chesapeake Bay estuary (Costanza and Greer 1995; Boesch et al. 2001; Kemp et al. 2005), where current SAV coverage is <15% of the 250,000 ha estimated to have existed historically (Stevenson and Confer 1978; Dennison et al. 1993; Orth et al. 2008).

Programs to restore SAV acreage to the Chesapeake Bay and its tributaries have been implemented to mitigate declines. However, these programs have resulted in minimal increases in SAV extent. Poor water and habitat

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quality at many restoration sites are likely the primary reasons for disappointing results (van Katwijk et al. 2009). Our goal in this paper is to assess the amounts and patterns of genetic diversity in the submersed aquatic plant species *Vallisneria americana* Michx. (Hydrocharitaceae) to begin to investigate the possibility that genetic factors are contributing to low restoration success rates (Frankel 1974; Frankham 1995a; Hughes et al. 2008). Genetic diversity can affect population persistence in dynamic environments (Lande and Shannon 1996) and the chances for successful establishment of restored populations (Williams 2001). Unfortunately, assessments of this type of diversity often are not directly included in management and restoration plans because it is hard to quantify without sophisticated equipment and substantial expense. Our intent is to provide a description of spatial patterns of genetic variation within and among populations of *V. americana* that can contribute to the design of restoration efforts.

Amongst SAV species, *V. americana* has suffered substantial population size declines in the northern freshwater reaches of the Chesapeake Bay and its tributaries (Kemp et al. 1983). *V. americana* is a cosmopolitan, dioecious, perennial macrophyte that is native to eastern North American freshwater and oligohaline habitats (Korschgen and Green 1988; Catling et al. 1994). The species reproduces sexually and vegetatively (Wilder 1974) and the relative frequency of the two reproductive modes is unknown. Distribution of *V. americana* is limited to habitats characterized by a maximum water depth of 7 m in clear water, substrates ranging from gravel to hard clay, water temperatures between 20 and 40°C, and salinity below 18ppt (Korschgen and Green 1988). It is further limited by turbidity, nutrient content in the water column, water pH, gas exchange, water current, and competition with other plant species and grazing by animals (Hunt 1963; Barko et al. 1982; Titus and Stephens 1983; Korschgen and Green 1988; Doering et al. 2001; Kemp et al. 2004; Jarvis and Moore 2008).

Full restoration of *V. americana* within the Chesapeake Bay will depend on linking both physical and biological factors (Allendorf and Luikart 2007). Previous investigations across a wide range of habitats have examined the abiotic growth requirements and ecology of *V. americana*. These include salinity (Doering et al. 2001; Kreiling et al. 2007; Boustany et al. 2010), light attenuation (Titus and Adams 1979; Korschgen et al. 1997; Kreiling et al. 2007; Boustany et al. 2010), temperature (Titus and Adams 1979), suspended nitrogen (Kreiling et al. 2007), germination requirements (Jarvis and Moore 2008), effects of competition (Titus and Stephens 1983), and sex-ratios and natural fecundity (Doust and Laporte 1991; Titus and Hoover 1991). Here we build on this previous knowledge and quantify the levels and patterns of genetic diversity

within and indirect measures of gene flow among naturally occurring sites supporting *V. americana* in the Chesapeake Bay.

Given the magnitude of decline in *V. americana* population size and extent in the Bay, we wanted to quantify the levels of genetic diversity and inbreeding overall and within remaining populations (Williams and Davis 1996; Williams 2001; Hufford and Mazer 2003) to know if levels were low enough to cause concern for survival and reproduction (Dudash 1990; Frankham 1995a; Gigord et al. 1998; Saccheri et al. 1998; Westemeier et al. 1998; Reed and Frankham 2003). We also wanted to know what amounts of genetic diversity are available because this diversity can affect probability of persistence of remaining populations, potential for unaided recovery, and selection of source material for propagation and planting. Unfortunately, there is no way to know how much genetic diversity there was prior to population size declines, nor exactly how much is enough to be safe from genetic concerns. We compare current levels of genetic diversity with those in other SAV species to understand if amounts of genetic diversity are substantially lower than expected such that they would cause concern for elevated levels of risk. We also wanted to understand patterns of differentiation because they provide insight into ecological and evolutionary processes that are relevant to restoration. For example, if populations are naturally highly differentiated, moving material among locations could have negative consequences due to outbreeding depression resulting from moving locally adapted individuals to less suitable locations (Montalvo and Ellstrand 2001). On the other hand, if historically high connectivity among populations of *V. americana* had been reduced or eliminated (Young et al. 1996), effective population size within habitat patches would be reduced, and the rate of inbreeding and genetic drift increased relative to historical conditions (Frankham 1995b, 1996). In this circumstance, knowledge of long-term patterns of gene flow can focus restoration efforts on locations that have potential for reestablishing natural movement among anthropogenically isolated sites. In total, the genetic data we present here provide useful guidance for the restoration community actively working with *V. americana* in the Chesapeake Bay.

Methods

Sampling localities and protocol

In 2007, 2008, and 2010, we sampled from 26 naturally occurring sites of *V. americana* present in tidal and non-tidal reaches of Chesapeake Bay tributaries (Table 1) to quantify patterns of allelic and genotypic diversity and

Table 1 Measures of genotypic and genetic diversity in populations of *Vallisneria americana* sampled from the Chesapeake Bay, North America

Population grouping	Sample locality	Code	N	G	Genotypic diversity	A	A _p	I	P	H _o	H _e	F _{is}	TPM
Northern Bay	Conford Point	CP	29	26	0.89	5.2	1	1.15	1.0	0.54	0.59	0.089	0.615
	Elk Neck	EN	30	23	0.76	5.5	1	1.22	0.9	0.64	0.60	-0.057	0.500
	Fishing Battery	FB	30	26	0.86	4.8	0	1.16	0.9	0.63	0.60	-0.044	0.082
	Sassafras River	SASS	30	29	0.97	5.8	5	1.24	0.9	0.61	0.61	0.004	0.285
Central Bay	Mariner Point	MP	30	24	0.79	4.6	0	1.20	0.9	0.62	0.63	0.003	0.002
	Dundee Creek	DC	30	30	1.00	5.5	1	1.12	1.0	0.58	0.61	0.052	0.313
	Chesapeake Bay Hot	CBH	25	16	0.63	5.1	0	1.24	1.0	0.65	0.64	-0.014	0.313
	Chesapeake Bay Cold	CBC	25	18	0.71	5.3	2	1.27	1.0	0.64	0.65	0.014	0.278
	Hawks Cove	HWC	29	27	0.93	5.8	3	1.32	1.0	0.67	0.66	-0.011	0.065
	Shallow Creek	SCN	30	6	0.17	3.1	0	0.92	0.9	0.50	0.57	0.138	0.014
	South Ferry Point	SFP	15	5	0.29	3.8	0	1.06	0.9	0.60	0.63	0.055	0.633
	Upper Potomac	Upper Potomac 1	TOUR1	15	3	0.14	2.1	0	0.59	0.7	0.57	0.45	-0.36
Upper Potomac 2		TOUR2	15	2	0.07	1.7	0	0.46	0.7	0.60	0.47	-0.667	N/A
Conococheague Creek		CON	12	2	0.09	1.6	0	0.38	0.5	0.45	0.35	-0.500	N/A
Hancock		HCK	25	8	0.29	3.2	0	0.79	0.7	0.48	0.45	-0.070	0.406
Williamsport		WSP	22	17	0.76	3.0	0	0.77	0.8	0.45	0.45	0.002	0.125
Brunswick		BWK	20	6	0.26	2.8	0	0.76	0.8	0.45	0.48	0.057	0.230
Point of Rocks		POR	33	13	0.38	2.6	0	0.74	0.7	0.49	0.45	-0.099	0.012
Whites Ferry		WF	20	12	0.58	2.9	0	0.75	0.8	0.50	0.44	-0.151	0.098
Lower Potomac	Pennyfield Lock	PL	30	1	0.00	1.5	0	0.35	0.6	0.50	0.50	N/A	N/A
	GW Parkway	GWP	30	26	0.86	4.2	0	0.89	1.0	0.39	0.46	0.160	0.862
	Piscataway Park	SWP	30	29	0.97	4.2	1	0.89	0.8	0.42	0.46	0.083	0.629
	Gunston Manor	GM	30	17	0.55	4.1	0	0.95	0.9	0.51	0.50	-0.014	0.545
	Leesylvania Park	LSP	30	26	0.86	5.0	0	1.06	1.0	0.42	0.52	0.193	0.839
Mattaponi	Aquia Landing	AL	30	30	1.00	5.5	1	1.07	1.0	0.42	0.51	0.193	0.862
	Horse Landing	HL	30	5	0.14	2.7	1	0.73	0.8	0.62	0.48	-0.356	0.320
Average			25.96	16.42	0.57	3.9	0.62	0.93	0.85	0.54	0.53	-0.052	N/A
SD			6.08	10.36	0.34	1.4	1.17	0.28	0.14	0.09	0.09	0.211	N/A

