Biological Conservation 179 (2014) 6–16

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

Effects of genetic diversity on conservation and restoration potential at individual, population, and regional scales

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ARTICLE INFO

Article history:
Received 25 February 2014
Received in revised form 13 August 2014
Accepted 20 August 2014

Keywords:
Freshwater aquatic plants
Genotypic diversity
Heterozygosity
Local adaptation
Opportunities for selection
Wild celery

ABSTRACT

All available evidence suggests that genetic diversity is important for ecological performance and resilience through the expression and variance of phenotypic traits. Genetic diversity is a multiscalar variable; it includes heterozygosity at the individual scale, genotypic diversity at the population scale, and local adaptation at the regional scale. The three scales of genetic diversity are predominantly studied in isolation to determine conservation status or restoration potential. However, synergisms among the three scales should enhance conservation and restoration assessments. We studied 49 genotypes of *Vallisneria americana*, a common freshwater submersed aquatic macrophyte that has seen catastrophic declines in its estuarine habitats. Two greenhouse experiments examined phenotypic traits and trade-offs and the effects of genetic diversity on the sustainability of *V. americana* populations. Clone size, an exploitative guerilla strategy, and plant height, a conservative phalanx strategy, were negatively associated, as were average turion size with clone size and turion abundance, suggesting that growth strategies trade-off to affect plant fitness. Leaf size and turion size were lower in individuals with lower heterozygosity. Early clonal expansion and flowering frequency were enhanced when genotypic richness was higher. Coefficients of variation revealed that opportunities for selection differed across *V. americana* source beds. We demonstrate that the three scales of genetic diversity work together to determine population performance and evolutionary potential. Thus, integrating the three aspects of genetic diversity is paramount in addressing the impacts of a changing world on the conservation and restoration potential of at-risk populations.

1. Introduction

Human-induced changes in natural ecosystems have caused unprecedented declines in biodiversity and have reduced the capacity of ecosystems to cope with short- and long-term environmental change (Griffin et al., 2009). Maintaining species diversity for preserving or restoring ecosystem processes is critical (Naeem et al., 2009). All available evidence indicates that it is equally important to preserve genetic diversity within species in terrestrial (e.g., Madritch and Hunter, 2002; Agashe, 2009; Ellers et al., 2011; McArt and Thaler, 2013) and aquatic (e.g., Duffy, 2006; Hughes and Stachowicz, 2009, 2011; Stachowicz et al., 2013) habitats. This topic is becoming increasingly important as populations are subjected to a variety of natural and human-driven environmental stresses that undermine the persistence and evolutionary potential of populations within landscapes. However, genetic diversity is often neglected in biodiversity conservation activities (Laikre et al., 2009).

Three aspects of genetic diversity affect population performance and ecosystem function: 1. Levels of heterozygosity within individuals (Dudash, 1990; Fenster and Dudash, 1994) that can be reduced due to non-random matings and can influence probabilities of survival (Ellstrand and Elam, 1993). 2. Levels of diversity between individuals (e.g., numbers of alleles, genotypes, and phenotypes; Biernacki and Lovett-Doust, 1997; Williams and Orth, 1998) that allow individuals to occupy different niches and promote population diversity (Vellend, 2006). 3. Adaptation of individuals to local environments (e.g., Montalvo and Ellstrand, 2000; Joshi et al., 2001; Hammerli and Reusch, 2002) that affect performance of individuals in local versus foreign environments (Kawecki and Ebert, 2004). The three aspects of genetic diversity act at different spatial scales, from the scale of the individual, to

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http://dx.doi.org/10.1016/j.biocon.2014.08.011
0006-3207/

the scale of a single population, to the scale of regions that encompass different environments.

Heterozygosity, genotypic diversity, and local adaptation are not mutually exclusive. Rather they may often act together to affect resilience of populations. For example, local adaptation may lead to increased isolation, which can incur an inbreeding cost (Verhoeven et al., 2011) and therefore lower heterozygosity with consequences to individual fitness. Lower individual fitness, in turn, may affect survival and hence genotypic diversity within populations. Therefore, for conservation and restoration of viable populations to be maximally effective, all scales of genetic diversity from individuals to across populations and regions need to be considered (Bischoff et al., 2010) to optimize population growth and survival. Indeed, in a literature survey that focused on 'heterozygosity', 'genotypic diversity', and 'local adaptation' as search terms, we found that 2378 abstracts of papers published in biological journals over the last decade (2003–2013; Proquest Biological Science Journals Database) included at least one of the terms. However, only 15 papers intentionally focused on the intersection of two of the three scales, and none addressed and integrated all three scales to address conservation or restoration issues.

We tested the role of the three scales of genetic diversity in the growth and reproduction of the submersed aquatic macrophyte *Vallisneria americana* (Michx.) (wild celery) in two controlled and well-replicated greenhouse experiments. *V. americana* is a key species in the functioning of freshwater systems for its ability to immobilize nutrients, accumulate metals, and trap sediments (Biernacki and Lovett-Doust, 1997; Benson et al., 2008) and for its provision of important food resources to aquatic fauna (Sponberg and Lodge, 2005). This dioecious plant is capable of both clonal growth and sexual reproduction (Titus and Stephens, 1983; Titus and Hoover, 1991; Korschgen et al., 1997; Lokker et al., 1997) and is used extensively in the restoration of aquatic systems (Korschgen and Green, 1988). However, little is known about the effects of genetic diversity on the restoration of *V. americana* populations or the conservation of the species (Lloyd et al., 2012; Marsden et al., 2013) despite observed wide variation in genetic diversity at local and regional scales within an estuary (Lloyd et al., 2011).

To examine the effects of genetic diversity on *V. americana* population performance and long-term sustainability, we first tested the hypothesis that genotypes invest in different functional strategies (Lovett-Doust, 1981; Sackville Hamilton et al., 1987) with trade-offs between traits that represent plant stature (leaf length and size), biomass accumulation (average turion size, total turion weight), clonal expansion (ramet production, rhizome length, turion abundance), and sexual reproduction (flowering frequency, length of flowering). Reliable estimation of phenotypic trait differences among genotypes is a necessary pre-requisite to test for the effects of heterozygosity, genotypic diversity and local adaptation on population performance. We then tested the hypothesis that heterozygosity affects an individual's response to high and low quality environments. We predicted that individuals with higher levels of heterozygosity would have higher rates of survival and growth than highly homozygous individuals (Chaves et al., 2011), especially in low quality sediments. At the population scale, we tested the hypothesis that genotypic and functional diversity of populations increases population growth and productivity. Higher productivity can result from two sources: chance presence of highly productive genotypes in more diverse populations or from complementarity in resource use by different genotypes (Hughes et al., 2008). At the regional scale, we tested the hypothesis that genotypes are locally adapted to their native environment. We first predicted that resident genotypes and populations would be more productive in their local habitat than foreign genotypes and populations (Kawecki and Ebert, 2004). We further predicted that opportunities for selection, or phenotypic variation across all genotypes (Crow, 1958), would be lower in benign habitats where phenotypes respond similarly to the environment, forming observable adaptive peaks (Wright, 1931). In contrast, in more spatially or temporally variable environments we expected opportunities for selection to be greater owing to higher variation in the response of phenotypes to variable environmental conditions. Through simultaneously studying the three scales of genetic diversity, we seek to highlight the importance of the three scales in conservation and restoration and increase knowledge for the conservation and restoration of a species that is critically important in estuarine functioning but has seen widespread population declines throughout its range (Kimber et al., 1995a,b; Korschgen et al., 1997; Lloyd et al., 2011).

