Biological Conservation 167 (2013) 203–214

Contents lists available at ScienceDirect

Biological Conservation

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

Genetic rescue versus outbreeding depression in *Vallisneria americana*: Implications for mixing seed sources for restoration

Brittany W. Marsden [a,*], Katharina A.M. Engelhardt [b], Maile C. Neel [c,d]

[a] Marine Estuarine Environmental Sciences, University of Maryland, 2102 Plant Sciences Building, College Park, MD 20742, United States
[b] University of Maryland, Center for Environmental Science Appalachian Laboratory, 301 Braddock Road, Frostburg, MD 21532, United States
[c] Department of Plant Science and Landscape Architecture, University of Maryland, 2102 Plant Sciences Building, College Park, MD 20742, United States
[d] Department of Entomology, University of Maryland, 2116 Plant Sciences Building, College Park, MD 20742, United States

ARTICLE INFO

Article history:
Received 18 January 2013
Received in revised form 1 August 2013
Accepted 8 August 2013

Keywords:
Chesapeake Bay
Inbreeding
Local adaptation
Plant population restoration
Relatedness
Submersed aquatic vegetation

ABSTRACT

The selection of seed stock for restoration remains a complex issue. Using local stock reduces the chances of outbreeding depression or genetic dilution, whereas mixing sources may increase diversity and counteract inbreeding depression. Evaluation of these opposing approaches remains difficult when planning a restoration project but is needed to increase chances of long-term population persistence. We evaluated seed production and germination success of seeds from controlled reproductive crosses of the submersed aquatic plant *Vallisneria americana* (wild celery) collected from populations throughout the Chesapeake Bay. We assessed differences in seeds, capsules, and germination success in three types of crosses: (1) individuals within-populations, (2) among-populations but within-genetically differentiated regions, and (3) among-regions. We observed population level differences in within-population and among-region crosses. Levels of genetic relatedness among individuals, genetic diversity within populations, or differentiation across populations did not predict reproductive success. Our data show that mixing sources from different populations and regions has both benefits and drawbacks. Thus, minimizing the risks of outbreeding and inbreeding depression, presented as a mostly dichotomous issue in the restoration literature, is not an either-or issue in *V. americana*.

1. Introduction

Two contradictory paradigms for selecting source materials create a major tension in restoration ecology. One approach argues for maintaining purity of local genetic stock by using propagules from one or a few sites in close proximity to a restoration site. The underlying hypothesis is that local stock is well adapted to environmental conditions of a site and will successfully establish with no risk of outbreeding depression from gene flow of non-local alleles (McKay et al., 2005; Montalvo and Ellstrand, 2000, 2001). Risk of restoration failure, however, can be high when source populations are small, have been isolated and drastically reduced in size, or have low diversity or low fitness due to inbreeding depression (Broadhurst et al., 2008; Weeks et al., 2011).

The alternative approach is to increase diversity and counteract local inbreeding by introducing genotypes from foreign source populations or by mixing genotypes from multiple populations (Broadhurst et al., 2008). Proponents argue that stock from multiple sources promotes persistence if associated phenotypes are adapted to a broader range of environmental conditions than individuals from any single population and mating among them following restoration results in heterosis (Broadhurst et al., 2008; Fenster and Dudash, 1994; Hughes et al., 2008; Weeks et al., 2011). Immediate negative consequences of such plantings arise if phenotypes are poorly adapted to local conditions and cannot survive and establish. Long-term consequences arise if reproduction between local and foreign stock is not possible or fitness of their offspring is compromised. Advocates of mixing propagules from many populations argue that benefits of increased diversity outweigh any potential negative consequences of outbreeding depression (Broadhurst et al., 2008) and argue that risks of outbreeding depression are overstated and unsubstantiated (Frankham et al., 2011; Weeks et al., 2011).

Inbreeding and outbreeding depression are increasingly presented as extreme dichotomous conditions. We argue here that degrees of differentiation among populations and inbreeding within populations are continuous gradients that vary independently. Managing the risks of using local or disparate sources of restoration stock, therefore, needs to account for the genetic context of natural source populations. In general, it appears that mixing slightly differentiated, inbred populations can lead to increased

* Corresponding author. Address: University of Maryland, 2102 Plant Sciences Building, College Park, MD 20742, United States. Tel.: +1 202 603 8277.
E-mail addresses: bwest1@umd.edu (B.W. Marsden), kengelhardt@umces.edu (K.A.M. Engelhardt), mneel@umd.edu (M.C. Neel).

0006-3207/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biocon.2013.08.012

fitness whereas mixing extremely differentiated, locally adapted populations can result in outbreeding depression (Forrest et al., 2011; Hereford, 2009; Hufford et al., 2012; Pickup et al., 2013; Waser, 1993). For example, recent studies by Forrest et al. (2011) and Hufford et al. (2012) found that plants crossed at intermediate-distances outperform within-population crosses in terms of germination success and survival while long-distance hybrids show signs of outbreeding depression. These studies furthermore concluded that spatial autocorrelation and genetic differentiation can be used to determine the optimal distances in which seeds can be mixed for restoration purposes (Forrest et al., 2011; Hufford et al., 2012). In another study, Pickup et al. (2013) found no evidence of outbreeding depression in crosses between pairs of populations across multiple generations, but they did detect evidence of heterosis. In contrast to previous studies, Pickup et al. (2013) found that heterosis was not limited to crosses between populations assigned to different genetic regions based on genetic dissimilarity. Therefore, empirical evidence for where natural populations lie along continua of genetic diversity and differentiation, and how that translates into risks for inbreeding or outbreeding depression, is essential to make informed decisions on what restoration stock to use to maximize fitness and long-term population persistence.

To assess the relative risks and benefits of these two restoration approaches, we evaluated reproductive success in terms of fruit size, seed number, seed size, and germination in controlled-environment crosses of individuals from within versus among 11 populations of the submersed aquatic plant species *Vallisneria americana* Michx. (wild celery; Family Hydrocharitaceae) in the Chesapeake Bay of eastern North America. These metrics were selected because seed supply is an important driver of initial establishment in restorations (Broadhurst et al., 2008) and they represent long-term potential for persistence and maintenance of genetic diversity via successful sexual reproduction. *Vallisneria americana* has characteristics and a history that would indicate a potential risk of both inbreeding depression and outbreeding depression. Once a dominant species influencing ecosystem function in freshwater and oligohaline portions of the Bay (e.g. Kemp et al., 2005), *V. americana* has greatly declined in abundance and distribution (Brush and Hilgartner, 2000) such that populations are a small fraction of their historical size (Orth and Moore, 1983). As a dioecious species, small populations have an elevated risk of lacking compatible mates and may suffer increased effects from mating among relatives. Genotypic diversity in 26 Chesapeake Bay populations varies greatly, ranging from 0 (populations consisting of one single clone) to 1 (populations made up of completely unique genotypes; Lloyd et al., 2011), a phenomenon also seen for other clonal aquatic species (Arnaud-Haond et al., 2010). This means that sites ranged from having no detectable sexual reproduction to no detectable asexual reproduction. Variation in genotypic diversity within populations is mirrored by microsatellite allelic variation, which ranges from 1.5 to 5.8 alleles/locus. Heterozygosity ranges from moderate heterozygote deficit (F_{IS} = 0.193), indicating potential risk of inbreeding, to large excess (F_{IS} = −0.667), indicating either recent bottlenecks or the presence of a heterozygote advantage. At the same time, evidence of genetic differentiation (Lloyd et al., 2011) and local adaptation (Engelhardt, Unpublished results) is accumulating. Assignment tests indicate four genetic regions in the Bay (Fig. 1), suggesting long-term limitations to gene flow among some populations and connections among others (Lloyd et al., 2011). Common garden experiments have demonstrated population level differences in growth rates and allocation of resources to leaf extension versus ramet production that are also mediated by the environment (Engelhardt, Unpublished results).