N number of sampled shoots, G unique genets, A average number of alleles (rarefied allelic diversity not shown), A_p number of private alleles, I Shannon’s information index, P proportion of polymorphic loci, H_o observed heterozygosity, H_e expected heterozygosity, F_{is} correlation of alleles within individuals within populations, TPM P value for Wilcoxon one tail for heterozygosity excess test using the two-phase model

Genotypic diversity = (G - 1)/(N - 1); F_{is} and TPM in bold typeface are significant at P < 0.01. Population groups were identified using the Structure analysis

historic gene flow. Collection sites were identified with the help of managers and scientists working within the Mid-Atlantic region of the USA. Sampling represented the geographical and ecological extent of the species in the Bay (Fig. 1). Other regions of the Bay are too deep or too saline to support this species. We sampled the Potomac River extensively because plant material from the river has been harvested in the past for use in restoration projects.

From each site, we collected ~30 shoots, each approximately 5–10 m apart. Samples were often taken blindly as the water was generally too turbid to see shoots, but the distances among samples were kept as consistent as possible given the natural variation in densities at sites. Latitude and

longitude coordinates were recorded for each sampled shoot using global positioning systems in all but three sites (CBH, CBC, CON). Shoot tissue was placed on ice and frozen at -80°C until DNA extraction and genotyping.

DNA extraction and genotyping

Genomic DNA was isolated and purified using methods described in (Burnett et al. 2009). We genotyped 11 microsatellite loci representing tri-nucleotide repeats from each sample using robust primers with specific amplification that were developed for the species (Burnett et al. 2009). Polymerase chain reactions (PCR) were performed

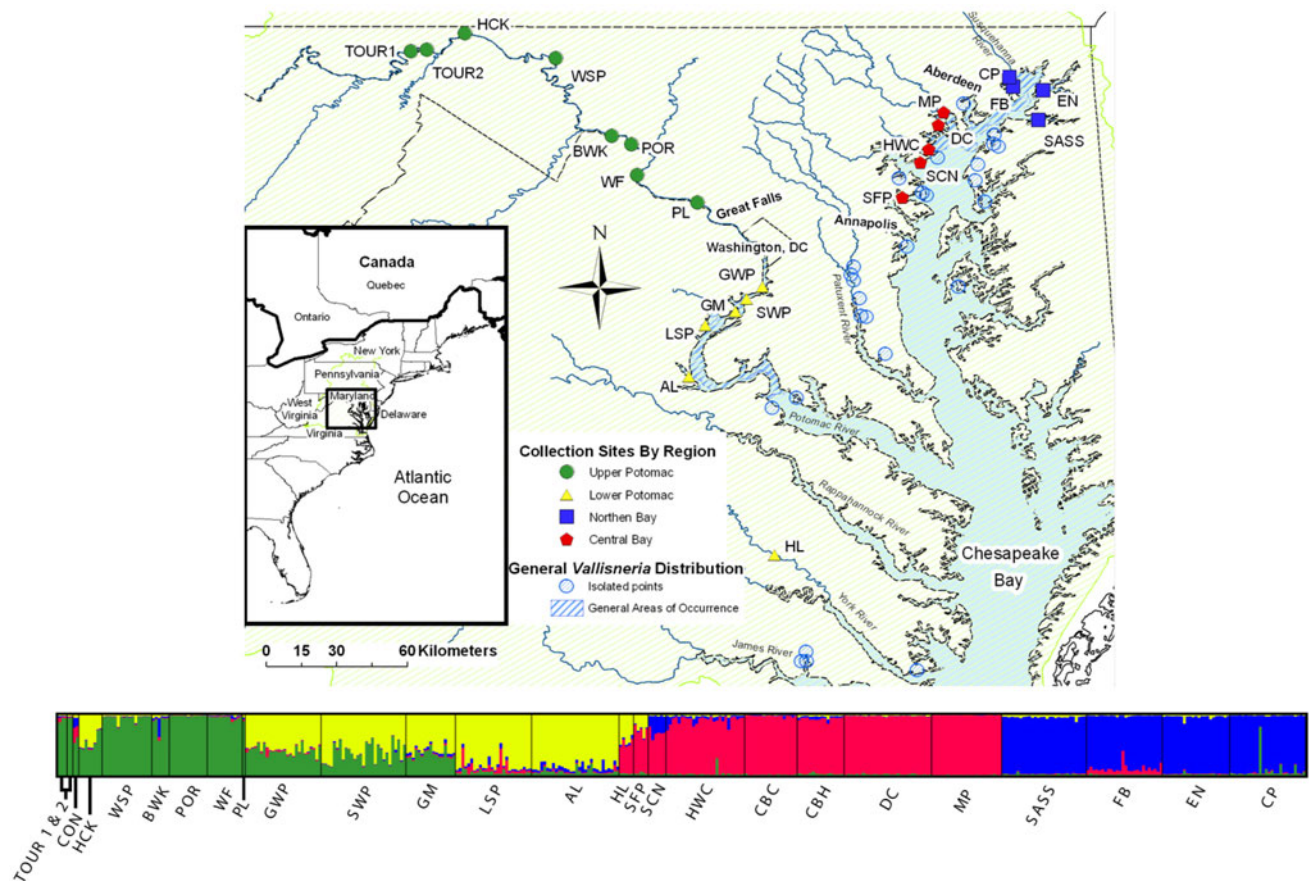


Fig. 1 Structure results (*bottom; colored bars*) for *Vallisneria americana* collection sites (*top; colored symbols*) visited in 2007, 2008, and 2010. Coloring of bars corresponds to coloring of symbols. When $K = 4$, collection sites from the upper Potomac, lower Potomac, central Bay, and northern Bay form four distinct groupings.

on an MJ Research PTC-200 Peltier Thermal Cycler using proprietary reagents in the TopTaq DNA Polymerase Kit (QIAGEN). Reaction conditions for all loci followed Burnett et al. (2009) with the exception of the locus *Vaa_m_AAG004*, for which we added dimethyl sulfoxide and Q-Solution (QIAGEN) to each reaction for optimal specificity. PCR products were separated and measured on an ABI 3730xl DNA Analyzer with GeneScanTM-500 ROXTM or 500 LIZTM Size Standard (Applied Biosystems) after tagging the PCR product with fluorescent labeled forward primers (Applied Biosystems). Peak data were then analyzed using Genemapper v3.7 (Applied Biosystems) and all allele calls were also visually inspected.