2. Materials and methods

2.1. Study area and development of plant repository

In June 2007, we harvested up to 30 *V. americana* shoots with attached roots ("samples") from each of 14 natural source beds located within the Chesapeake Bay (Lloyd et al., 2011). When the extent of the population was limited, we sampled fewer than 30 shoots for a total of 403 shoots harvested. Because *V. americana* populations may be composed of one clone or many different genotypes (Lloyd et al., 2011), we sampled every 5–10 meters to determine the extent of clones and to maximize the sampling of different genotypes (Lloyd et al., 2011). Sampled populations varied in extent and cover and differed in environmental conditions (Table S1). We placed each *V. americana* sample into individual 2.4 l containers (diameter: 16 cm; height: 15.5 cm) filled 10 cm deep with steam-sterilized (Slack Associates, Inc. Model 1964) Chesapeake Bay sediments. Steam sterilization killed any propagules (seeds or turions) that could have contaminated the propagation containers if left in the sediments. Microorganisms were reintroduced by planting the *V. americana* field samples. The plants were grown for two years in the Appalachian Laboratory greenhouse to minimize environmental carryover from the field (Hughes and Stachowicz, 2009). As genotypes matured, we recorded the sex of each genotype to estimate the sex ratio of each source bed (Table S1). After senescence, we harvested turions and stored them in wet sterilized sand at 4 °C until planting the following spring.

Using 11 DNA microsatellite loci designed specifically for this species (Burnett et al., 2009), we identified genets from leaf cuttings and assigned *V. americana* samples to genotypes (Lloyd et al., 2011). In spring 2009, the *V. americana* repository of known genotypes consisted of 161 genets (out of 403 shoots harvested in the field) and 8000 turions.

2.2. Monoculture experiment

We planted 49 cloned genotypes of *V. americana* in 516 greenhouse mesocosms (4.7-l; diameter: 20.7 cm, height: 16.5 cm) in March 2009 to quantify traits, determine trade-offs among growth and reproductive strategies, and test for the effects of heterozygosity on individual performance. Genotypes originating from the HWC (17 genotypes), SWP (20 genotypes), and 12 additional source beds (one genotype each) were planted in monoculture in a replicated ($n = 12$ for each SWP and HWC genotype and $n = 6$ for all other genotypes), competition-free, and controlled greenhouse environment. We focused on HWC and SWP genotypes because the HWC and SWP source beds were diverse and yielded multiple genotypes for use in experiments. The source beds were associated with different environmental conditions

(HWC = sheltered habitat with sandy substrate, SWP = high energy riverine environment with gravel substrate), allowing us to test for local adaptation. The twelve additional genotypes increased the breadth of genetic diversity within the experiment.

We weighed and measured the length (from base to tip) and width (diameter at the base) of each *V. americana* turion to account for initial turion size in statistical tests. We then planted one turion in the middle of each experimental unit. We randomly placed experimental units on 6 greenhouse benches in 2 sets of orthogonal blocks (tables and zones) to account for any potential effects of microclimate within the greenhouse. A misting system ensured that all experimental units were periodically flushed of any accumulated nutrients. Algae that accumulated were hand-picked or flushed out.

We measured morphological and life history traits of the 49 *V. americana* genotypes during the 2009 growing season. Morphological traits included length, width, and area of *V. americana* leaves measured every 6 weeks. Growth traits included number of ramets and distances between ramets measured every 6 weeks, and turion size, weight and abundance measured in November 2009 after plant senescence. Life history traits included timing and frequency of emergence of male and female flowers from which we calculated the length of flowering for each genotype.

2.3. Diversity experiment

To test for the effect of genotypic diversity on population performance, we planted 120 experimental mesocosms (20-l; diameter: 28.5 cm, height: 36.0 cm) in March 2009 with experimental populations that differed in the number and source of genotypes (“diversity experiment”). We randomly drew 2, 4, and 8 genotypes from the pool of 49 genotypes that were also grown in monoculture to produce experimental populations composed of SWP genotypes, HWC genotypes or a mix of all 14 source beds. Both sexes of dioecious *V. americana* were equally represented in the genotype pool such that each sex had an equal chance of being selected during the random draw. Thus, the experimental populations included any combination of sexes. We planted the experimental populations as turions at a density of 8 individuals per mesocosm. Genotypes were evenly distributed such that four turions per genotype were planted in the 2-genotype treatment, two turions per genotype in the 4-genotype treatment, and one turion per genotype in the 8-genotype treatment. We measured the length of all turions to account for potential effects of initial turion size. To ensure consistent initial spacing, we planted turions in a circle (diameter = radius of the container) around the container center. At 6 weeks, we replanted 40 individuals that had failed to grow (4% of the original 960 individuals representing 18 genotypes from 8 source beds). To account for potential microclimatic differences within the greenhouse, we randomly assigned 120 experimental units to 30 wheeled platforms (“blocks”; 4 units per block) that were themselves randomly placed within the greenhouse and moved during the experiment to limit microclimate effects.

We counted number of ramets and measured longest leaf length at 6, 12, and 24 weeks after planting. We documented the timing of every flowering event. In November 2009, we harvested, counted and weighed turions from each experimental unit and measured the length of the longest turion per experimental unit using high precision calipers (±1 mm).

2.4. Effects of local adaptation

Local adaptation results in adaptive peaks (Wright, 1931) representing maximized growth and reproduction in local conditions. However, environments are typically less than optimal or consistent and will confer some level of stress, with fitness consequences for individuals. Environmental stress is hard to quantify because it requires some idea of the optimal habitat for an organism and measurement of the departure from optimal conditions, which is usually not possible. One solution is to measure responses of organisms to different environments to determine their relative fitness (Fischer et al., 2000).