We predicted that if local adaptation is strong, crosses within populations would produce more, higher quality seeds that germinate than crosses among populations within genetic regions, which, likewise, would be more successful than crosses among regions. Alternatively, we expected that crosses between individuals from different populations would yield higher trait values if inbreeding in populations is relieved. To move beyond simple dichotomous comparisons of within versus among population crosses, we explicitly tested if reproductive success was affected by degree of relatedness among individuals, amount of genetic diversity within populations, or differentiation among populations.

2. Methods

2.1. Collection locations and protocol

We sampled *V. americana* in summer 2007 from tidal and non-tidal reaches of Chesapeake Bay tributaries (Lloyd et al., 2011), collecting ~30 shoots, 5–10 m apart, from 11 populations. Individuals from the populations were propagated in estuarine sediment at the University of Maryland Center for Environmental Science Appalachian Laboratory greenhouse. Shoots had previously been genotyped at 10 microsatellite loci (Burnett et al., 2009; Lloyd et al., 2011) and grouped into four regions based on minimal deviations from both Hardy-Weinberg and linkage equilibrium (Fig. 1). Regions were designated as the North-Chesapeake (including CP, EN, FB, and SASS), Mid-Chesapeake (DC, HWC, MP, and SFP), Potomac River (MATTA and SWP), and York River (HL).

In order to produce replicates of genotypes that had little field condition legacy we cloned all collected plants ($n \approx 330$) over multiple seasons in a common environment (Kawecki and Ebert, 2004). Genotype sex was determined by production of staminate versus pistillate flowers. To clone the samples we harvested turions after senescence in fall 2007, stored them in 4 °C water in the dark, and planted multiple turions of each genotype in 2008. Turions were again harvested at the end of the growing season.

2.2. Reproductive crosses

In 2009, we planted turions from 2008 in separate containers. We planted ~6 replicates for each unique female and male genotype. Maternal turion size (length and width) was measured for a subset of the planted genotypes (n = 15). Reproductive crosses were designed to include males and females (1) from within the same population, (2) from different populations within the same genetic region, and (3) from different populations from different regions. Replication of crosses was limited by timing and quantity of male and female flowers. *Vallisneria americana* pollen is only viable for a few days (McFarland and Shafer, 2008), and we found that female flowers were only receptive for ~24 h. These limitations precluded a full factorial design of within-versus-among population crosses. Therefore, we emphasized within-population crosses (n = 158) as well as crosses that included females from each population pollinated by males representing two distinct populations and genetic regions – HWC from the Mid-Chesapeake Region (n = 113) and SWP from the Potomac River region (n = 94; Table 1). In sum, 300 crosses were produced that involved the use of 71 unique female and 50 unique male *V. americana* genotypes.

As plants bloomed, female flowers were hand pollinated by dipping the blooms into buoyant male inflorescences. Only one male genotype was used per female replicate to ensure unambiguous attribution of paternity. Even though plants produce multiple flowers per reproductive event, just one was pollinated per replicate bucket. Various fathers were used to pollinate different replicates of the same female genotype. Successful pollination led to the production of a single fruit per cross. We harvested mature fruits in October and measured fruit and seed traits. Fruits are cylindrical

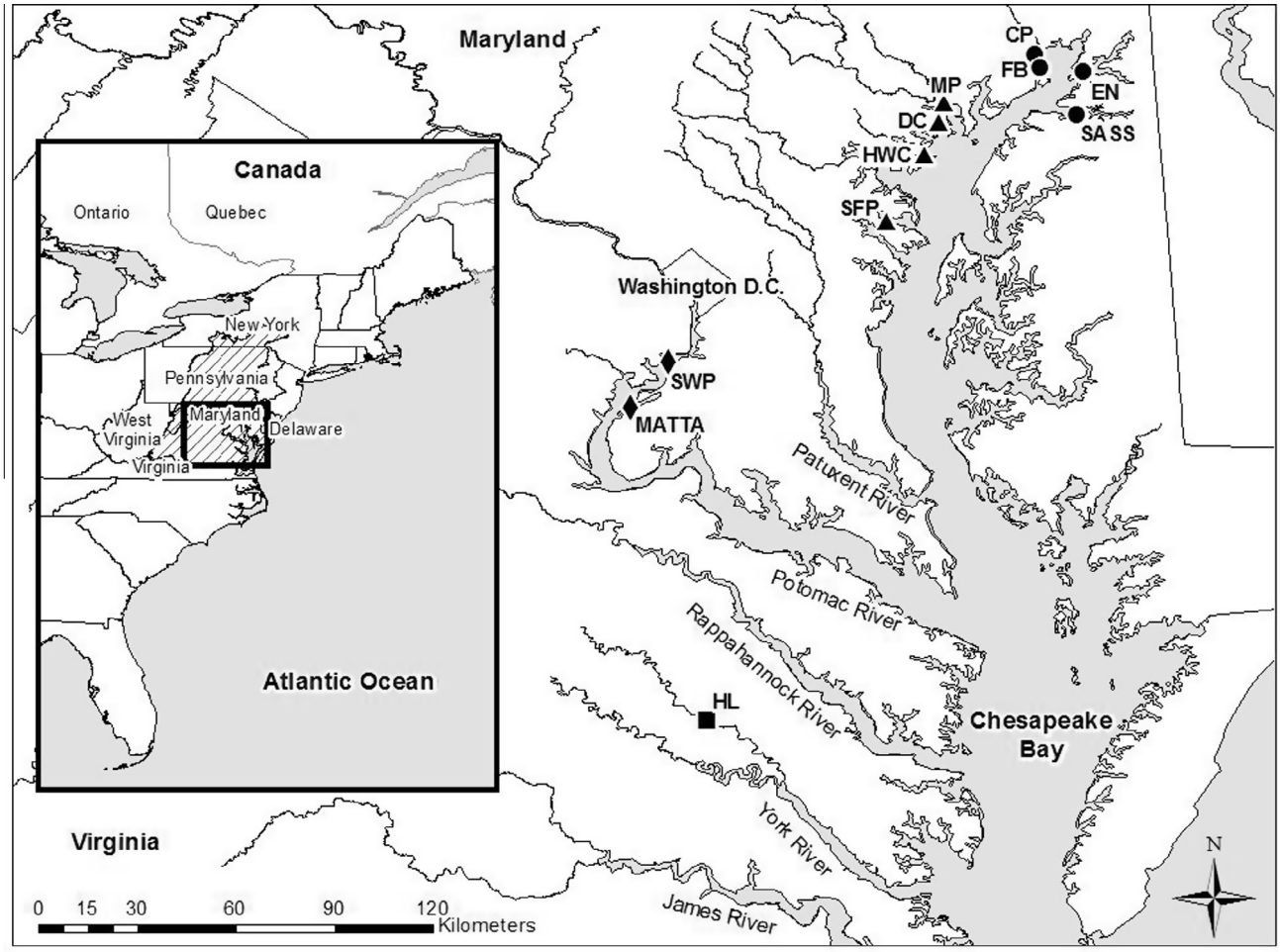

Fig. 1. *Vallisneria americana* collection locations in the Chesapeake Bay. Population abbreviations are as follows: Concord Point, Susquehanna Flats, MD (CP), Elk Neck, Elk River, MD (EN), Fishing Battery, Susquehanna Flats, MD (FB), Sassafras River, MD (SASS), Dundee Creek, Gunpowder River, MD (DC), Rocky Point Hawks Cove, Back River, MD (HWC), Mariner Point, Gunpowder River, MD (MP), South Ferry Point, Magothy River, MD (SFP), Mattawoman Creek, Potomac River, MD (MATTA), Piscataway Park, Potomac River, MD (SWP), and Horse Landing, Mattaponi River, VA (HL). Regional assignments to the North-Chesapeake (circle), Mid-Chesapeake (triangle), Potomac River (diamond), and York River (square) were based on previous population genetic analysis (Lloyd et al., 2011).

Table 1
Replication (rep.) numbers of controlled *Vallisneria americana* reproductive crosses by maternal (rows) and paternal (columns) population sources nested within four genetic regions of the Chesapeake Bay.