Ambiguity in calls resulting from human or PCR error can result in individuals being misclassified and cascading errors in subsequent analyses. For quality control purposes we reran every ambiguous call up to three times (as necessary). If after three attempts the sample was still ambiguous, the alleles were coded as missing data. In addition, we confirmed genotype calls by re-extracting

PL was excluded from the analysis due to low genotypic diversity. Sites not shown are CON (near WSP), and CBH/CHC (near DC). Dark blue hashed areas represent general and isolated areas where *Vallisneria* occurs in the Bay (Moore et al. 2000)

DNA from 32 samples, rerunning all PCRs and re-genotyping at all loci. These samples were chosen because together they were present across all eight 96 well plates used in the initial fragment analysis. This confirmatory process was completed several months after the initial analysis of the raw data and scoring was done without looking at the initial scores. We detected no allele scoring differences in any of these samples.

Genotypic diversity

We detected clones within and across sites by identifying identical multilocus genotypes using the program GenClone v2.0 (Arnaud-Haond and Belkhir 2007). Because mutation and scoring errors can lead to individuals originating from the same sexual reproductive event having different genotypes we used Genodive v2.0b17 (Meirmans and Van Tienderen 2004) to quantify pairwise differences in alleles among all individuals. Genodive calculates a distance matrix based on the minimum number of mutation steps that

are needed to transform the genotype of one individual into the genotype of the other, summed over all loci. Individuals with distances below a threshold in the distance matrix (threshold = 11) were considered to represent the same genet (Rogstad et al. 2002; Meirmans and Van Tienderen 2004). This threshold represents the minimum number of mutation steps that is needed to transform the genotype of one individual into the genotype of another and was chosen because it was prior to the point of inflection in the distribution number of clones. Beyond this threshold, genotypes that were different at multiple loci would be identified as one genet, which we considered inappropriate. We compared genets identified using this method with those that would be identified using complete multilocus matches and found 66 individuals differed due to 3–6 base pair mutation at a single locus and 25 individuals were missing data at one locus but matched exactly at all nine other loci. Thus, everything we identified as a clone was also identified when exact multilocus matches were required, but we lumped 91 ramets with another genotype that would be identified as unique if missing data or the mutations were coded separately.

We assessed the probability that shoots with identical genotypes were members of the same clone rather than occurring by chance by using P_{gen} (Parks and Werth 1993) to estimate the probability of the occurrence of each genotype based on allele frequencies in each population. We then calculated the probability of sampling a second occurrence of each genotype given the number of genets sampled using P_{sec} (Parks and Werth 1993). These calculations were done using the program GenClone. For each site, the proportion of unique genotypes was calculated as $(G - 1)/(N - 1)$, where G is the number of unique genotypes and N is the total number of shoots sampled (Pleasants and Wendel 1989; Arnaud-Haond et al. 2007). For subsequent analyses, each genet within a population was represented by only one shoot (ramet).

The dispersal of vegetative tissues across long distances has been documented in other submersed aquatics (Langeland 1996; Fér and Hroudová 2008), providing the possibility for sharing of *V. americana* genotypes among sites. To assess the extent of such sharing we pooled all samples, and quantified shared genotypes among sites in Genodive. As with the within-population comparisons, everything we determined to be a clone was an exact multilocus match.

Measures of genetic diversity

For all loci, observed number of alleles (A_n), expected (H_e) and observed (H_o) heterozygosity, proportion of polymorphic loci (P), and private alleles (A_p) within each of the 26 collection sites and across all sites combined were

calculated using GDA v1.1 (Lewis and Zaykin 2001). To compare allelic diversity among collection sites and regions, we controlled for varying sample size by conducting a rarefaction analysis using the program HP-Rare v1.0 (Kalinowski 2004, 2005); rarefied estimates were not used in other analyses. Shannon's information index (I) was calculated using PopGene v1.32 (Yeh et al. 1997).

Wright's F_{is} was calculated for the global dataset using the estimator f (Weir and Cockerham 1984) in GDA to test for site-level deviations from Hardy–Weinberg equilibrium. Significance of F_{is} was tested by obtaining confidence limits around each estimate generated by 1000 bootstraps in GDA. Significant departures from Hardy–Weinberg equilibrium can indicate a departure from random breeding.

We examined each site that had more than two genotypes for presence of a recent genetic bottleneck using a test for heterozygote excess in the program Bottleneck v 1.2.02 (Cornuet and Luikart 1996). Bottleneck computes heterozygote excess as the difference between expected heterozygosity (H_e) and heterozygosity expected at equilibrium (H_{eq}) for each site from the number of alleles given the sample size (Cornuet and Luikart 1996). Significance of the difference between H_e and H_{eq} was tested using a one-tailed Wilcoxon's sign rank test under a two-phase mutation model which provides results intermediate between an infinite allele model and a stepwise mutation model that are considered to be most appropriate for microsatellites (Di Rienzo et al. 1994).

Population differentiation

We assessed patterns of genetic differentiation in three complementary ways. First we used the program Structurama v1.0 (Huelsenbeck and Andolfatto 2007) to identify theoretical a posteriori 'populations' from our collection of sites based on minimal deviations from both Hardy–Weinberg and linkage equilibrium as in Pritchard et al. (2000). Structurama differs from the program Structure (Pritchard et al. 2000) in that the number of theoretical populations is included as a parameter in the model and a posterior distribution of the probabilities of each number is generated. Prior number of populations and expected number of populations were set as random variables. The sampler was run for 1,000,000 generations and sampled every 25 generations for a total of 40,000 samples. Four heated chains (temperature = 0.1) were used in the analysis. Data were summarized after discarding 10,000 burn-in samples. We chose the mean partition value as the number of theoretical populations (K) containing the highest posterior probability. Because Structurama lacks clearly interpretable visualization of individual assignments we used Structure v2.3.2 (Pritchard et al. 2000) to assess

distinctiveness of theoretical populations (Berryman 2002) by assigning individuals to the number of populations inferred by Structurama. Structure was run assuming prior admixture, with 1,000,000 steps in the Bayesian sampler, using a burn-in of 50,000 steps. The analysis was run 10 times, and the best run was selected based on the highest likelihood score.

To provide a general overview of site-level differentiation, we calculated global and pairwise estimates of Wright's F_{st} , using Weir and Cockerham's (1984) estimate θ as calculated in GDA. Significance was assessed by generating confidence limits derived from 1000 bootstrap samples. All θ values were normalized to account for the theoretical maximum value and thus allow for future comparison across studies (Hedrick 2005; Meirmans 2006) using the program Genodive (Meirmans and Van Tienderen 2004). There is no significance test for these normalized values (Meirmans 2006). To account for potential limitations of F_{st} in quantifying differentiation (Hedrick 2005; Jost 2008), we also calculated pairwise and global values of Jost's (2008) measure of genetic differentiation, D , using Chao et al.'s (2008) estimate D_{est_Chao} in SMOGD v 1.2.5 (Crawford 2009). Significance was assessed by generating confidence limits derived from 1000 bootstrap samples in SMOGD.

We tested for relationships between linearized pairwise F_{st} ($F_{st}/(1 - F_{st})$) (Slatkin 1995) among sites and two different geographic distances using a Mantel test as implemented by the program IBDWS v3.16 (Jensen et al. 2005). Significance was assessed using 1,000 randomizations in IBDWS. We used pairwise Euclidean geographic distances calculated from the GPS coordinates collected in the field, and the shortest distance over water among paired sites using Pathmatrix v1.1 (Ray 2005). Euclidian distance is potentially realistic for seed dispersal by waterfowl that can fly over land whereas the weighted geographic distances are more realistic for water-dispersed pollen.