Of the many environmental factors we could have manipulated (e.g., light, currents, heavy metals, herbivory, competitors), we focused on sediments because nutrients within the interstitial spaces of sediment are an important factor for aquatic plants and determine plant survival, growth, and reproduction (Lehmann et al., 1997). The higher energy environment of the SWP source bed supports sediments of coarse gravelly texture with a large number of shells and 1–2 cm diameter rocks. The sheltered HWC source bed, on the other hand, supports sediment that is sandy in texture with no coarse components. Coarse textured sediment immobilizes and stores fewer nutrients (McDaniel et al., 2009), therefore supporting lower plant growth. We therefore determined that the SWP genotypes originated from a more stressful environment (higher energy, fewer nutrients in the sediment) and predicted that the SWP sediment would be the more stressful habitat compared to the HWC sediment.

To test for a home-site advantage in local adaptation, we conducted the monoculture and diversity experiments in two sediments (SWP and HWC) that were native (SWP genotypes planted in SWP sediment and HWC genotypes planted in HWC sediment) or foreign (SWP genotypes planted in HWC sediment and HWC genotypes planted in SWP sediment). We collected the two sediment sources from the two source bed locations, dried and then steam-sterilized the sediments, and filled each experimental mesocosm with homogenized sediment to 10 cm depth and dechlorinated water to the top of the mesocosms. Each genotype × sediment treatment was replicated 6 times and each diversity × sediment treatment was replicated 3 times. Genotypes and experimental populations from the 12 other sampled source beds were grown only in the HWC sediment to decrease the size of the experiment and because neither SWP nor HWC was their native sediment.

2.5. Data analysis

We transformed variables to meet the assumptions of normality. We tested for covariation between variables using Pearson correlations, linear regression, and logistic regression. Significance was always assessed at $\alpha = 0.05$ and Bonferroni corrections were applied. We used SAS 9.1 (SAS Institute, Cary, NC, USA) and R version 3.1.0 for statistical analyses.

We tested for differences among genotypes in the monoculture experiment using Analysis of Covariance (ANCOVA, Type III sums of squares) and Chi-square analysis and then tested for trade-offs among phenotypic traits (leaf length and area, turion production, ramet production, clonal expansion, date of flowering, number of days flowering, number of flowers, and consistency of flower production) using Pearsson correlations. We used the average trait value across the six replicates planted in the HWC sediment because not all genotypes were planted in the SWP sediment. Because more energy is stored in bigger turions, we accounted for initial differences in turion size among genotypes using initial turion area (length × width) as a measure of turion size. A second covariate was individual heterozygosity (Lloyd et al., 2011), which was used to test for effects of heterozygosity on performance of genotypes. The ANCOVA of the monoculture experiment also included sediment (HWC or SWP source bed locations) as main factors.

Genotypic diversity affects population performance when phenotypic traits differ among genotypes (Ellers et al., 2011) and

dissimilar genotypes are complementary in their use of the environment. Alternatively, when genotypic identity affects population productivity (Vellend et al., 2010), population performance is driven predominantly by presence of genotypes that grow faster or produce more turions or flowers under competitive conditions. Several different measures of functional diversity are available for describing functional dissimilarity among populations or communities (Pla et al., 2012). Using FunctDiv (Lepš et al., 2006), we calculated several indices and chose the Rao index to represent the data. The Rao index is a generalized form of the Simpson index of diversity, because it uses the relative abundance of species (or genotypes) to calculate a trait community (or population) weighted mean (Rao, 1982; Ricotta and Moretti, 2011). Using the Rao index, we calculated phenotypic dissimilarity of each experimental population weighted by relative abundance of genotypes. We then used this measure to assess the relationship between functional phenotypic diversity and population performance. We used trait means across genotype treatments, which effectively assumes no plastic phenotypic response to environmental conditions. As our inputs, we used the relative abundances of genotypes initially planted in each experimental mesocosm and the measured traits of each genotype in monoculture. Selection of traits for inclusion in a functional diversity index can greatly influence results and therefore need to be chosen carefully. We calculated functional diversity for different ecological strategies including horizontal vegetative expansion (distance between ramets and number of ramets), productivity (number and weight of tubers), vertical growth (leaf length and area), and sexual reproduction (length of flowering and frequency of flowering). The functional diversity of each strategy was individually correlated with population performance. Functional diversity was also calculated collectively across all strategies and correlated with population performance.

In the diversity experiment, we used Analysis of Variance (ANOVA) to test for the effects of richness (2, 4 and 8 genotypes), population (HWC, SWP or Mixed), and sediment (HWC or SWP) in a randomized block. We used presence/absence of individual genotypes to test for the effects of specific genotypes on population responses. Infrequent genotypes (planted in <10 experimental units) were not included owing to insufficient representation. Because of this constraint, the effects of some genotypes that were superior performers in the monoculture experiment could not be assessed in the diversity experiment. We regressed each of the response variables measured in the experimental populations by the Rao index that we calculated for each trait in the monoculture experiment to determine whether functional diversity accounts for any variation in experimental population performance.

Microclimatic variation within the greenhouse had a strong effect on phenotypic variation in the monoculture experiment and population performance in the diversity experiment (Tables S2 and S4). We therefore accounted for microclimatic effects using two orthogonal greenhouse blocks in statistical models. Initial turion size differed across genotypes (ANOVA; $F_{48,447} = 4.77$, $P < 0.001$) but was not a significant covariate in models. Thus, turion size was not accounted for in the final model.

3. Results

3.1. Phenotypic differences and trade-offs

Genotype strongly influenced morphology, clonal expansion, turion biomass production, and sexual reproduction in the monoculture experiment (Table S2). Most traits were positively associated. For example, leaf area was positively associated with total turion weight per clone ($r = 0.42$, $P = 0.003$), average turion weight per clone ($r = 0.57$, $P < 0.001$), rhizome length ($r = 0.35$, $P = 0.017$), number of days that a clone flowered ($r = 0.37$, $P = 0.011$) and the number of times a clone flowered ($r = 0.35$, $P = 0.015$). In contrast, leaf area was negatively associated with number of ramets produced ($r = -0.31$, $P = 0.03$). Similarly, average turion weight per clone was negatively associated with the number of ramets ($r = -0.38$, $P = 0.008$) and the number of turions produced per clone ($r = -0.52$, $P < 0.001$).

Male genotypes showed strong positive associations within and between vegetative and flowering attributes. For example, leaf length was positively associated with the proportion of clones that reproduced within each genotype ($r = 0.41$, $P = 0.049$), the length of the flowering season ($r = 0.49$, $P = 0.014$), and the number of flowers produced within the growing season ($r = 0.54$, $P = 0.006$), whereas female genotypes had no such associations.

Taller plants flowered earlier (Pearson correlation; $r = -0.36$, $P < 0.001$) and increased the odds that a plant became reproductively mature and produced flowers during the growing season (Logistic regression; intercept = −3.5484; slope = 0.1296, $P < 0.0$ 01; odds ratio $CI_{95\%} = 1.124–1.321$). Logistic regression showed that the critical leaf length for sexual reproduction differed among source beds, ranging from 24 cm in the HWC genotypes to 37 cm in the SWP genotypes.