	Region	Population	Paternal Source											Total Rep
			North-Chesapeake Bay				Mid-Chesapeake Bay				Potomac River		York River	
			CP	EN	FB	SASS	DC	HWC	MP	SFP	MATTA	SWP	HL	
Maternal Source	North-Chesapeake Bay	CP	5	–	–	–	–	2	–	–	–	4	–	11
		EN	–	13	–	–	–	3	–	–	–	3	–	19
		FB	–	–	12	–	–	7	–	–	–	8	–	27
		SASS	–	–	–	26	–	14	–	–	–	7	–	47
	Mid–Chesapeake Bay	DC	–	–	–	–	3	3	–	–	–	4	–	10
		HWC	–	–	–	–	–	56	–	–	–	42	-	98
		MP	–	–	–	–	–	9	19	–	–	5	-	33
		SFP	–	–	–	–	–	4	–	3	–	2	-	9
			–	–	–	–	–							
	Potomac River	MATTA	–	–	–	–	–	5	–	–	8	5	-	18
		SWP	–	–	–	–	–	5	–	–	–	9	-	14
	York River	HL	–	–	–	–	–	5	–	–	–	5	4	14
	Total Rep.		5	13	12	26	3	113	19	3	8	94	4	300

capsules that contain hundreds of small, dark seeds embedded in a clear gelatinous matrix. We measured capsule length and width to calculate capsule area. We counted the number of seeds in every capsule and calculated average length per cross from 10 randomly chosen seeds. Seeds were stored in tap water in the dark at 4 °C until germination trials.

In January 2010, we assessed germinability of 10 randomly selected seeds from each harvested fruit by planting seeds in Petri dishes. To remove orientation effects on germination, we stabilized the seeds in a horizontal orientation in 0.2% agar covered with a thin layer of dechlorinated tap water (Baskin and Baskin, 1998). We randomly placed Petri dishes in a growth chamber at 30 °C with a 12 h light-dark cycle at ~200 μmol m^{-2} s^{-1} of fluorescent light, conditions found to be optimal for *V. americana* germination in previous research (Jarvis and Moore, 2007). Water was added daily to compensate for evaporation and the locations of petri dishes were re-randomized weekly. We monitored germination, defined as emergence of the radicle at least 1 mm from the seed coat (Jarvis and Moore, 2007), daily for 30 days and calculated percent of successful germination events per cross.

2.3. Estimating relatedness

Variation in degree of genetic relatedness among crossed individuals can be a source of uncontrolled variation, especially in species with large ranges in genotypic diversity and broad distribution of a few clones (Lloyd et al., 2011). Because full diallel crosses were not possible we wanted to account for the effect that relatedness might have on seed production and germination between any two crossed individuals. Randomly crossing more or less related individuals within or among populations or regions may bias our results. In absence of known pedigree information, estimated relatedness can be used to understand the genetic component of phenotypic similarity (see Appendix A). To account for effects of this variation on reproductive success we used multilocus genotypes (Lloyd et al., 2011) to calculate Wang's (2002) estimator of pairwise relatedness between crossed individuals. We chose Wang's estimator because Monte-Carlo simulations (Table A1) indicated it had the lowest variance and minimal bias across various relationship categories (Van de Casteele et al., 2001). Relatedness ranges from 0 (unrelated) to 1 (identical clones). Sometimes Wang's relatedness estimates are negative, which is also interpreted as unrelated (Wang, 2002). Pairwise relatedness was included as a random factor in all subsequent data analysis.

2.4. Statistical analysis

Statistical analyses on reproductive fitness were performed using The SAS® System for Windows (SAS Institute, Inc.). We used nested one-way ANOVAs with the Satterthwaite approximation to account for unequal sample variances to determine if capsule area, seed count, or seed length differed between regions in the within-population crosses. Population source was treated as a random effect nested within region. Pairwise relatedness was included as a random effect. Likewise, one-way ANOVA was used to test for differences among populations in the within-population crosses, followed by post-hoc Tukey–Kramer tests. Differences in germination by region and population were examined with chi-square tests of independence.

In the within population crosses we used Spearman rank correlation (R Project v2.12.2, 2011) to quantify relationships between variation in seed trait variables with one another as well as with genetic diversity and differentiation metrics. Specifically, capsule area, seed count, seed length, and germination success were compared with the genotypic diversity (the proportion of unique genotypes found in a population), average number of alleles, number of private alleles, observed and expected heterozygosities of each population, the average population relatedness of all individuals originally sampled from each population in the Chesapeake Bay, and the average relatedness among only the crossed individuals. We estimated relatedness among populations by averaging relatedness estimates for pairwise comparisons of genotypes collected from different populations (Table 2). Average among population relatedness was compared to Hedrick's heterozygosity-corrected measure of population divergence (G'_{ST}; Hedrick, 2005) as calculated from the program SMOGD (Crawford, 2010; Table 2). Hedrick's G'_{ST} is a derivative of Wright's F_{ST} that is more appropriate for comparisons of loci that have different mutation rates, like microsatellites. To conserve family-wise error rates among multiple correlation comparisons, Bonferroni corrections were applied.

To quantify effects of mixing sources on capsule area, seed count, or seed length, we performed a suite of statistical analyses on crosses that used only HWC or SWP pollen. First, we used one-way ANOVAs and Tukey–Kramer tests to test for differences in fruit and seed traits in crosses classified as either within-population (e.g., HWC × HWC), among-population within the same region (e.g., SFP × HWC), or among-region (SASS × HWC). Using the same analyses, we also tested for differences in fruit and seed traits across all pairwise population combinations. We then used two-way ANOVAs on data from HWC- and SWP-pollinated crosses to determine whether interactions between maternal and paternal population sources could be observed.

The effects of different pollen sources on fruit and seed production were assessed using one-way ANOVA on mothers crossed with pollen from either within their population, from HWC, or from SWP. Contrasts within mothers were compared using *F*-tests to determine whether differences in seed or capsule production by pollen source exist. Differences in germination in the among-population crosses were examined with Chi Square tests of independence.

Maternal turion size was only collected for 15 of the 71 maternal genotypes used in crosses, spanning five Chesapeake Bay populations (DC, HWC, SFP, SWP, and MP). One-way ANOVA on this subset of the data found that maternal turion length was not significantly different among populations (ANOVA; $F_{4,10} = 1.71$; $p = 0.224$) or regions (ANOVA; $F_{1,13} = 2.61$; $p = 0.130$). Overall, 78 of the 300 crosses used flowers from these maternal genotypes, so we also used Spearman rank correlation (R Project v2.12.2, 2011) to determine if there were significant relationships between average maternal turion length and the capsule area, seed count, seed length, and percent germination resulting from crosses using these individuals. There were no significant correlations. Therefore, maternal turion size was not used as a covariate in the analyses.

3. Results

3.1. Within-population crosses

Of the 300 capsules produced, within-population crosses yielded 138 capsules, with an average length of 9.5 ± 0.2 cm (2.0–17.9 cm) and width of 3.0 ± 0.1 mm (1.3–5.2 mm). On average these capsules produced 137.7 ± 6.5 seeds (0–385 seeds), with lengths averaging 2.6 ± 0.02 mm (1.91–3.20 mm).

We observed no seed trait differences in within-population crosses among the four genetic regions. Despite lack of regional differences, individual populations differed from one another in capsule area (ANOVA; $F_{10,63.8} = 2.29$; $p = 0.023$) and seed count ($F_{10,63.4} = 2.51$; $p = 0.013$; Fig. 2). The SFP and HL within-population crosses exhibited the lowest values in multiple traits (Fig. 2). Germination also varied by population ($X^2_{10,1530} = 74.44$; $p < 0.001$), but not by region (Fig. 3). At the extremes, seeds from crosses

Table 2
Genetic relatedness among *Vallisneria americana* individuals within or among populations and differentiation of population within and among four genetically defined regions of the Chesapeake Bay. Hedrick's heterozygosity-corrected measure of divergence (G'_{ST}) is above the diagonal (white), the average Wang pairwise relatedness measure for individuals in populations is on the diagonal (dark gray) and the mean relatedness of all pairs of individuals among the specified populations is below the diagonal (light grey). Relatedness estimates above zero are in bold. Population abbreviations are defined in Fig. 1.