We used principal components analysis (PCA) on the variance–covariance matrix of allele frequencies, using Genodive, to understand the distribution of variance among sampled locations that is a function of variation in allelic composition. PCA provides a different perspective from the Structurama/Structure analyses because it represents the relative degree of genetic similarity among sites in a continuous rather than categorical framework.

Estimates of gene flow among populations

Because coalescent-based methods can provide more accurate and powerful estimates of migration than classical frequentist estimates (Rosenberg and Nordborg 2002; Holsinger and Weir 2009), we quantified migration among population groupings using Migrate-n v3.2.6 (Beerli and

Felsenstein 1999, 2001; Beerli 2006). Migrate-n employs a likelihood method of parameter estimation utilizing coalescent theory to estimate asymmetric migration among populations under an equilibrium model that assumes migration has been constant over time (Beerli and Felsenstein 1999). Estimating migration among all sites would require estimating 462 parameters. To estimate a reasonable number of parameters given our data, we limited migration to four groupings based on results from the Structurama/Structure analyses and geographic proximity of sites. The HL locality was difficult to assign to a group in Structure (Fig. 1) due to assignment probabilities being split between groupings and geographic distance from other sites; it therefore was excluded from this analysis.

Migrate-n was run with the following parameters. Data were treated under a Brownian motion mutational model where mutation rate was calculated as a random variable from the data and missing alleles were discarded. The Bayesian sampler started from a random genealogy with a full migration model, where both migration rate (M) and population size (θ) were free to vary. The sampler utilized uniform priors for both M and θ . To reduce the size of the tree-space explored by the samples, the priors were constrained based on exploratory analyses between 0 and 4.5 with delta = 0.01 for θ , and 0–150 with delta = 30 for M each with 500 bins. Four parallel chains with a swap interval of 1.0 were run with heating values of 10, 7, 4, and 1. One long chain of 80,000 recorded steps was sampled every 20 steps, for a total of 1,600,000 sampled parameters values. Subsequent posterior distributions were summarized after a burn-in of 10,000 steps. The burnin value was selected following examination of exploratory data analyses. Convergence of the run was assessed using effective sample size calculated in migrate-n.

The number of immigrants per generation (Nm) was estimated as $4Nm_j = M_{ij} \times \theta_j$, where θ_j is the effective population size of the recipient population and M_{ij} is the migration rate from population i to population j .

Results

Genetic diversity

We sampled a total of 675 shoots, representing 427 unique genotypes. Within each of 26 locations, we sampled an average of 26.0 shoots (Table 1). A median of 68% of sampled shoots within sites represented unique genets, but the proportion of shoots representing multiple genets varied from 0.00 to 1.000 (Table 1). Eight of nine sites upstream from and including PL in the Potomac River and site HL in the Mattaponi River were particularly low in genotypic diversity, with genotypic diversity ranging

between 0 and 0.38 of sampled shoots being unique genets (Table 1). Site PL was the most extreme, with all 30 samples representing a single genotype. Two exceptions to the trend of low genotypic diversity upstream of PL in the Potomac River were WF and WSP that had clonal diversity values of 0.58 and 0.76, respectively.

Five genotypes were shared among sites within the upper Potomac River (Table 2). Two of these genotypes dominated multiple sites, often comprising 53–100% of sampled shoots. Those two genotypes spanned large geographic distances; one genotype covered approximately 160 river km and the other was present across 132 river km. We found no genotypes shared among other sites within the Chesapeake Bay.

The probability of recovering any given genotype by chance ranged from 5.63×10^{-16} to 5.75×10^{-7} (SD = 3.97×10^{-8}). The probability of finding a second occurrence of each genotype, given the number of genets sampled, ranged from 2.37×10^{-13} to 2.45×10^{-4} (SD = 1.70×10^{-5}). The genotypes that spanned large geographic distances in the Potomac River ranged in the probability of occurrence from 6.5×10^{-11} to 1.5×10^{-7} and in the probability of re-sampling one of those genotypes from 2.75×10^{-8} to 6.57×10^{-5} (Table 2). Thus we consider these identical genotypes to be clones that resulted from the same sexual reproduction even.

Many loci showed departure from Hardy–Weinberg equilibrium; however, the degree of deviation was often minimal (Table 3). The locus AAGX013 showed significant departure from HWE, and also had a large amount of missing data (31.92%); therefore, it was excluded from subsequent analyses. The amount of missing data in the remaining 10 loci was negligible, averaging 0.84% and ranging from 0.23 to 2.35%.

The proportion of polymorphic loci within sites was 0.854 (SD = 0.139). The average number of alleles per locus across all sites combined was 8.70 (SD = 4.08) and within sites was 3.91 (SD = 1.40). When we standardized by number of genets, the number of alleles among sites was similar indicating that genotypic diversity largely controlled allelic diversity. Between one and five private

Table 3 Genetic diversity of individual loci averaged over all *V. americana* populations

	A	H_o	H_e	F_{is}	Percent missing data
Locus					
AAGX071	10	0.681	0.753	0.095*	0.7
AAGX051	16	0.789	0.865	0.087*	0.94
AAGX012	6	0.406	0.441	0.078*	0.23
ATG002	10	0.723	0.771	0.062*	0.23
AAGX030	5	0.312	0.350	0.107*	0.23
M49	14	0.607	0.694	0.124*	0.47
M13	9	0.631	0.807	0.218*	1.64
AAG002	4	0.547	0.568	0.036	1.17
M16	4	0.082	0.084	0.017	0.47
AAG004	9	0.580	0.688	0.156*	2.35
Average	8.700	0.536	0.602	0.109	0.843
SD	4.084	0.213	0.244	0.058	0.703
Excluded locus					
AAGX013*	7	0.152	0.582	0.740*	31.92

A total number of alleles, H_o observed heterozygosity, H_e expected heterozygosity, F_{is} correlation of alleles within individuals within populations

* $P < 0.05$

alleles were found in nine populations. Seven of the sites with private alleles were in the main stem of the Chesapeake Bay (Table 1). Sites with private alleles were also relatively high in genotypic diversity (>18 genets). None of the sites with low genotypic diversity in the Potomac River had private alleles.

Observed heterozygosity was high at all sites (avg $H_o = 0.535$; SD = 0.086). Nine sites departed significantly from Hardy–Weinberg equilibrium (Table 1); six sites had more heterozygotes than expected (EN, Tour1, Tour2, CON, WF, and HL) and three had fewer heterozygotes (GWP, AL, LSP; Table 1). Shannon’s information index was similar among all sites except the HL site, and those sampled in the Potomac River above Great Falls, MD (Table 1).

Table 2 Number of *V. americana* shoots, and P_{gen} and P_{sec} of each genet (Parks AND Werth 1993) that are shared among sites on the main stem of the Potomac River

Genotype	Tour 1 (n = 15)	Tour 2 (n = 15)	HCK (n = 25)	WSP (n = 22)	BWK (n = 20)	POR (n = 33)	WF (n = 20)	PL (n = 30)	P_{gen}	P_{sec}
1	8	12	12	1	7	2			1.55×10^{-9}	6.61×10^{-7}
2				6	9	15		30	1.54×10^{-7}	6.57×10^{-5}
3			1	3					6.47×10^{-11}	2.76×10^{-8}
4			3	1					1.93×10^{-9}	8.25×10^{-7}
5						1	5		8.85×10^{-10}	3.78×10^{-7}

Sites are ordered from upstream (left) to downstream

Based on analysis with the program Bottleneck (Cornuet and Luikart 1996), 3 of the 24 sites we could analyze (MP, SCN, and POR) showed evidence that H_e significantly exceeds H_{eq} , which suggests that they have undergone recent genetic bottlenecks (Table 1). Of the sites in the lower Potomac with significant F_{is} , two of these sites supported only two genotypes and thus did not have the minimum number of samples to run Bottleneck; the third only met the minimum requirement of three genotypes. Lack of a significant bottleneck for this site could easily have been due to the small sample size.