Flower production differed among genotypes ($\chi^2 = 165.43$, df = 49, $P < 0.001$), ranging from 0% to 80% of replicate ramets planted. The SWP flowering season overlapped completely with that of HWC. Most genotypes completed flowering in <28 days across all replicate plants. Exceptions were male genotypes SASS24 (59.0 ± 7.6 days), HWC23 (40.4 ± 11.0 days), and HWC11 (33.3 ± 10.6 days). Male plants had longer flowering seasons than female plants (means = 15.0 ± 1.9 days and 7.4 ± 1.8 days, respectively; ANOVA; $F_{1,159} = 5.75$, $P = 0.02$).

3.2. Heterozygosity

SWP genotypes had a lower proportion of heterozygous loci (0.415 ± 0.026, range = 0.2–0.6) than HWC genotypes (0.647 ± 0.026 loci, range = 0.3–0.9; ANOVA, $F_{1,68} = 57.63$, $P < 0.0$ 01). Heterozygosity is a continuous variable such that its effects on plant growth and reproduction need to be interpreted as a regression slope in an ANCOVA that accounts for independent effects of sediment and source bed on plant response (Table S3). Turion weight was higher in individuals with higher heterozygosity (Fig. 2). Similarly, leaf area, width and length increased with heterozygosity. However, leaf area increased with heterozygosity only in individuals from the SWP source bed (Fig. 2), leading to a significant heterozygosity by source bed interaction (B × H in Table S3).

3.3. Genotypic diversity

Genotypic diversity was an important factor in ramet production only in week 6, when the 8-genotype treatment produced a higher number of ramets than either the 4- or the 2-genotype treatments (Fig. 3). Length of flowering increased with genotypic diversity, where the 8-genotype treatment flowered longer than the 4- and the 2-genotype treatments (Fig. 3). Length of flowering was positively correlated with number of flowers produced within each experimental population ($r = 0.88$, $P < 0.001$). The number of experimental units with both female and male flower production increased with genotypic diversity (Fig. 3; $\chi^2 = 19.98$, df = 8, $P = 0.01$). Conversely, units lacking flower production decreased with genotypic diversity (Fig. 3). Even though either sex had an equal chance of being drawn for inclusion in experimental populations, almost half of the mesocosms in each diversity treatment supported the production of only male flowers; female flowers were the only flowers observed in 12% of all experimental units.

Population performance measures were all higher when both sexes flowered compared to when one sex flowered and especially when neither sex flowered as observed in higher turion biomass (ANOVA, $F_{3,115} = 6.27$, $P < 0.001$), longer turions ($F_{3,116} = 2.93$, $P = 0.04$), more flowers ($F_{3,116} = 51.20$, $P < 0.001$), and longer flowering period ($F_{3,116} = 31.05$, $P < 0.001$).

SWP experimental populations produced new ramets at a faster rate than the HWC and Mixed populations through week 12, but ramet production was higher for the HWC populations in week 24 (Table S4). Turion biomass and count were higher in HWC experimental populations than the SWP and the Mixed populations. The HWC and Mixed populations produced longer turions than the SWP experimental populations. Length of flowering season was more than twice as long for the HWC and Mixed experimental populations than the SWP populations. Similarly, the number of flowers produced was twice as high in the HWC and Mixed experimental populations than the SWP population.

Functional diversity increased with genotypic diversity when functional strategies (flower production, leaf size, tuber production, and clone size) were considered individually or were combined as a group ($P < 0.001$ in all cases). The HWC and Mixed experimental populations were functionally more diverse in flower production than the SWP populations ($P = 0.001$) but the opposite was observed for clone size ($P = 0.002$) where the SWP populations were functionally more diverse. The HWC experimental populations were functionally less diverse in turion production than the SWP and Mixed populations ($P < 0.001$), whereas the SWP and HWC populations were both functionally less diverse in leaf size than the Mixed populations ($P < 0.001$). Given these differences across source beds, when all functional traits were averaged in one Rao index, the SWP, HWC and Mixed experimental populations did not differ in functional diversity.

Even though functional diversity increased with genotypic diversity, functional diversity was not an important variable in explaining variation in the performance of experimental populations. In 35 regressions between seven response variables (turion weight and count, ramet count in weeks 6, 12, and 24, rhizome length, leaf length, and flower count) and five Rao indices (flower production, leaf size, turion production, clone size, all traits combined), none of the regression models were significant after Bonferroni correction, and the best model only explained 10% of the variation in population performance. Instead, the presence of individual genotypes affected all experimental population measures, and the direction and strength of effects varied by genotype (Table S4).

3.4. Local adaptation

In the monoculture experiment, total rhizome length was the most affected by differences in sediment, followed by ramet number in weeks 6 and 12. Leaf width was the least affected by sediment (Tables S2 and S3). SWP sediment was the inferior sediment type, supporting less growth and fewer reproductive events. For example, the HWC sediment supported plants with leaves or rhizomes that were on average twice as long (Fig. 1) and produced more turions (Fig. 1), twice as much turion weight, and twice as many flowers compared to the SWP sediment (Table 1). Genotypes performed better in the SWP sediment in a few cases, leading to a significant genotype × sediment interaction