Region		North-Chesapeake Bay				Mid-Chesapeake Bay				Potomac River		York River
	Pop	CP	EN	FB	SASS	DC	HWC	MP	SFP	MATTA	SWP	HL
North-Chesapeake Bay	CP	**0.03**	0.03	<0.01	0.02	0.08	0.07	0.09	0.13	0.05	0.09	0.21
	EN	<-0.01	**0.11**	0.04	0.02	0.07	0.05	0.12	0.12	0.03	0.07	0.22
	FB	**0.05**	**0.02**	**0.08**	0.04	0.06	0.03	0.09	0.10	0.02	0.09	0.19
	SASS	-0.04	-0.02	-0.03	-0.03	0.10	0.08	0.09	0.12	<0.01	0.08	0.19
Mid-Chesapeake Bay	DC	-0.16	-0.16	-0.11	-0.19	-0.05	0.015	0.02	0.05	0.06	0.12	0.22
	HWC	-0.16	-0.13	-0.12	-0.17	-0.13	**0.05**	0.02	0.07	0.02	0.13	0.15
	MP	-0.16	-0.20	-0.12	-0.18	-0.09	-0.10	-0.01	0.10	0.10	0.19	0.20
	SFP	-0.18	-0.12	-0.10	-0.14	-0.06	-0.19	-0.12	**0.14**	0.09	0.15	0.19
Potomac River	MATTA	-0.03	**0.02**	-0.05	-0.02	-0.10	-0.16	-0.19	-0.02	**0.29**	<0.01	0.02
	SWP	-0.10	-0.02	-0.07	-0.05	-0.16	-0.19	-0.12	-0.11	**0.16**	**0.18**	0.16
York River	HL	-0.12	-0.12	-0.11	-0.09	-0.17	-0.05	-0.10	-0.07	**0.11**	-0.04	**0.51**

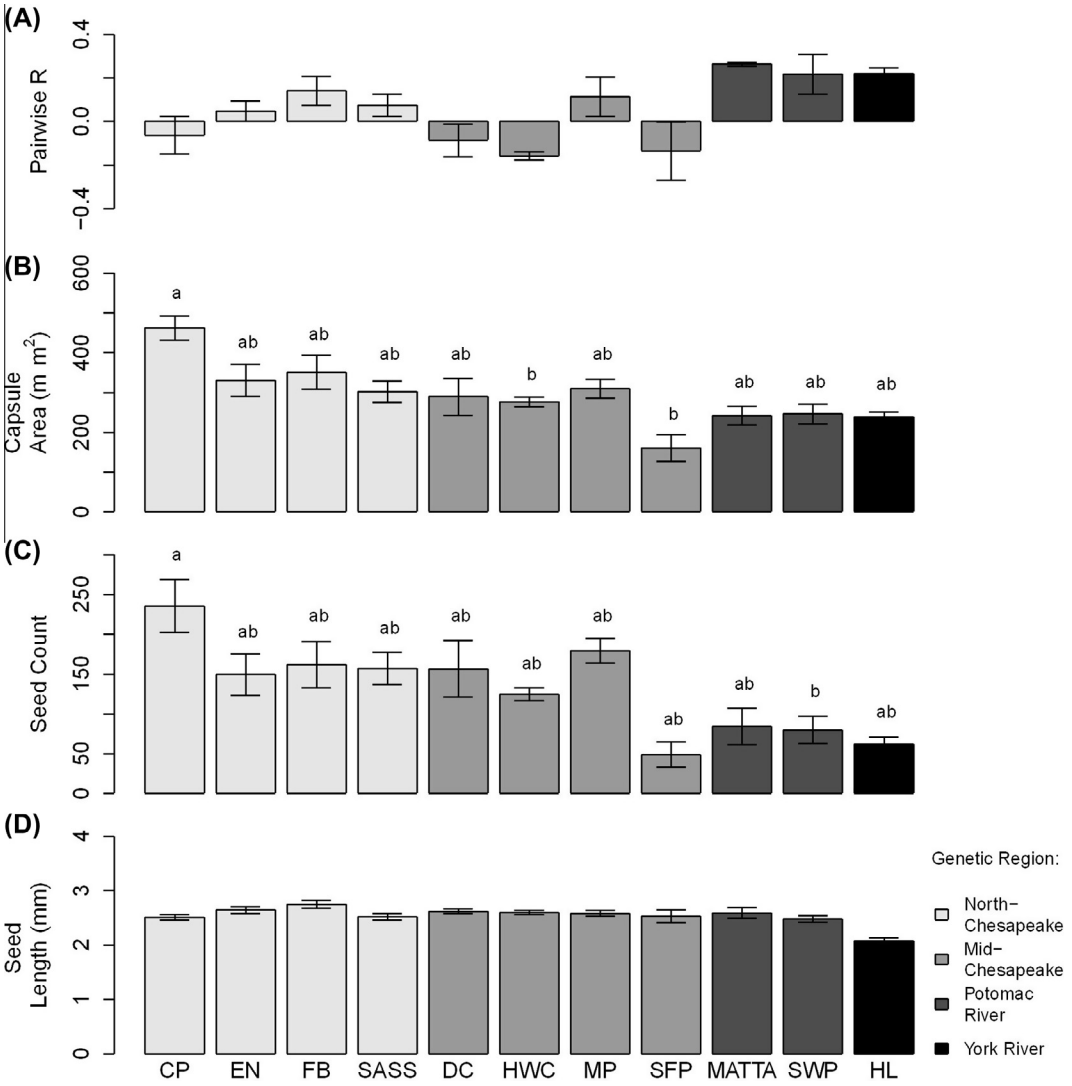

Fig. 2. Population means and standard errors of (A) pairwise relatedness between crossed individuals, (B) capsule area, (C) seed count, and (D) seed length from Chesapeake Bay within-population *V. americana* crosses. Different letters in panels b and c denote significant differences between pairs of means at the 0.05 level based on ANOVAs with the Satterthwaite approximation to account for unequal sample variances and posthoc Tukey–Kramer tests. ANOVAs were not used to assess differences in relatedness. Light gray indicates populations from the North-Chesapeake Region, gray indicates populations from the Mid-Chesapeake Region, dark gray indicates populations from the Potomac River, and black indicates populations from the York River.