Population differentiation

Bayesian clustering analysis as implemented by Structurama indicated that there are four genetic subdivisions in the 26 sampled locations of *V. americana* in the Chesapeake Bay ($\text{Pr}[K = 4|X] = 0.9993$). When Structure was run assuming $K = 4$ to visualize individual clusters three primary divisions were noted: northern Bay localities, central Bay localities, and Potomac River localities (Fig. 1). A further subdivision between the upper and lower Potomac River was identified. Mixed population assignments of individuals provide evidence of similarity among all members of the upper Potomac and several lower Potomac sites (GWP, SWP, GM). The sites LSP and AL had low probability of assignment into the upper Potomac localities (Fig. 1). The Potomac River sites also have a very small degree of admixture with the central Bay sites, which is most evident in LSP (Fig. 1). Site HL from the Mattaponi River was difficult to assign, with assignment probabilities being split between the Potomac group and the central Bay group.

Overall, we observed moderate levels of global genetic differentiation among all sites combined ($\theta = 0.114$, 95% CI = 0.081–0.152). The PL location was excluded from these analyses because it is not possible to calculate F_{st} or D for a site with only one sample. Within regions identified in Structure, the median pairwise values of θ among sites ranged from ~ 0.020 in the upper and central Bay, to 0.043 among sites in the lower Potomac, to 0.10 in the upper Potomac. The median pairwise θ value of sites from different regions was 0.114 and the range was from 0.013 to 0.32. Thus, the pairwise differences among sites from the upper Potomac (range was from -0.02 to 0.31) were similar to differences among other sites from different regions. The global D_{est_Chao} (0.124, 95% CI = 0.008–0.352) was slightly higher than θ . The median pairwise D_{est_Chao} among regions was 0.07. Within region median values of D_{est_Chao} were lower than those observed with θ (northern Bay = 0.02; central Bay = 0.01; upper Potomac = 0.01; lower Potomac = 0.009), and indicate that differentiation within regions was substantially lower than among regions.

There were significant relationships between genetic distance and both straight-line ($r = 0.39$; $P < 0.001$) and weighted ($r = 0.59$; $P < 0.001$) distances (Fig. 2) for all sites combined. Relationships with both geographic distances were also significant in the upper (straight-line: $r = 0.41$; $P < 0.001$; weighted: $r = 0.47$; $P < 0.001$) and lower Potomac River (straight-line: $r = 0.69$; $P < 0.001$; weighted: $r = 0.93$; $P < 0.001$) groups. In the northern Chesapeake Bay, neither measure of geographic distance provided a significant correlation. The central Chesapeake

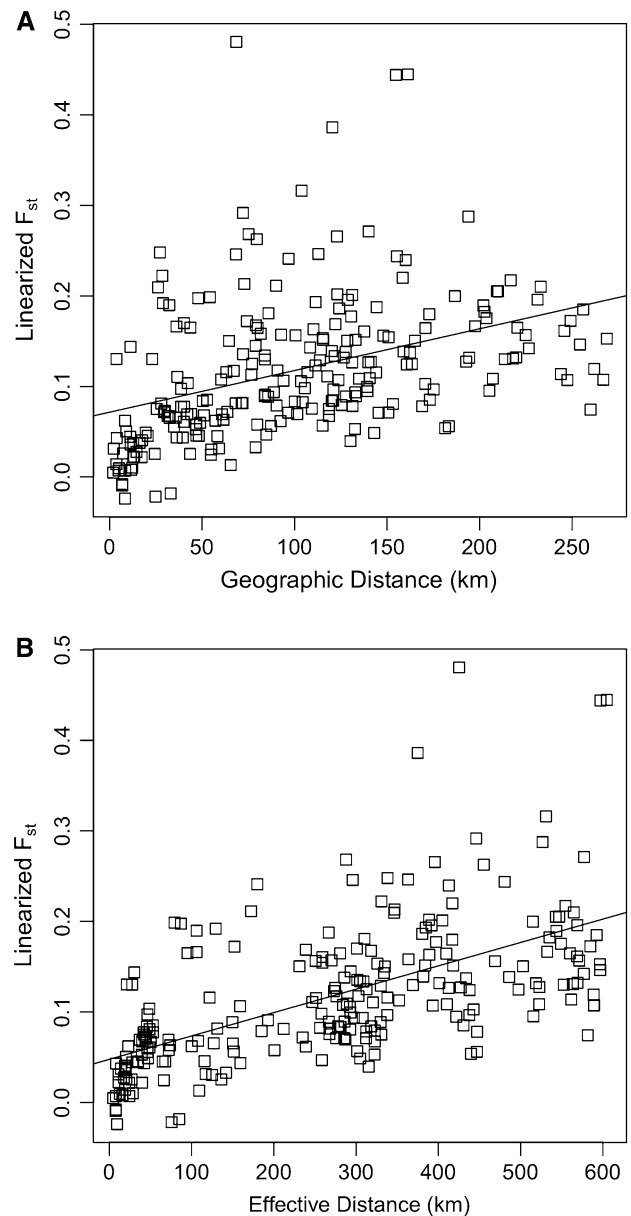


Fig. 2 Linearized F_{st} ($F_{st}/(1 - F_{st})$) (Slatkin 1995) genetic distance regressed against **a** Euclidean geographic distance and **b** the shortest distance over water among collection sites (weighted geographic distance)

Bay tended to have larger genetic distances among sites relative to the northern Chesapeake Bay (distance table not shown); however, the correlation was not significant for either distance measure.

The PCA on the variance–covariance matrix of allele frequencies showed that allelic composition was generally more similar within than among the four geographic regions within the Chesapeake Bay identified in the Structure analysis (Fig. 3). The first axis explained 27.58% of the variance in allele frequencies and captured differences among the regions. The second axis explained 18.65% of the variance and was driven primarily by two sites with extremely low genotypic diversity ($G = 2$ in CON and $G = 1$ in PL). Both populations were distinct due to chance fixation of some alleles and the fact that given small number of genets present in each site, allele frequencies are by necessity limited to a small range of values, and those values happened to be higher than those in other populations. The alleles that were fixed in these sites were also present in other sites but the resulting large differences in allele frequency placed CON and PL away from all other sites, and compressed the remaining sites into a small portion of Axis 2 (Fig. 3).

Migration

Effective sample size, a measure of convergence, exceeded 1000 samples for all parameters. The number of migrants per generation ($4Nm$) among the four groups identified