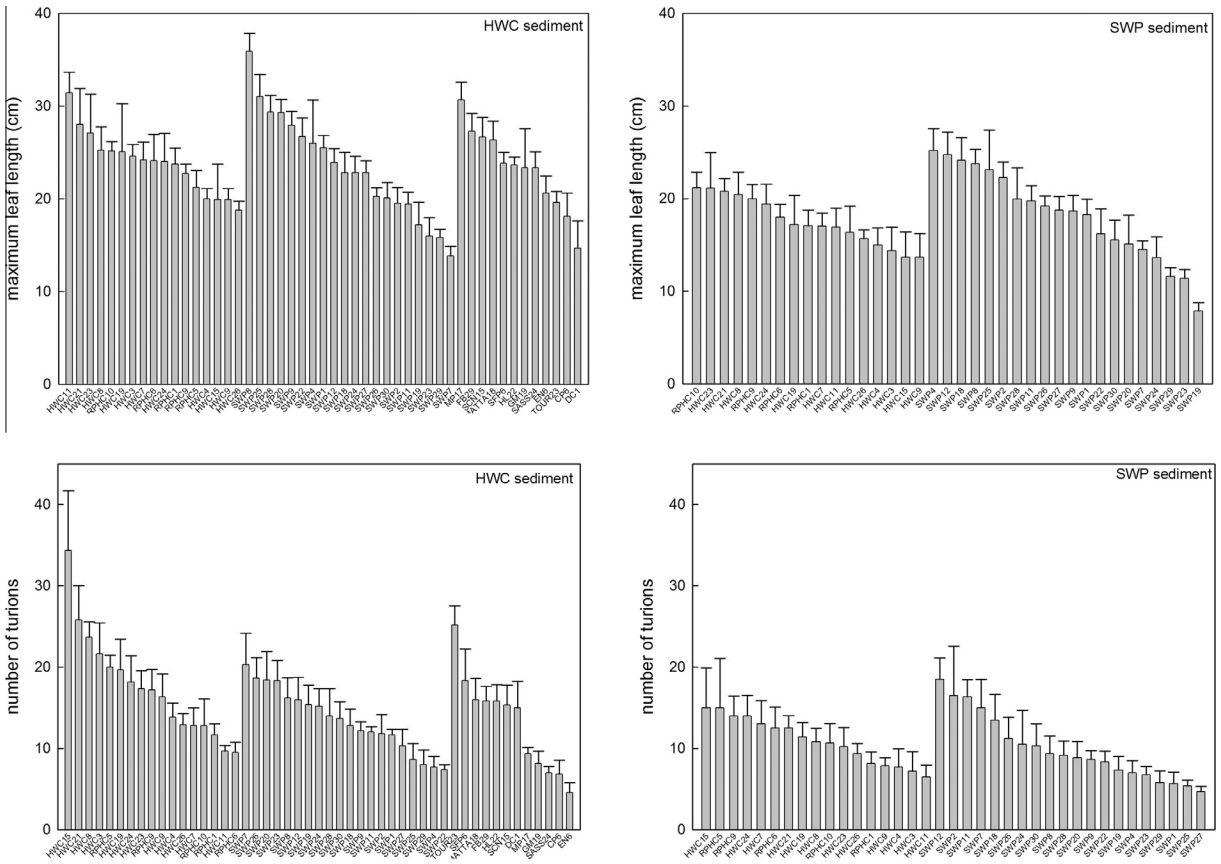

Fig. 1. Means and standard errors of maximum leaf length (top row) and number of turions (bottom row) produced within one growing season for each of the 49 genotypes planted in HWC sediment (left) and 37 genotypes planted in SWP (right) sediment.

Table 1
Means, standard deviations (STD) and coefficients of variation (CV) of HWC and SWP source beds growing in HWC and SWP sediments. Genotype treatment means from the monoculture experiment are used here as replicates, where $n_{HWC} = 17$ and $n_{SWP} = 20$). CVs were ranked for each trait measured and rankings summed for each source bed × sediment treatment to highlight the differences in variation among treatments. Higher rankings correspond to lower CVs.

	HWC source bed								SWP source bed							
	HWC sediment				SWP sediment				HWC sediment				SWP sediment			
Trait	MEAN	STD	CV	Rank CV	MEAN	STD	CV	Rank CV	MEAN	STD	CV	Rank CV	MEAN	STD	CV	Rank CV
Turion weight (g)	1.48	0.41	0.277	3	0.76	0.14	0.184	4	1.14	0.33	0.289	2	0.69	0.4	0.580	1
Turion count	17.49	6.41	0.366	2	10.93	2.81	0.257	4	13.43	3.88	0.289	3	9.94	4.06	0.408	1
Ramet count	12.21	3.29	0.269	2	7.93	1.92	0.242	4	9.44	2.71	0.287	1	6.21	1.51	0.243	3
Rhizome length (mm)	55.51	15.1	0.272	4	29.32	8.78	0.299	3	38.14	12.4	0.324	2	17.89	5.87	0.328	1
Leaf area (cm^2)	14.15	2.41	0.170	4	9.35	2.29	0.244	3	12.14	3.9	0.321	2	8.388	3.48	0.414	1
Leaf length (cm)	23.83	3.27	0.137	4	17.52	2.58	0.147	3	23.31	5.72	0.245	2	18.17	4.91	0.270	1
Leaf width (mm)	5.94	0.7	0.118	4	5.28	0.72	0.136	2	5.13	0.66	0.129	3	4.43	0.9	0.203	1
Flower count	5.15	2.82	0.548	3	1.76	1.89	1.074	2	2.8	1.19	0.425	4	1.5	1.64	1.093	1
# days flowering	11.37	11.02	0.97	4	5.43	12.32	2.27	2	2.95	6.62	2.24	3	0.90	2.88	3.2	1
Sum of ranks				30				27				22				11

(Table S2), but better performance in SWP sediments was not specific to the origin of the source bed.

On average, HWC genotypes were more productive than SWP genotypes in HWC sediment (Table 1). In contrast, coefficients of variation (CV), calculated across genotype treatment means, were generally highest in SWP genotypes, especially when they were planted in the native SWP sediment (Table 1, Fig. 1). One exception was the CV of the maximum number of ramets produced within the growing season, which was higher in the HWC sediment than the SWP sediment for both the HWC and SWP source beds (Table 1). The HWC population, on the other hand, had generally lower CVs in both the native and the foreign sediments compared to the SWP population (Table 1, Fig. 1). In contrast to the monoculture experiment, sediment source was only important in affecting turion length in the diversity experiment (Table S4).

4. Discussion

Genetic diversity effects on individual and population performance were evident at all three levels we examined: 1. Differences in fitness were observed across levels of heterozygosity, which differed across source beds. 2. Performance of experimental populations responded to genotypic diversity and genotypic identity, but not to functional diversity. 3. The HWC source bed performed better across both native and foreign sediments, but the SWP source bed supported higher variability in phenotypes, suggesting differences in evolutionary potential. Our study therefore highlights the importance of examining all three levels of genetic diversity to rigorously evaluate population performance for conservation or restoration. We also highlight that, in addition to genetic diversity, population source and environmental conditions affect the performance of populations. Thus, the effect size of the three aspects of genetic diversity will vary depending on ecological and evolutionary context.