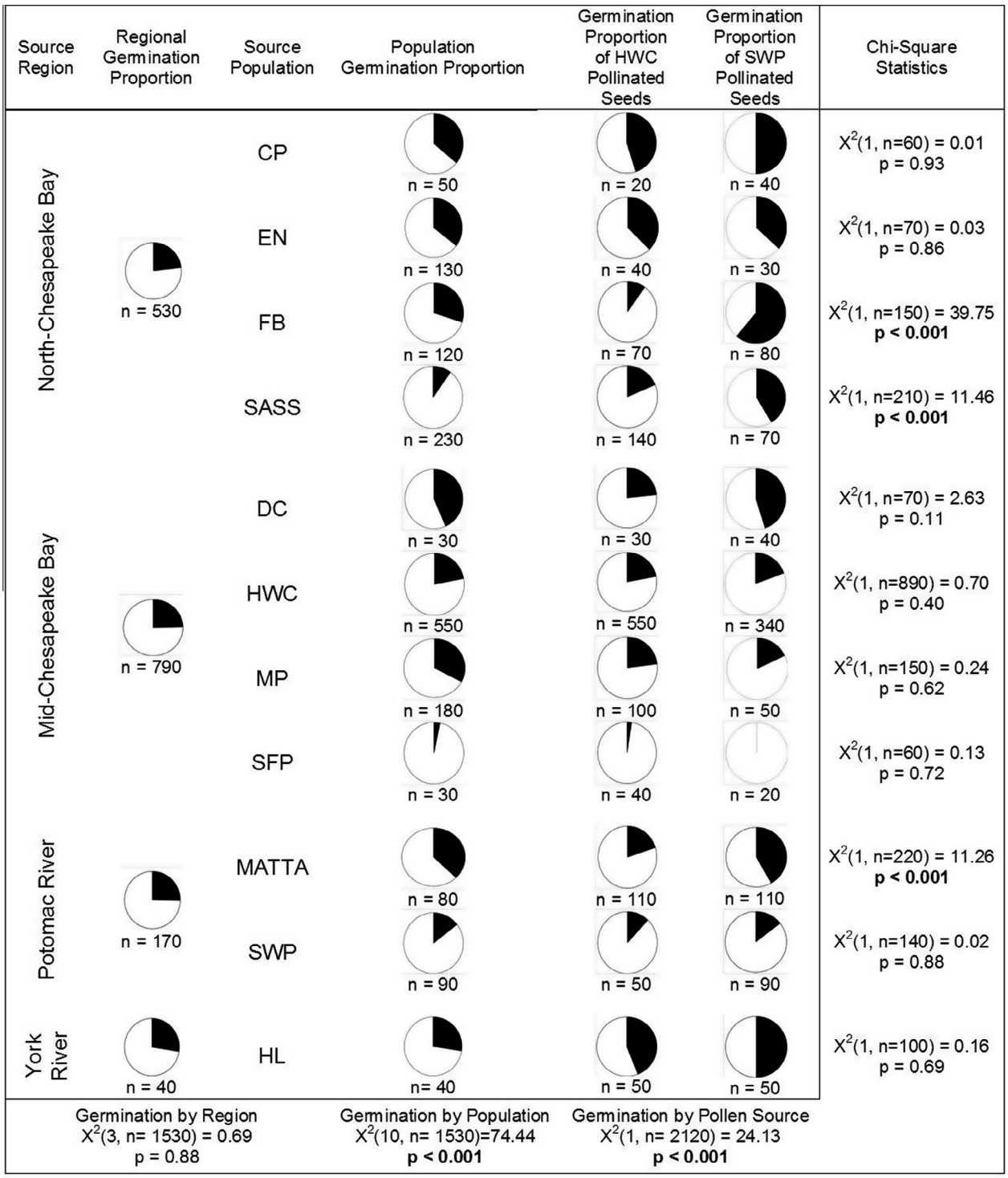

Fig. 3. Proportion of successfully germinated *V. americana* (10 seeds per cross) pollinated within each region and population as well as from either HWC or SWP sources. Chi Square tests of independence were used to determine if germination count varied significantly by region, population, or by HWC versus SWP pollen source. Black designates successful germination and white designates unsuccessful germination.

within SFP (3%), SASS (10%), and SWP (14%) germinated poorly whereas DC and MATTA had the highest germination success (43% and 38%, respectively).

Even after correcting for multiple comparisons with the Bonferroni correction, there are significant positive correlations between capsule area and seed count ($r_s = 0.79$, $p < 0.001$), capsule area and percent germination ($r_s = 0.29$, $p < 0.001$), and seed count and percent germination ($r_s = 0.28$, $p < 0.001$). The average relatedness estimate for each population was positively correlated with the average relatedness of individuals used in the crosses (Table 3), indicating that crossed individuals represented their source populations. Without correcting for multiple comparisons, both estimates of population relatedness were negatively correlated with some genetic diversity metrics (Table 3), however, genotypic diversity, average number of alleles per population, and the observed or expected heterozygosity of each population were not correlated with

Table 3
Spearman Rank Correlation coefficients among measures of relatedness for crossed individuals, average population relatedness, population genetic diversity metrics (from Lloyd et al., 2011), and average seed trait variables from within each *Vallisneria americana* population from the Chesapeake Bay. Correlation coefficients significant at the 0.05 level without correction for multiple comparisons are designated boldface. Superscripts denote changes to correlation coefficients after correcting for family-wise error rates.

	Crossed Individual's R	Average Population R	Genotypic Diversity	*A*	A_p	H_o	H_e
Crossed Individual's R	–	0.65	−0.35	−0.61	−0.21	−0.12	**−0.72**[a]
Average Population R	0.65	–	**−0.69**[a]	−0.78[a]	−0.40	0.05	−0.49
Capsule Area	−0.27	−0.58	0.23	0.47	0.08	0.08	−0.13
Seed Count	−0.26	**−0.74**[b]	0.40	0.50	0.16	−0.05	−0.07
Seed Length	−0.29	−0.31	0.08	0.43	−0.28	0.52	0.50
% Germination	−0.03	−0.20	0.16	0.17	−0.08	−0.02	−0.24

R = Wang's (2002) estimator of relatedness; A = average number of alleles; A_p = number of private alleles; H_o = observed heterozygosity; H_e = expected heterozygosity. Genotypic diversity = (G − 1)/(N − 1).

[a] These correlation coefficients are no longer significant after controlling for family-wise error rate with the Bonferroni correction across the 10 comparisons between the 5 genetic metrics and the 2 estimated relatedness metrics.

[b] This correlation coefficient is no longer significant after controlling for family-wise error rate with the Bonferroni correction across the 28 comparisons between seed traits variables and genetic metrics.

reproductive traits (Table 3). Average population relatedness was negatively correlated only with seed count (Table 3). However, after controlling for family-wise error rates among the multiple comparisons, these correlations are no longer significant. Thus, we observed no consistent association between reproductive variables and relatedness values for sampled Chesapeake Bay *V. americana*.