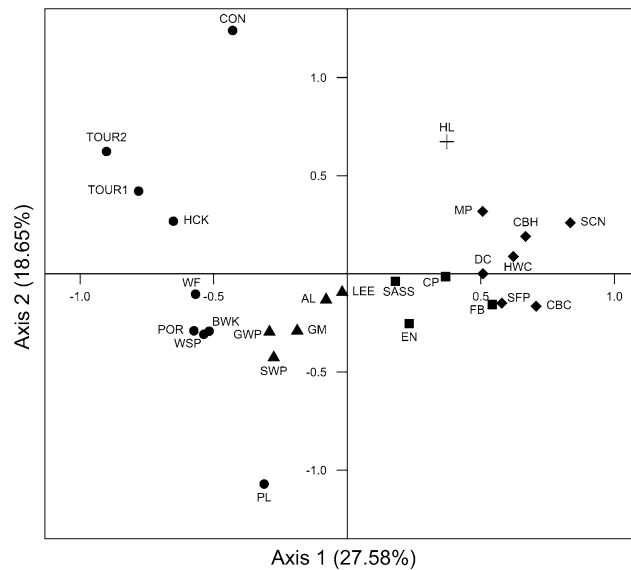


Fig. 3 Principal components analysis of the covariance matrix of allele frequencies. Axis 1: Eigenvalue = 0.29, percent of variation explained = 27.58; and axis 2: Eigen value = 0.19, percent of variation explained = 18.65. Symbols represent the four genetic regions within the Chesapeake Bay

using Structure and geographic proximity varied from 7.69 to 29.91 (Fig. 4). The upper Potomac River population grouping was largely isolated from all other populations. The lower Potomac River population grouping had apparent migrant exchange with both the northern and central population groupings with relatively equal frequency ($4Nm = 25.41$ – 29.91). The northern Chesapeake Bay received nearly the same number of migrants from ($4Nm = 28.14$; CI = 23.21 – 32.96) as it contributed to ($4Nm = 21.29$; CI = 17.06 – 26.24 ; Fig. 4) the central Chesapeake Bay. In contrast, the upper Potomac River appeared to share more migrants with the lower Potomac ($4Nm = 17.39$; CI = 12.44 – 21.62) than the lower Potomac shared with the upper Potomac ($4Nm = 9.91$; CI = 7.67 – 13.61), but the confidence intervals in these estimates overlapped to a small degree.

Discussion

Overall, most sites of *V. americana* in the Chesapeake Bay support a diversity of genotypes and alleles, and most are not highly inbred. This is good news for the future of the species in the Bay because high genetic diversity increases a population’s capacity to persist under variable environmental conditions (Frankham 1995a; Procaccini and Piazzi

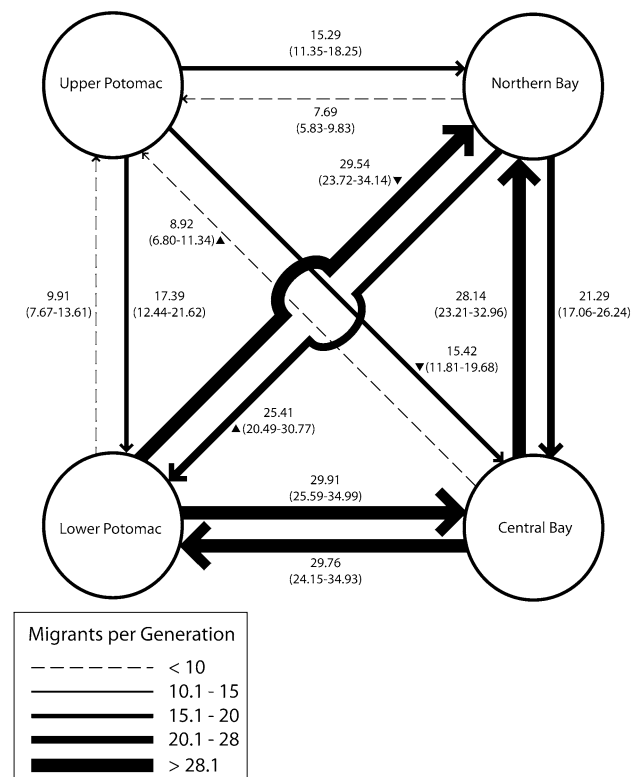


Fig. 4 Per generation bidirectional migration rates ($4Nm$) among the four population grouping recovered from analysis in Migrate-n

2001; Williams 2001; Reed and Frankham 2003) and to adapt to novel conditions (Frankham 2005; Lavergne and Molofsky 2007; Barrett and Schluter 2008). The genotypically diverse sites can also serve as sources of material for restoring *V. americana* to currently unoccupied sites. The geographic structuring of genetic diversity we documented is important to consider if movement of propagules around the Bay is proposed. Despite the relatively positive general outlook, evidence for recent bottlenecks in three sites, signs of inbreeding at three sites, and low genotypic diversity in the upper Potomac River raise concern for long-term effects of the previous population declines.

Genetic diversity

Species level allelic richness in the Chesapeake Bay and its tributaries was on par with what has been found in other SAV species from throughout the world, which ranges from 2 to 18 alleles per locus (Reusch et al. 1999b, 2000; Rhode and Duffy 2004; Pollux et al. 2007; van Dijk et al. 2009; Campanella et al. 2010). Our site-level allelic richness was also mostly within the typical ranges of values found in these same studies of other SAV species (2.3–10.5 alleles per locus). The three exceptions that had particularly low allelic richness (1.5–1.7 alleles/locus) supported only 1 or 2 unique genotypes each (Table 1). Beyond these extreme cases, lower allelic diversity was associated with lower genotypic diversity, typically with <30% of sampled shoots in low allelic diversity sites being unique genets.

Evidence of recent bottlenecks based on heterozygote excess in three sites (MP, SCN, and POR) and the significant inbreeding coefficients in three sites in the lower Potomac River (GWP, LSP, AL; Table 1) cause some concern. However, widespread inbreeding was not observed despite low levels of genotypic diversity (and therefore effective population size). The dioecious mating system of *V. americana* enforces outcrossing and may explain why inbreeding was not more prevalent. Determining the full implications of apparent bottlenecks and inbreeding requires understanding their fitness consequences, which is beyond the scope of this study.

One of our more striking results is that genotypic diversity ranged from 0 to 1.0, meaning that sites ranged from being monoclonal to every sampled shoot being distinct. It also means sites range from having no detectable sexual reproduction to no detectable asexual reproduction. Such variation in mating structure across this same spatial scale is not common in aquatic species but has been documented in *Typha minima* Hoppe (Till-Bottraud et al. 2010) and in *Posidonia oceanica* Delile (0.1–0.97; Arnaud-Haond et al. 2010). The general paradigm that *Vallisneria* populations are maintained primarily by vegetative reproduction

(e.g., McFarland and Shafer 2008) is not supported by our data.

The sites with low genotypic diversity relative to other *V. americana* locations in the Bay are those in the upper Potomac River, site HL in the Mattaponi River, and sites SCN and SFP in the central Chesapeake Bay. Variation in levels of genotypic diversity among sites is interesting because of the advantages typically associated with high genotypic diversity and for the insights into the potential mechanisms that might have caused these sites to have fewer, more extensive clones than other sites in the Bay. Higher genotypic diversity has been correlated with increased resistance to periodic stressors and more resilience after climatic extremes in experimental settings (Hughes and Stachowicz 2004; Reusch et al. 2005; Hughes and Stachowicz 2009) and with increased survival of transplants (Procaccini and Piazzini 2001). Thus, although sites in the upper Potomac River support extensive cover, the few highly successful genotypes may not provide the genetic variation necessary to withstand novel perturbations or adapt to future conditions. It is important to note that the effect of genotypic diversity on the stability of SAV beds is still unclear. At least some field observations indicated higher mortality in more genetically diverse populations of *P. oceanica* (Arnaud-Haond et al. 2010). Further, sedimentation rate was a stronger predictor of shoot mortality in *P. oceanica* than were genetic diversity or even demographic parameters (Arnaud-Haond et al. 2010).

Clearly, at extreme levels of disturbance that exceed physiological tolerances, no amount of genetic diversity will be sufficient to withstand or overcome perturbations, and environmental factors become more important. Short of such extremes, it is plausible that a limited number of genotypes will be sufficiently resistant to survive perturbations, which would result in less genotypically diverse populations in high disturbance sites. Conversely, low genotypic diversity in more stable sites has been explained as resulting from one genotype becoming dominant. Periodic or fluctuating disturbance could foster more genotypic diversity if survival and fitness of genotypes differed across conditions (Hammerli and Reusch 2003). The patterns observed in any particular case will depend on the magnitude and frequency of disturbance and the interaction between that disturbance and genotypic or phenotypic abilities to withstand it. Without monitoring over time, it is not possible to know if low genotypic diversity is a signature of past environmental perturbations that have left only tolerant genotypes or the result of stochastic losses.