4.1. Genotypic differences and trade-offs

A prerequisite for observing effects of genetic diversity on individual and population performance is that genotypes must differ in vegetative and reproductive traits. The greenhouse experiments indeed showed that *V. americana* genotypes varied substantially in leaf morphology, turion production, clonal expansion and sexual

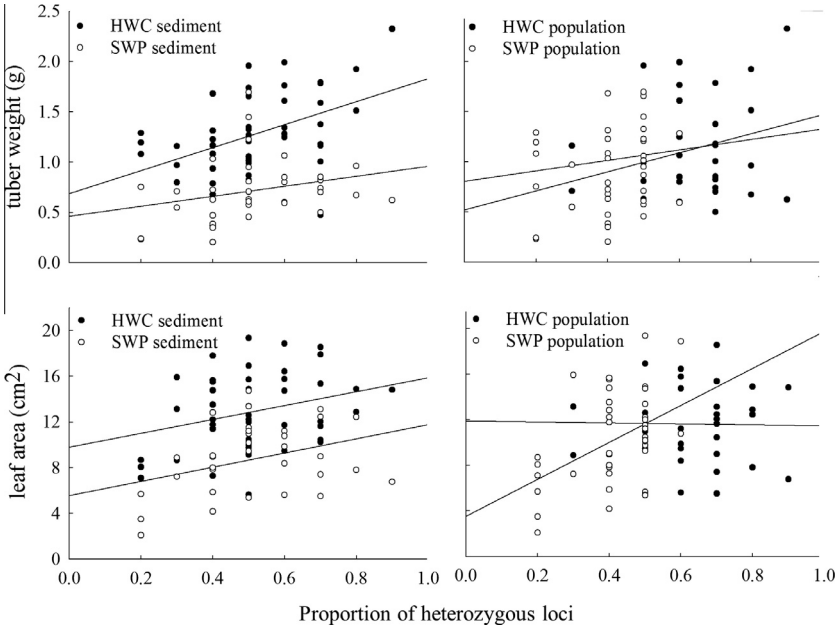

Fig. 2. The relationship between heterozygosity and plant traits. Slopes are compared between two sediments (HWC and SWP; first column) and two populations (HWC and SWP; second column). See Table S3 for results of statistical tests.

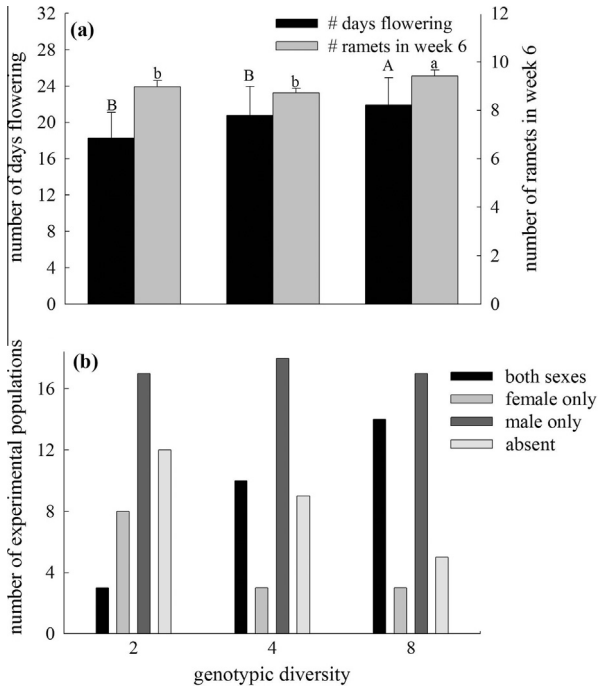

Fig. 3. (a) The effect of genotypic richness on ramet production and length of flower production (see Table S4 for results of statistical tests). Different uppercase or lowercase letters denote differences in length of flower production or ramet production, respectively. (b) Frequency of producing both sexes, neither sex, or either male or female flowers in experimental populations ($\chi^2 = 19.98$, df = 8, $P = 0.01$).

reproduction. These traits may be selected to be more advantageous in certain aquatic environments. For example, thin and relatively short leaves with smaller leaf area may be more advantageous in river currents, such as the SWP environment within the Potomac River (Table 1). In contrast, plants with taller and wider leaves may have an advantage over small-stature plants in turbid environments by allowing greater access to the water column and its light and carbon resources. Indeed, leaf length and area were positively associated with flowering frequency and length, where ramets that grew taller during the growing season had increased odds of producing flowers. Taller *V. americana* plants during the growing season were also positively associated with higher total turion biomass and average turion size per clone at the end of the growing season, similar to observations made on the submersed aquatic species *Potamogeton crispus* (Xie and Yu, 2011). The ability to acquire water column resources through a larger plant stature appears to affect the amount of carbohydrate resources that *V. americana* can invest in vegetative and sexual reproduction. Thus, resource allocation into large ramets has tremendous fitness consequences as it enhances long-term viability and the rapid and successful establishment of next year's plant beds (Zhou and Wang, 2012).

However, investment in one growth strategy, such as large plants, is often associated with costs to other traits. Such trade-offs are prevalent in clonal plants that face reproductive trade-offs between foraging, reproduction, dispersal and growth (Gardner and Mangel, 1999). For example, a clonal plant may invest in many widely-spaced ramets that can colonize new areas and escape resource-poor sites (a guerilla strategy) or a plant may produce few but large ramets that are competitive for limiting resources (a phalanx strategy; Lovett-Doust, 1981). Thus, some genotypes expand rapidly as clones but others are locally more competitive (Sackville Hamilton et al., 1987). Indeed, plant stature, reflecting the ability of a plant to capture water column resources, was negatively associated with ramet abundance, which reflects the ability of a plant to expand vegetatively into new areas. Thus, *V. americana* plants may indeed be investing in some form of guerilla (many spreading ramets that are small in stature) growth, or, alternatively, in phalanx (few but tall plants) growth. This trade-off may have tremendous consequences for plant fitness as each strategy has its benefits and costs in different environments.

In addition, plants that produced many turions, typically produced smaller turions suggesting a classic trade-off between investing in few but large, or many but small offspring (the reproductive ecology hypothesis, Crosby and Latta, 2013). In contrast, the classic trade-off between flower production and vegetative growth (the cost of reproduction hypothesis, Obeso, 2002) was not observed suggesting that sexual reproduction has no measurable costs and that *V. americana* plants focus predominantly on survival and growth to maximize fitness (Thiele et al., 2009).

4.2. Levels of heterozygosity

Vegetative production of turions is critical for population persistence (Korschgen et al., 1997) because plants in the Chesapeake Bay senesce during winter. Turion biomass indicates how much energy is stored and available in the following year to produce ramets and therefore new photosynthetic tissue. In this study, individuals with lower heterozygosity produced less turion biomass, suggesting that heterozygosity may have an influence on the annual persistence of populations.

Plant stature (height and leaf area) enhance a plant's ability to escape potentially light limiting environments by either allowing a plant to grow closer to the water surface where light conditions are more favorable or by allowing a plant to gather more light across the entire leaf surface, both important factors in turbid estuarine environments. Plant stature is furthermore important for *V. americana* to attain reproductive maturity, which is ultimately important for genetic recombination and dispersal via seeds. Because leaf length and area were lower in individuals with lower heterozygosity (Table S3; see also Montalvo et al., 1997), heterozygosity may influence the ability of populations to withstand turbid conditions common within the estuarine environment, and to expand, potentially into more favorable conditions, via seed dispersal.