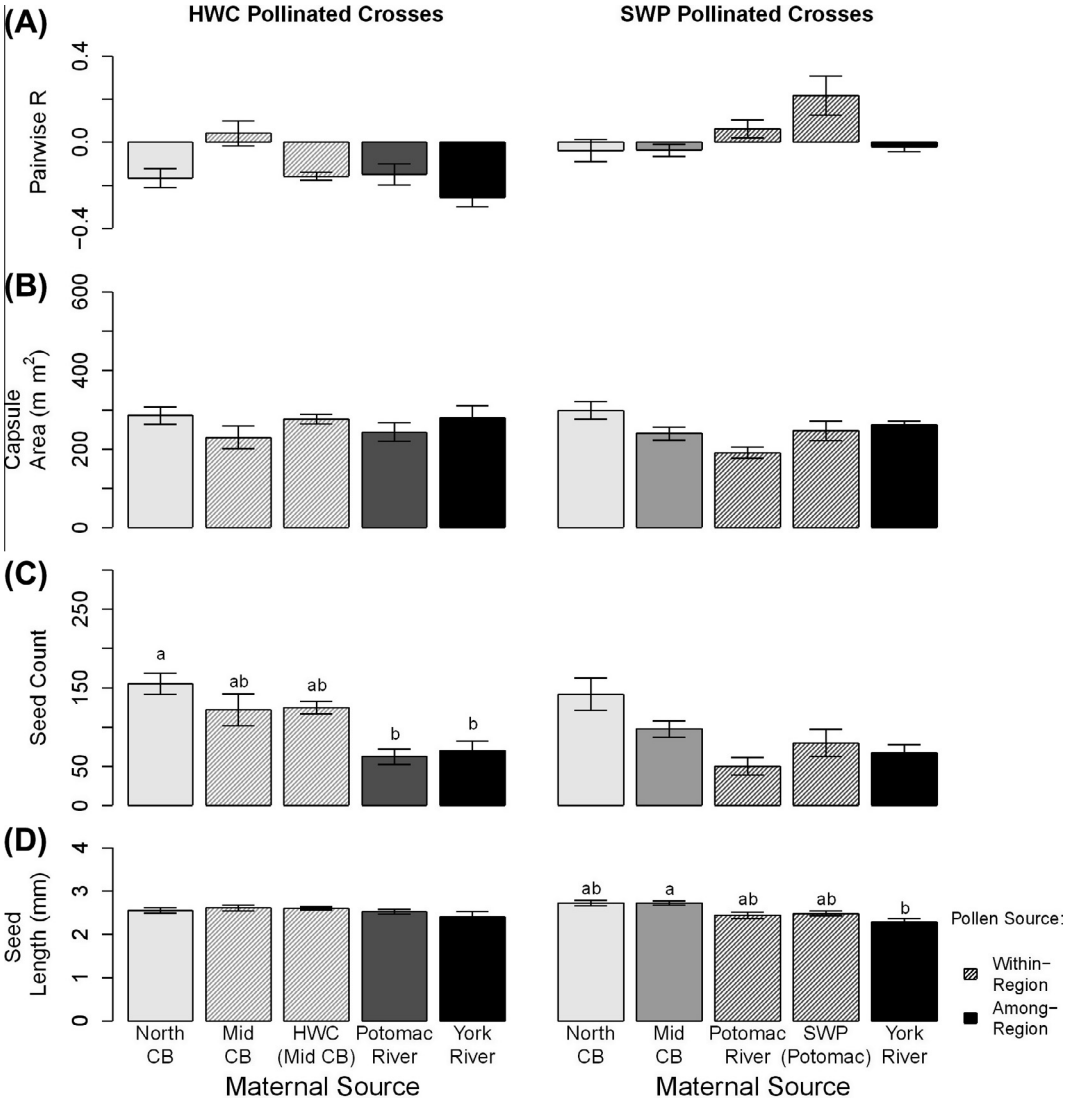

Fig. 4. Means and standard errors of (A) pairwise relatedness between crossed individuals, (B) capsule area, (C) seed count, and (D) seed length from Chesapeake Bay (CB) *V. americana* crosses pollinated by either HWC or SWP pollen, grouped by maternal region. Different letters in panels c and d denote significant differences between pairs of means at the 0.05 level based on ANOVAs with the Satterthwaite approximation to account for unequal sample variances and posthoc Tukey–Kramer tests. Lack of letters denotes no observed significant differences. ANOVAs were not used to assess differences in relatedness.

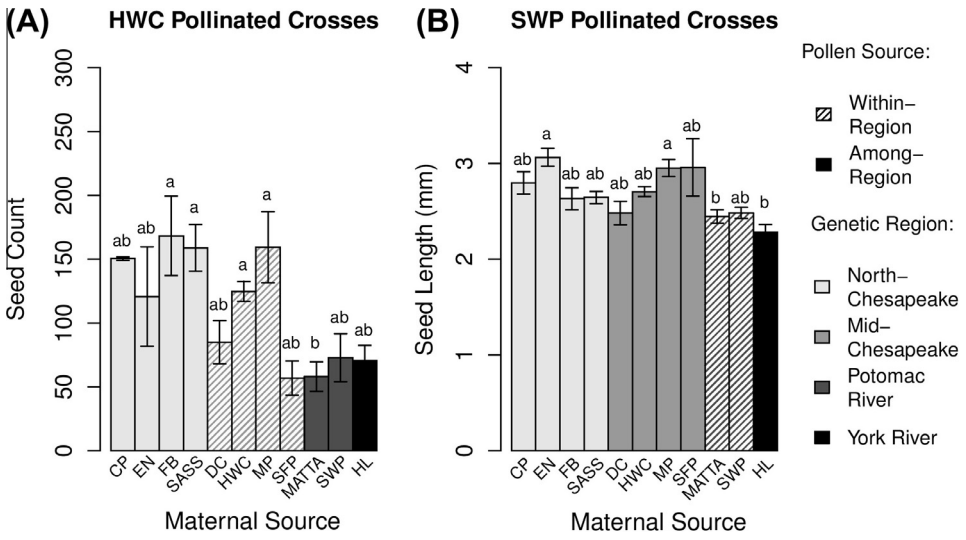

Fig. 5. Means and standard errors of (A) seed count from Chesapeake Bay *V. americana* crosses pollinated by HWCpollen and (B) seed length from Chesapeake Bay *V. americana* crosses pollinated by SWP pollen. Results are grouped by maternal population. Different letters denote significant differences between pairs of means at the 0.05 level based on ANOVAs with White's heteroscedasticity correction and posthoc Tukey–Kramer tests.

3.2. Among-population crosses

The among-population crosses produced 138 capsules with average lengths of 8.7 ± 0.2 cm (2.0–16.8 cm) and widths of 2.9 ± 0.04 mm (1.3–5.0 mm). On average, capsules produced 111.3 ± 4.9 seeds (0–307), with lengths averaging 2.60 ± 0.02 mm (1.91–3.46 mm).

HWC-pollinated crosses differed in seed count whereas SWP-pollinated crosses differed in seed length (Figs. 4 and 5). At the regional level, maternal sources from the North-Chesapeake region pollinated by HWC (from the Mid-Chesapeake region) produced more seeds than the other among-region crosses (ANOVA; $F_{4,63.9} = 4.55$; $p = 0.003$; Fig. 4). Likewise, maternal sources from the Mid-Chesapeake pollinated by SWP (from the Potomac River) produced longer seeds than York-Potomac crosses (ANOVA; $F_{4,51} = 4.13$; $p = 0.006$; Fig. 4). Region-level ANOVAs masked subtler differences in seed count and seed length between specific population combinations (Fig. 5). However, no one cross type consistently outperformed the others (Fig. 5).

Although certain combinations of regions or populations differed in capsule and seed production, no interactions between maternal and paternal population source on capsule area, seed count, and seed length were observed. Maternal population source accounted for some variation observed in seed count ($F_{10,75.6} = 3.43$; $p = 0.001$) and seed length ($F_{10,109} = 2.69$; $p = 0.006$). Regardless of pollen source, crosses involving mothers from populations in the North-Chesapeake typically produced many large seeds whereas crosses involving mothers from MATTA and HL consistently produced fewer, shorter seeds. However, comparison of fruit and seed production from crosses from a single maternal source and three different pollen sources (within-population, HWC, or SWP) revealed significant paternal effects in seed count (ANOVA; $F_{30,276} = 12.22$; $p < 0.001$) and seed length ($F_{30,271} = 4.49$; $p < 0.001$; Fig. 6). Some populations crossed with SWP pollen were outperformed by the within-population or HWC-pollinated crosses, while other populations did better with SWP pollen (Fig. 6). Thus, capsule and seed production tended to be population specific and differences were not consistent enough to produce an overall paternal-maternal interaction.

Germination success among crosses was also population specific. For example, SFP mothers crossed with either HWC (within-region) or SWP pollen (among-region) had 2.5% and 0% germination success, respectively, whereas offspring of CP mothers had high germination rates regardless of paternal source. Germination was higher overall for SWP-pollinated crosses than for HWC-pollinated crosses ($X^2_{1,2120} = 24.13$; $p < 0.001$; Fig. 3), but this was largely driven by a few specific cases (Fig. 3). Germination success did not differ between crosses that occurred within-versus-among genetically defined regions. In contrast, within (32% germination) versus among (39% germination) population crosses differed ($X^2_{1,3000} = 5.17$; $p = 0.023$).

Not surprisingly, among population relatedness was negatively correlated with levels of population differentiation ($r_s = -0.461$, $p < 0.001$). Individuals from populations in the North-Chesapeake or the Potomac River regions had the highest levels of relatedness to one another and these regions had the lowest differentiation among populations (Table 2). Despite similar levels of relatedness and differentiation, populations from the North-Chesapeake tended to produce many large seeds whereas Potomac River populations had less robust seed production (Figs. 2, 4 and 5).