In addition to having low genotypic diversity, multiple sites along the upper Potomac River shared the same genotype (Table 2). The geographic extent of the five shared genotypes is remarkable: two of them extended a

distance of 130 and 160 river km, and the remaining three genotypes covered distances of 50 river km. The probability of recovering the specific genotypes by chance if they were not identical by descent given global allele frequencies is astronomically small 10^{-7} to 10^{-11} (Parks and Werth 1993), and the probability of finding a second occurrence of each genotype, given the number of genets sampled, is 10^{-5} to 10^{-8} (Parks and Werth 1993). A typical mutation rate of microsatellite loci ($\sim 10^{-3}$ to 10^{-4} per allele per generation; Thuillet et al. 2002; Vigouroux et al. 2002) does provide the possibility that these genotypes are merely identical in state (Mank and Avise 2003); however, it is highly unlikely that mutation events simultaneously produced identical individuals across such a large geographic range. Although a large proportion of studied angiosperm species exhibit clonality that extends across more than one location (Ellstrand and Roose 1987), extremely large clonal extent is rare. Examples of the larger known clonal extents include a single *Populus tremuloides* Michx. clone that covers an area of roughly 43 ha (Mitton and Grant 1996), and several submersed aquatic species that are known to have clones that extend >5 km (Reusch et al. 1999a; Ruggiero et al. 2002). Most studies of other SAV species indicate that clones are primarily limited to within individual sites (Titus and Hoover 1991; Campanella et al. 2010) with extents typically limited to the scale of ~ 18 m (Becheler et al. 2010), to 78 m (Arnaud-Haond et al. 2010), to ~ 250 m (Zipperle et al. 2009).

Vegetative expansion of *V. americana* through rhizomes is generally limited to within a few meters of the parent plant (Titus and Hoover 1991). Maximum seasonal lateral growth of *V. americana* from the upper Potomac River genotypes is 60 cm under greenhouse conditions (Engelhardt, unpublished data). At this ideal growth rate it would take roughly 260,000 years to grow 130–160 km, and even supposing growth occurred from a central location outward, it would take 130,000 years to traverse that distance. It is unlikely that habitat necessary to allow this vegetative growth would have been sufficiently continuous and stable throughout the stretch of the river for such a long period of time. Thus, although lateral vegetative growth within sites could potentially lead to local dominance by one or a few genotypes, it is highly improbable that lateral growth alone is responsible for genotypes extending 50–160 km along the Potomac River.

The question, then, is how did these few genotypes come to extend and dominate over such large areas? Specific mechanisms could include passive stochastic loss and colonization, deterministic processes based on competitive ability, selective advantages due to environmental tolerance of particular genotypes, or a combination of passive and deterministic processes. Passive processes could include initial chance colonization by few genotypes that

expanded in place, or stochastic loss of genotypes within sites followed by repeated recolonization by a small number of genotypes. More deterministic processes include selection in response to abiotic factors or competition. If particular genotypes were resistant to abiotic stressors, they would become dominant as other genotypes were eliminated. Dominance by a few clones could also result if downstream sites were colonized by a small number of competitively superior vegetative propagules from upstream populations, widespread dominance of a limited number of genotypes would result. We offer these mechanisms as possible explanations; our current data are not sufficient to infer mechanism but are more consistent with some possibilities than others, and clearly point to the need for further experiments.

Tubers of *V. americana* are generally negatively buoyant, but they can become positively buoyant if attached to shoot fragments (Titus and Hoover 1991). The extensive clones we observed in the Upper Potomac River could have originated from dislodged shoots and tubers that were carried downstream in floods (Fér and Hroudová 2008). Flooding events sufficiently extreme to cause scouring are common in the Potomac River and removal of individuals from suitable habitat would create opportunities for expansion of chance colonists. It is likely that upstream populations have either had low diversity due to founder events, or that diversity has been lost from small, isolated sites. Once upstream populations have low genotypic diversity, opportunities to gain new diversity would be limited due to unidirectional water flow from headwaters to mouth. Large distances from other major bodies of water yield small chances of recolonization from sources other than nearby low diversity sites (Chen et al. 2007). The process could generate a positive feedback loop in that as particular genotypes become more dominant, they become more likely to be source material for additional colonizations. An additional consequence of low genotypic diversity that may in turn facilitate dominance of a few genotypes is the reduced probability of having both males and females, which limits sexual reproduction. Existing clones could have higher potential to spread and occupy larger areas than they might in populations that also had sexually produced propagules. We have no quantitative data on sex ratios but we have observed fertile fruits at all sites, indicating some sexual reproduction is occurring. However, for the same level of search effort, we found substantially fewer fruits at many of the upper Potomac River sites than we found in other locations throughout the Bay.

Another explanation that we considered to possibly explain widespread dominance was the introduction of competitively superior genotypes into the Potomac River via restoration or other activities, or through natural

mechanisms such as ingestion and dispersal of tubers via waterfowl. We know of no restoration activities within any of these regions. Additionally, many of the sites visited were not easily accessible, which would hinder the inadvertent introduction by humans through recreational activities such as boating or through activities such as dumping of aquaria.

It is most likely that the unprecedented size of the large *V. americana* clones in the Potomac River has resulted from a combination of local spread via rhizomes and repeated longer distance dispersal of tubers during storm events. Clearly, much still needs to be learned regarding dispersal of vegetative propagules from parent populations (Titus and Hoover 1991). Regardless of the mechanisms, lower genotypic and allelic diversity in the upper Potomac River sites compared to other localities in the Bay suggests that they should be considered cautiously as source material for restoration plantings. Sampling shoots from even widespread locations is highly likely to yield the same genotype. If the upper Potomac River were used as a source for restoration, using seed rather than vegetative material would improve chances of representing more genetic diversity and of including both male and females in restoration plantings.

Genetic differentiation and migration

The overall patterns of genetic differentiation among sites in the Bay related strongly to geographic distance (both straight line and weighted and is indicative of equilibrium between genetic drift and gene flow (Hutchison and Templeton 1999). Beyond coarse geographic trends, Structure analysis indicated the Chesapeake Bay can be broken into four genetic regions. These subdivisions roughly correspond to regions of differing salinity. The northern Chesapeake Bay is oligohaline and the central Chesapeake Bay is oligohaline to seasonally mesohaline (Pritchard 1952). Sites in the lower Potomac River are oligohaline and are strongly tidally influenced while the upper Potomac River is entirely freshwater. Such environmental differences can increase isolation among populations (Keeley 1979; Stanton et al. 1997; Doebeli and Dieckmann 2003), influence patterns of occurrence and hybridization (Crain et al. 2004; Blum et al. 2010), and drive adaptation to local conditions (Clausen et al. 1941; Antonovics and Bradshaw 1970; Linhart and Grant 1996; Antonovics 2006).

The admixture among the regions implies at least historic gene flow among sites, and results from the full Migrate-n analysis show evidence of some exchange between the two regions within the Potomac River (Fig. 4). Even with this admixture, the level of substructuring we detected is surprising given the potential for the Bay to

represent one large, hydrologically connected unit (e.g., van Dijk et al. 2009). The degree of substructuring is greater than has been found in other studies at similar scales (Campanella et al. 2010).