In contrast, number of ramets and associated total rhizome length, which are both important for clonal expansion and thus population density and stability, were best explained in our experimental monocultures by population source not heterozygosity. This lack of a relationship between heterozygosity and measures of clonal expansion differs from those observed in *Zostera marina* (Williams, 2001) and *Quercus chrysolepis* (Montalvo et al., 1997), where heterozygosity was associated with greater shoot production and larger clone size, respectively. Nevertheless, lower heterozygosity and, hence, higher levels of inbreeding (Heschel and Paige, 1995; Carr and Dudash, 2003; Markert et al., 2010), appear to affect the sustainability of *V. americana* populations through the production of fewer turions and plants of smaller stature.

The disadvantage of inbred individuals is especially manifested in stressful habitats, where fitness consequences can be pronounced (Markert et al., 2010; Fox and Reed, 2011). In our case, however, associations of heterozygosity with plant performance were approximately equal between sediments as indicated by a nonsignificant interaction (parallel slopes) between heterozygosity and sediment (Fig. 2, Table S3). This suggests that, even though plants were more stressed in SWP sediment (smaller plants, fewer ramets, shorter rhizomes, less turion biomass), the effects of heterozygosity were not more pronounced than in less stressful conditions.

4.3. Genotypic diversity

Differences observed among the 49 genotypes should affect performance of populations, where resource exploitation should be greater and production higher in populations that support a variety of different genotypes (Hughes and Stachowicz, 2011). The Rao index of functional diversity showed that experimental populations planted with more genotypes were indeed functionally more diverse. However, this higher functional diversity did not translate measurably into higher performance of experimental populations, which either suggests that functional diversity does not affect performance, an important trait was not measured, or perhaps the wrong trait combinations were used to predict performance at the population level. Submersed aquatic macrophytes are known to compete strongly among species that differ in traits (e.g., Engelhardt and Ritchie, 2002). Intraspecific competitive interactions are likely to be even stronger because trait similarity, and therefore overlap in resource use, is greater among genotypes within a species. Thus, even though we cannot rule out the possibility that other functional traits or trait combinations would be more predictive of performance, our current results indicate that functional diversity is not a dominant driver of population performance at the spatial and temporal scale of the experimental populations. Rather, specific genotypes with specific traits drive performance (Table S4). For example, variance in ramet and turion production was better explained by population source and presence of specific genotypes than the number of genotypes planted (Table S4). Thus, fast expanding genotypes ultimately occupy the most resource space and drive population performance. These genotypes were also superior genotypes in the monoculture experiment.

Length of flowering and chances of both sexes flowering within the same population increased whereas the frequency of no flower production decreased with genotypic diversity (Fig. 3). These responses to genotypic diversity are important for seed production and the chances of recombining with other genotypes from within or outside the local population. Similarly, Williams (2001) showed that genetic diversity at the individual and population levels influenced flowering and seed germination in *Z. marina*. Other studies observed earlier flowering and increased seed production when genetic diversity was higher (Schaal and Levin, 1976; Oostermeijer et al., 1994). The response we observed was a classic allee effect, where higher genotypic diversity increases the chance that prolific flower producers (Table S4) are selected. For example, some genotypes, such as RPHC6, SASS24, and SFP6 produced flowers in over 80% of all genets planted, while others, such as CP6, HL22, and SWP18, produced no flowers at all within the entire growing season. The 8- and 4-genotype treatments were respectively 4 and 2 times as likely to include a prolific flower producer than the 2-genotype treatment; thus, frequency of flowering should be expected to increase with genotypic diversity, as was observed.

Genotypic diversity of *V. americana* populations in the Chesapeake Bay varied from 0 (dominated by one clone) to 1 (all sampled individuals were genetically different; Lloyd et al., 2011; Table S1). Populations with higher genotypic diversity (>0.75; Lloyd et al., 2011) supported both sexes at 0.5 or higher sex ratios (Table S1). Because one male can pollinate many females, successful seed production of a population will depend on the presence of both sexes but especially maternal genotypes. However, despite both sexes being represented equally in the pool of genotypes used in the experiments, only 35% of all experimental populations supported the flowering of both sexes; 50% of the populations supported the production of pollen only (Fig. 3). This mate limitation can be overcome by increasing genotypic diversity, which increases the chance that more maternal genotypes are selected by chance. In populations that are biased towards male genotypes (e.g., SASS, DC, SWP, TOUR, and SCN; Table S1), restoration designs need to be especially concerned about increasing genotypic diversity to increase the chance that female genotypes will be selected.

4.4. Local adaptation

Our data support the prediction that the coarser SWP sediment is the more stressful environment for aquatic plants (McDaniel et al., 2009) and would therefore support lower plant growth (Lehmann et al., 1997). Growth, biomass production, and vegetative expansion were reduced in SWP compared to HWC sediments (Table 1). Fifty-one percent of plants failed to sexually reproduce in the SWP sediment because they did not reach the height necessary for successful reproduction. Failure to reproduce reduces mate availability, which has implications for the genetic structure of populations (Honnay and Jacquemyn, 2008). This can in some cases result in lower heterozygosities (Ohtani et al., 2005).

HWC genotypes enjoyed a home-site advantage; they grew better in their native sediment than the foreign SWP genotypes and they performed more poorly in the foreign SWP sediment (Table 1). This pattern needs to hold for other populations and under more conditions to conclusively demonstrate local adaptation (Kawecki and Ebert, 2004). SWP genotypes showed an entirely different pattern; they were slightly less robust in the native sediment compared to HWC genotypes (Table 1) and benefitted from exposure to the foreign but more favorable HWC sediment. These opposing responses of HWC and SWP genotypes to HWC and SWP sediments demonstrate that home field advantage may not be symmetric in many cases. Individuals from favorable sites will not perform as well when exposed to less favorable conditions, which is the case for HWC genotypes. However, individuals in a poor quality site, such as SWP, may be selected for in terms of their ability to cope with stressful conditions (poor quality sediments, high energy environment) and so will have home field advantage. However, the adaptations to poor conditions do not preclude individuals taking advantage of luxury. Therefore, local adaptation is likely to be a factor driving plant growth in *V. americana* given differences in sediment quality, light availability and energy exposure we can observe at different sites. However, habitat quality is an overriding factor that affects all genotypes.