4. Discussion

4.1. Risk of inbreeding and outbreeding depression

Most restoration practitioners would agree that benefits and risks of genetic rescue (alleviation of inbreeding and recovery of genetic diversity; Frankham, 2010; Frankham et al., 2011) versus outbreeding depression (Broadhurst et al., 2008; Frankham et al., 2011; McKay et al., 2005) must both be considered. Differences in opinions arise regarding which risks are higher and more pervasive. Increasingly, advocates of restoration strategies that involve mixing sources suggest that risks of outbreeding depression are overemphasized and poorly supported (Frankham et al., 2011; Weeks et al., 2011). In contrast to the simplistic dichotomous framework for dominance of one risk over the other, we find that neither has overwhelmingly strong or consistent effects in *V. americana* from the Chesapeake Bay. The fact that many within-population crosses were more successful than among-population crosses provides evidence for local adaptation and concerns over outbreeding depression. In contrast, more and larger seed production with higher rates of germination in some among-population crosses

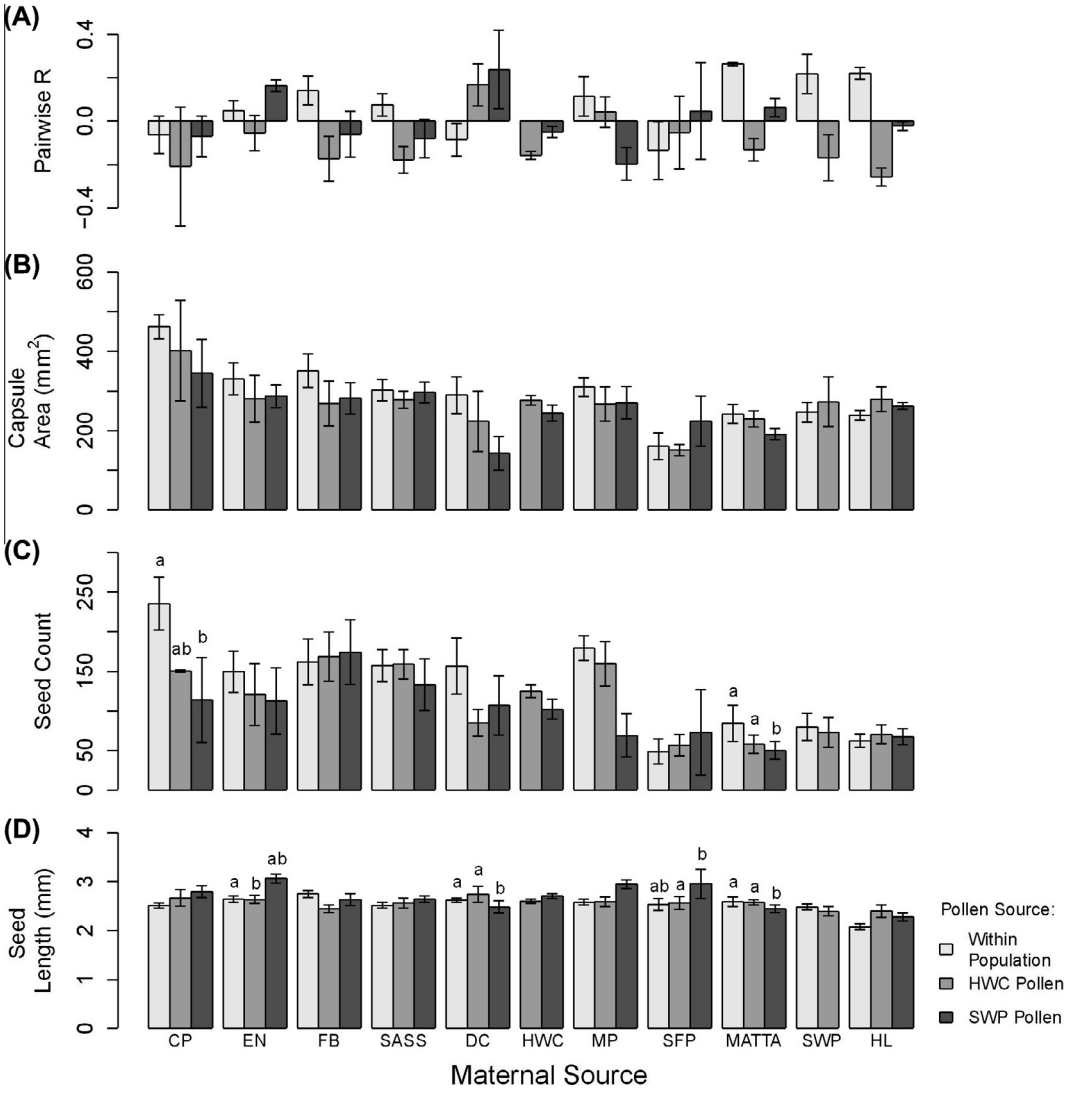

Fig. 6. Means and standard errors of (A) pairwise relatedness between crossed individuals, (B) capsule area, (C) seed count, and (D) seed length from Chesapeake Bay *V. americana* crosses pollinated within-populations or with HWC or SWP pollen, grouped by maternal population. Different letters in panels (C and D) denote significant differences between pairs of means within a maternal population at the 0.05 level based on ANOVAs with White's heteroscedasticity correction and posthoc *F*-tests with comparison-wise error rates. Lack of letters denotes no observed significant differences. ANOVAs were not used to assess differences in relatedness.

indicate potential genetic rescue. It is disconcerting that none of the easily measured aspects of genetic diversity were useful in predicting which populations might need genetic rescue and which would be at risk of outbreeding depression. Rather, we see a complicated picture in which reproductive success varies independently of measured genetic diversity and relatedness.

Frankham et al. (2011) suggest that risk of outbreeding depression in crosses among populations is heightened when populations have fixed chromosomal differences, have had limited gene flow during the past 500 years, or inhabit different environments. Information from previous studies on *V. americana* in the Chesapeake Bay shows differentiation among populations. The four genetic regions in the Bay (Fig. 1) suggest long-term limitation to gene flow between populations assigned to different regions and connections among populations within regions (Lloyd et al., 2011). Populations exist in a variety of environmental conditions (e.g. salinity, turbidity, sediment composition) and there is mounting evidence of local adaptation to specific habitats (Engelhardt, Unpublished results). At the same time, populations of SAV in the Chesapeake Bay declined precipitously in the 1960's from reductions in light availability due to eutrophication and increased turbidity, such that by the 1970's beds were reduced to a fraction of their historical size (Orth and Moore, 1983). SAV now occupy isolated patches in the Bay, a situation that is known to increase risk of reducing genetic diversity and experiencing inbreeding depression (Aguilar et al., 2008; Ellstrand and Elam, 1993; Frankham et al., 2011). For such populations, genetic rescue by reestablishing gene flow or by supplementing individuals from more genetically diverse populations (Frankham, 2010) is often suggested.

Weeks et al. (2011) proposes that when population divergence is low, translocation of individuals among populations can occur without the need to go through a risk-assessment for outbreeding depression. However, they do not define 'low divergence' and instead offer a few case studies of species with very different life histories. In our experiments, populations with lower measures of divergence (e.g. G'_{ST} = 0.09 between CP and SWP and G'_{ST} < 0.01 between MATTA and SWP) produced significantly fewer seeds than when crossed within-populations (Fig. 6). Likewise, when we control for family-wise error rate, none of the population genetic diversity metrics were correlated with reproductive output traits

(Table 3). Thus, low population divergence based on neutral genetic markers may not be the best predictor of successful population mixing.

Correlation between levels of genetic diversity and fitness may be weak if the genetic markers used to estimate genetic diversity are neutral, genetic variation is nonaddative, or there is differential selection on the measured traits (Reed and Frankham, 2001; 2003). Despite these theoretical limitations, a large body of literature suggests that genetic diversity estimates from neutral markers like allozymes and microsatellites, are good proxies for population fitness and adaptive potential (Merilä and Crnokrak, 2001; Reed and Frankham, 2003; Reynolds et al., 2012a). For example, despite differences in magnitude between quantitative traits and measures of genetic differentiation, Merilä and Crnokrak (2001) found the measures were positively correlated, suggesting that divergence in neutral markers may be indicative of the degree of genetic differentiation in quantitative traits. Likewise, Hufford et al. (2012) were able to use molecular marker data to predict the scale of outbreeding depression while other studies have found measures of genetic diversity, like level of inbreeding and number of alleles, were consistent predictors of heterosis when mixing individuals from different populations (Pickup et al., 2013). Studies like these have led to the creation of plant restoration guidelines for the translocation of individuals that rely primarily on levels of genetic diversity and differentiation (e.g. Weeks et al., 2011). However, the results presented here as well as in other studies (reviewed in Reed and Frankham, 2001) find low correlation between molecular markers and measured traits, suggesting that molecular markers alone cannot be used to predict population fitness and potential for population persistence.

Because among-population and among-region crosses did not consistently outperform within-population or within-region crosses in seed production (Figs. 4 and 5) there is no strong evidence of genetic rescue benefits. Specific population combinations, however, had reduced or enhanced reproductive output. Unfortunately, seed production was not predicted by any genetic metrics that sometimes indicate outbreeding or inbreeding depression risk. For *V. americana*, therefore, common-garden or field based experiments that cross individuals among populations are needed to assess potential outbreeding depression and rescue effects prior to restoration.