The level of differentiation we observed among sites within each region is similar to levels documented from hydrologically connected populations of several *Vallisneria* species ($G_{st} = 0.02–0.06$; Lokker et al. 1994; Chen et al. 2007) and other seagrass populations sampled from similar spatial scales (Campanella et al. 2010). When sites are pooled, the degree of genetic differentiation between the north and central Chesapeake Bay ($D_{est_Chao} = 0.060$) is at the upper range of the levels documented among connected sites. Levels of differentiation among sample sites in different regions are more similar to those found in isolated water bodies: $F_{st} = 0.132–0.202$ and $G_{st} = 0.457$ (Laushman 1993; Wang et al. 2010). Interestingly, the amount of gene flow between the north and central localities estimated by Migrate-n is theoretically enough ($4Nm = 21.29–28.14$) to swamp out genetic differentiation among populations. If successful migration among populations is sufficiently common (e.g., >1 migrant per generation), genetic subdivision is not likely to occur (Wright 1931; Slatkin 1981, 1985, 1987). Several factors could be influencing the observed patterns of gene flow among the populations. Coalescent-based analyses integrate estimates of migration and effective population size over $4N_e$ generations (Kingman 1982a, b). A disconnect between current patterns of genetic differentiation and the amount of historic gene flow among populations could exist (Sork et al. 1999). In addition, genetic differentiation can occur in presence of substantial gene flow (Morrell et al. 2003). In cases where extreme environmental heterogeneity exists among sites, reproductive isolation can develop and be sustained even in the face of genetic exchange among populations (Caisse and Antonovics 1978; Antonovics 2006).

We interpret the inferred regions cautiously because sampling from a continuous population with local mating structure can yield ‘populations’ using the program Structure (Schwartz and McKelvey 2008). However, most sites we sampled in the northern and central Bay were from discrete beds that are isolated from other beds by depth and salinity beyond the limits of tolerance for *Vallisneria*. Thus, although they would have been more extensive historically, it is not likely that many of the now isolated beds would ever have been continuous. In contrast, the upper Potomac River is probably best considered one extensive relatively continuous population with a combination of extensive vegetative dispersal and of sexual reproduction among spatially proximal individuals. Within the upper Potomac, F_{st} and Jost’s D values (Table 1) reflect local mating structure while the extensive distribution of some

genotypes (Table 2) indicate connectivity over large distances that is not reflected in other statistics calculated including only one representative of each genotype. There are no extensive natural physical barriers along this part of the river, and there is no abrupt environmental change. There are several small dams that cause 1–2 km breaks in the distribution of *Vallisneria* by increasing sediment deposition immediately upstream and causing extensive scouring immediately below. In contrast, differences in F_{st} and Jost's D between the upper and lower Potomac are more similar to those in between other regions, and no genotypes are shared. The major environmental difference between two parts of the river is the tidal influence in the lower reaches of the river that is absent above Great Falls, MD. More intensive sampling between our existing sampling locations is needed to elucidate finer scale patterns of population structure, clonal diversity, and clonal extent, which are necessary to understand spatial mating and dispersal structure.

Implications for restoration

Goals for 'restoration' can range from simply returning vegetation to a site, to full-scale ecological restoration. Ecological restoration is defined as, "an intentional activity that initiates or accelerates the recovery of an ecosystem with respect to its health, integrity and sustainability" (Society for Ecological Restoration International Science and Policy Working Group 2004). This definition requires, the restored ecosystem to be self-sustaining and be sufficiently resilient to endure the normal periodic stress events in the local environment. (http://www.ser.org/content/ecological_restoration_primer.asp#5). There are three main paradigms for selecting material for revegetation efforts.

1. Select a few particularly well performing genotypes for a particular set of criteria and propagate those genotypes in a manner similar to development of cultivars in agriculture and horticulture. This approach lends itself to efficient commercial production of source material and development of material with resistance to known pests or pathogens or with characteristics that meet specific needs. Planting one or a few genotypes over broad areas may be successful in the short-term but provides no raw material for evolution to changing abiotic conditions or novel pathogens. Although it is sometime applied in revegetation project, it is generally not considered acceptable in ecological restoration.

2. Select propagules such that amounts and types of genetic diversity in restored populations reflect those found in surrounding natural populations. This approach recognizes the importance of local adaptation and uses local genetic stock. A major goal is to prevent founder events in the restoration process that can occur during collection, cultivation or planting so that future evolutionary potential

is maintained. At the same time, propagule sources can be selected based on spatial proximity or habitat similarity (van Katwijk et al. 2009) between the source and reference site that are deemed to be sufficiently local. This approach can be problematic if individual sites are genetically depauperate and or inbred, but prevents planting maladapted stock or causing genetic pollution of local populations (McKay et al. 2005). However, the presence of local adaptation is not documented for most species and the spatial scale at which such adaptations may occur is likely to be idiosyncratic. Unnecessarily restricting source material for widespread species with little or no local adaptation can severely hamper restoration efforts (Broadhurst et al. 2008).

3. Use large numbers of propagules of diverse origin, letting natural selection sort out appropriate genotypes for a particular site (Broadhurst et al. 2008). This approach is suggested for relatively common, widespread species that have long-distance dispersal abilities but that are now fragmented and in which individual remnants do not support much remaining diversity or in which inbreeding depression may be causing reduced fitness. Such an approach is also suggested for large-scale regional restoration efforts in which sufficient propagules may not exist within small isolated fragments. Advocates of this approach suggest that the genetic diversity of the source material is as important as or more important than being 'local.' Inappropriate use of genetic stocks in environments to which they are not adapted can substantially impact the success of restored populations (Montalvo et al. 1997; Hufford and Mazer 2003). Restoration failure may result when the foreign genetic stock provisions resources at inappropriate times (Jones et al. 2001), is maladapted to local conditions (McKay et al. 2005), or contributes to outbreeding depression (Templeton 1997; Montalvo and Ellstrand 2001; Potts et al. 2003).

Although they provide insight into only the one aspect of genetic diversity, our results inform aspects of each of these potential approaches. We found that levels of genotypic and allelic diversity at most sites are high and can serve as source populations for restoration material. Exceptions include upper Potomac River sites (e.g., HCK, POR, WF), and two sites in the central Bay (SCN, SFP). Low diversity in sites and presence of shared genotypes among sites in the upper Potomac River also cautions against the use of that region for source material without prior thought and understanding of the potential implications of low diversity collections. On the other hand, the widespread genotypes in the low diversity sites could be candidates for intensive propagation if their dominance was shown to relate to superior competitive ability that confers resistance to environmental stressors affecting the Potomac River. We do not advocate approaches that reduce

genetic diversity, but as part of a comprehensive restoration program, having genotypes that can withstand and even flourish under stressful conditions could be beneficial. Our current data only provide a starting point for investigation of such possibilities.

Based on the diversity we observed, we found no compelling evidence for the need for genetic rescue of any population through introduction of genotypes or the need to mix genotypes in restoration plantings (Hedrick and Fredrickson 2010). We have no way of knowing the original levels of genetic diversity in the Bay, but, despite extensive population size declines, there is no evidence of catastrophic losses in that most remaining sites are not genetically depauperate or homogeneous. Confirmation of this assertion requires comparing fitness in apparently bottlenecked populations with populations that have no indication of severe reduction.

The spatial substructuring we detected among sites in the northern and central Bay suggests that caution should be used in moving propagules to locations distant from their source. It is also necessary to more thoroughly understand the population structure within the Potomac River to determine the scales at which there is genetic interaction from dispersal of vegetative propagules, pollen, and seed. Specifically, we suggest that movement of propagules for restoration activities be limited to within each of the four primary geographic areas that are related to environmental factors, in particular salinity. We find no strong evidence against moving propagules within regions. Our data do not allow us to assess the degree to which the genetic differences we detected indicate adaptation to local environmental conditions. We are just beginning to conduct experiments to determine whether there is evidence for local adaptation within these regions and if there are fitness consequences of crossing individuals from different regions. Until more investigations relating these patterns with fitness are completed, it is prudent to be cautious and carefully select plant material from within one of the genetic regions.

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