Despite observing a strong sediment effect in the monoculture experiment, only turion length showed the anticipated response to sediment (HWC > SWP) in the diversity experiment. The lack of a clear sediment effect in the diversity experiment may be caused, in part, by compensatory dynamics, whereby superior performers compensate for poor performers under stressful conditions. Thus, the variability of genotypic responses we observed within the monoculture experiment could be driving population performance when genotypes are mixed. However, the lack of an experimental population response based on functional diversity refutes this hypothesis, as do our results showing that all genotypes on average performed more poorly in SWP sediments. Alternatively, different resources such as nutrients and light may have been limiting in the monoculture and diversity experiments (Peace and Grubb, 1982), which differed in mesocosm size and number of individuals planted. The sediment surface was twice and water volume five times higher in the diversity experiment than the monoculture experiment, and the number of individuals planted was 8 times higher in the diversity experiment. Light was most likely limiting in the diversity experiment through shading by neighboring ramets and therefore the difference in sediment quality was not a driving factor. The single clone planted in the monoculture experiment, on the other hand, would have been less affected by the light environment because photosynthate was transported, at least partially, between interconnected ramets.

Therefore, nutrients were most likely the limiting resource in the monoculture experiment.

Plants in both greenhouse experiments responded to location within the greenhouse. Microclimates are often present in greenhouses, where some areas may be lighter or warmer depending on the location of evaporative coolers and shading effects of greenhouse walls. This response shows that *V. americana* appears to be plastic in its response to even small environmental differences as noted by French and Moore (2003). Thus, it is likely that plasticity, in combination with general habitat quality effects, were masking effects of local adaptation.

4.5. Opportunities for selection

Even though local adaptation is typically reflected in population means, the variability around population means – the coefficient of variation (CV) – is also important to understand opportunities for future selection of a population (Crow, 1958). A narrow range in genotypic responses to an environment across individuals may be advantageous in a constant environment but would confer fewer opportunities for selection to act on than a wider range. Breadth in genotypic responses is especially important for stressful and variable environments where a wide range of genotypic responses is necessary for a population to survive, expand vegetatively, and reproduce sexually (Waller et al., 2008).

Variation across replicates of a single genotype (Fig. 1) quantifies phenotypic plasticity that confers resistance to perturbation (Fajardo and Piper, 2011) whereas among genotype CVs (Table 1) represent the range of genotypic responses, and hence opportunities for selection within a population (Crow, 1958; Waller et al., 2008). The monoculture data demonstrate that phenotypic plasticity (Fig. 1) indeed varied two-fold among genotypes, where the phenotypic variation in some genotypes (e.g., HWC15) appeared to be consistently higher within and among the two sediment environments. However, although genotypes varied in phenotypic plasticity, no consistent patterns emerged among source beds. The monoculture data further demonstrate that CV of responses across all SWP genotypes is higher than across HWC genotypes in both native and foreign sediments (Table 1, Fig. 1), suggesting that opportunities for selection in general are higher for the SWP source bed.

Our data offer some insights into the hypothesis that genotypic responses to the environment become constrained when the environment selects for advantageous and against deleterious alleles or traits (Carr and Dudash, 2003). Individuals in the HWC population had a lower range of responses but higher overall fitness in both sediment types, suggesting that selection may have acted on this population, constraining responses towards phenotypes with higher and consistent productivity. The range of responses is approximately the same in native and foreign sediments, suggesting the population has few opportunities to respond to new environments and that phenotypes may indeed reside close to an optimum. In contrast, the SWP population has lower average fitness, perhaps either because some genotypes are more inbred (they had lower overall heterozygosity) or because adaptive peaks have not been reached (Verhoeven et al., 2011). However, the SWP population can withstand environmental variability through its higher range of individual responses in both sediments. We hypothesize that greater opportunities for selection in the SWP population may be maintained as a consequence of the more stressful environment found at the SWP location. Thus, even though the HWC population on average outperformed the SWP population in both sediment environments (Table 1) owing to HWC's consistency in performance, some individual SWP genotypes outperformed the most productive HWC genotype in one or both sediments (Fig. 1). The SWP environment is more variable because the coarse sediment is less mixed than the HWC sediment and therefore provides greater heterogeneity in microenvironmental conditions. We therefore hypothesize that variable environments require a wider range of responses for population persistence (Waller et al., 2008) and do not allow selection to optimize fitness around narrow adaptive peaks (Wright, 1931). To gain further insights into this hypothesis, more populations from a broader stress gradient would need to be sampled and transplanted in native and foreign environments.

5. Conclusions

Coastal ecosystems are considered to be among the most vulnerable systems to global change and biodiversity loss (Sala et al., 2000). Hence, assessing the current state of diversity and ways to protect, maintain or restore existing or dwindling resources are paramount for effective management of coastal resources. Our research shows that population performance is affected by three scales of genetic diversity: heterozygosity, genotypic diversity, local adaptation. Effect size of the three scales of genetic diversity will vary depending on population source and environmental conditions. Thus, managers are challenged to assess the relative effects of the three scales of diversity on long-term sustainability of managed populations and accordingly prioritize management actions. We offer several guidelines.

1. Because *V. americana* plants invest in either ramet size or clone size, managers need to assess which functional strategy would be best suited for the managed environment. For example, low stature ramets that spread fast vegetatively may be most successful in high energy environments, whereas ramets that invest in large leaves may be less successful in such environments. The opposite may be true for turbid conditions.
2. In most cases, managers will want to avoid low levels of heterozygosity (inbreeding) to maximize the vigor of individuals.
3. When introductions are warranted, managers will want to introduce locally adapted individuals. If local individuals are not available, however, sources from similar habitats and climates should be selected (Marsden et al., 2013).
4. In all cases, managers will want to maximize genotypic diversity to enhance early establishment and reproductive potential. However, phenotypic diversity will need to be matched to the habitat. In dynamic habitats where environmental conditions vary around a stationary mean (e.g., variation in turbidity, with constant long-term average), genotypes need to be selected with high phenotypic plasticity (that is, high coefficient of variation within genotypes). In environments where the environment is likely to be nonstationary and environmental change is directional (e.g., rising sea level, salinity and/or temperature), plant material will need to be selected to also encompass a range of phenotypes (that is, high coefficients of variation among genotypes). In benign habitats with relatively narrow fluctuations in environmental conditions, the best management strategy will be to select phenotypes that are best suited for the environment but are relatively similar in their phenotypic responses to the environment.

Synergisms across all three scales of genetic diversity have the capability to greatly affect population performance and evolutionary potential and therefore the conservation and restoration of populations. Coastal managers need to optimize genetic diversity across all three scales of genetic diversity to maximize the long-term sustainability of populations managers are tasked to protect or restore.

Acknowledgements

We thank Rebecca Byzon, Kate Chavanak, Jason Granberg, Stephanie Lewis, Katie Parsh, Ann Stark, and Brittany West Marsden for help with initial set-up, measurements, and harvest. Robert Burnett was instrumental in developing a microsatellite library for *V. americana* and helped with the genotyping of samples. Kathy Heil helped us in the literature survey. We thank anonymous reviewers for their thoughtful comments. NOAA's Sea Grant Program in Maryland provided partial financial support (Grant NA05OAR4171042 SA7528085-V).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocon.2014.08.011.

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