Although our results provide valuable insights, experiments were limited to fitness effects manifested early in the *V. americana* life cycle under benign greenhouse conditions. Our results were further limited to seed production traits, but there may be differences in correlations between genetic differentiation metrics and morphological versus life history traits (e.g. Merilä and Crnokrak, 2001). While we found no correlation between seed production and measures of genetic diversity and differentiation, it is possible that the morphological traits that have already demonstrated population level differences in growth rates and allocation of resources to leaf extension versus ramet production (Engelhardt, Unpublished results) may be better correlated with genetic diversity. Furthermore, fitness effects of both inbreeding and outbreeding are often greater in later life stages (Holtsford and Ellstrand, 1990; Husband and Schemske, 1996) and in subsequent generations (Broadhurst et al., 2008; Edmands, 2007; Huff et al., 2011) as well as under stressful conditions (Carr and Dudash, 1995; Crnokrak and Roff, 1999; Keller, 1998; Murren and Dudash, 2012). This research focused specifically on sexual reproductive fitness because of its importance in establishing diverse populations post-restoration. Research on other macrophytes has demonstrated that genetically diverse assemblages do better in terms of plant productivity in both stressed and non-stressed environments (e.g. Reusch et al., 2005; Reynolds et al., 2012a).

4.2. Additional factors affecting reproductive output

Vallisneria americana reproduces vegetatively and sexually (McFarland and Shafer, 2008), and we see evidence that suggests a trade-off between seed production and allocation to vegetative expansion or turion production. For example, Lloyd et al. (2011) found that in most populations >70% of samples were unique genotypes, but some populations consisted of a single clone. Only 29% of the sampled genotypes from the SFP population were unique, but relatedness estimates were low (Fig. 2) indicating high *in situ* vegetative reproduction and low inbreeding during sexual reproduction. Despite no indication of inbreeding, this population had poor seed production (Fig. 2). It did, however, rank high relative to the other populations in turion production (Engelhardt, Unpublished results), producing a mean of 18 turions per replicate clone ($n = 6$) within one growing season. In contrast, 89% of genotypes in the CP population were unique (Lloyd et al., 2011). Crosses involving CP mothers had higher seed production than average (Fig. 2), yet the mean number of turions per clonal replicate within one growing season was <7 (Engelhardt, Unpublished results). These observations suggest an inverse relationship between vegetative and sexual reproductive fitness, irrespective of the degree of relatedness among crossed individuals. Furthermore, in other aquatic plants there is evidence of tradeoffs between sexual and asexual reproduction that are mediated by the environment (e.g. Prati and Schmid, 2000; Xie and Yu, 2011). The presence of stressful environments or increased competition may lead to an increase in sexual allocation of resources relative to asexual reproduction (e.g. Prati and Schmid, 2000). Alternatively, the submersed macrophyte *Potamogeton crispus* produces turions of greater mass in nutrient-poor sediment compared with plants grown in nutrient-rich sediment (Xie and Yu, 2011). If our populations are genetically adapted to reproduce dominantly by either sexual or asexual reproduction under low stress conditions, then our seed production data may be biased since all plants were grown in a stress-free greenhouse environment. How these reproductive tradeoffs interact with and influence genetic diversity and population persistence over time are key future research topics.

4.3. Implications for restoration

Our objective was to evaluate relative risks and benefits of using local versus non-local plantings in restoration as indicated by *V. americana* seed production and germination success. Restoration of aquatic species in the Chesapeake Bay typically involves planting locally sourced material, including whole individuals harvested from beds in the same tributary, individuals reared from seeds harvested from nearby beds, or individuals from repositories that were initially established from local populations (Lloyd et al., 2012). Reynolds et al. (2012b) demonstrated that *Zostera marina* seeds harvested from multiple parents from nearby beds can preserve genetic diversity in restored sites with no signs of inbreeding depression in either donor or restored sites. Lloyd et al. (2012) found that current *V. americana* restoration techniques generally reflect the genetic diversity found in natural populations in the Chesapeake Bay. We see no strong argument against local sourcing in this case because most populations are not inbred based on microsatellite markers, and population level differences in seed production (Fig. 2) and germination (Fig. 3) suggest potential for local adaptations or differences in compatibility among populations. Similarities in seed production and germination between crosses that occurred within-regions (Fig. 4) indicate that movement within-regions does not substantially affect local adaptation if it exists. Additionally, very few of the among-population crosses

were substantially better than within-population crosses, indicating little benefit from genetic rescue. Some specific populations were consistently weak (e.g. HL, SFP) or had low replication (e.g. DC, SFP) and thus warrant further investigation.

In summary, the accumulating evidence for *V. americana* in the Chesapeake Bay is that most remnant populations are diverse in terms of the number of genotypes and alleles and do not suffer from heterozygote deficiencies (Lloyd et al., 2011). Although we do see evidence of population level differences in morphology and reproductive success, we do not see systematic patterns that indicate widespread inbreeding or outbreeding depression. Increasing SAV coverage is a worldwide restoration goal because of the vital ecosystem services they provide (Orth and Moore, 1983). Therefore, even though risk of outbreeding depression is low for *V. americana* in the Chesapeake Bay, there is little evidence of inbreeding and the potential cost of losing local adaptations outweighs the potential benefits of mixing multiple sources when attempting to increase coverage. The most disconcerting finding was that the performance of populations and crosses was not consistently explained by easily quantified genetic diversity, differentiation, and relatedness metrics suggested for assessing risk of inbreeding versus outbreeding depression. The degrees of differentiation among populations and inbreeding within populations fall along continuous gradients that vary independently. This finding highlights the need of identifying better metrics or methods to help conservation practitioners efficiently select restoration stock that best balances the risks of inbreeding/outbreeding depression, which are not as dichotomous as previously suggested, while providing the most benefit in terms of genetic rescue and long-term persistence.

Acknowledgements

We thank Deirdre Griffin and Ryan Blaustein (UMD) for data collection, Katie Chavanak (UMCES) for greenhouse assistance, Dr. Joe Hereford (UMD) and Dr. Robert Hilderbrand (UMCES) for statistical analysis support, and funding from Washington Biologists' Field Club, Maryland SeaGrant, UMD College Park, UMCES Appalachian Laboratory, and US Environmental Protection Agency's Science to Achieve Results Fellowship. Also, two anonymous reviewers for valuable comments on an earlier version of the manuscript.

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.2013.08.012.

References

Aguilar, R., Quesada, M., Ashworth, L., Herrerias-Diego, Y., Lobo, J., 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Mol. Ecol. 17, 5177–5188.

Arnaud-Haond, S., Marbà, N., Diaz-Almela, E., Serrão, E.A., Duarte, C.M., 2010. Comparative analysis of stability – genetic diversity in seagrass (*Posidonia oceanica*) meadows yields unexpected results. Estuaries Coast. 33, 878–889.

Baskin, C.C., Baskin, J.M., 1998. Ecologically meaningful germination studies. In: Baskin, C.C., Baskin, J.M. (Eds.), Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, London, pp. 5–26.

Broadhurst, L.M., Lowe, A., Coates, D.J., Cunningham, S.A., McDonald, M., Vesk, P.A., Yates, C., 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. Evol. Appl. 1, 587–597.

Brush, G.S., Hilgartner, W.B., 2000. Paleoecology of submerged macrophytes in the upper Chesapeake Bay. Ecol. Monogr. 70, 645–667.

Burnett, R.K., Lloyd, M.W., Engelhardt, K.A.M., Neel, M.C., 2009. Development of 11 polymorphic microsatellite markers in a macrophyte of conservation concern, *Vallisneria americana* Michaux (Hydrocharitaceae). Mol. Ecol. Resour. 9, 1427–1429.

Carr, D.E., Dudash, M.R., 1995. Inbreeding depression under a competitive regime in *Mimulus guttatus* – consequences for potential male and female function. Heredity 75, 437–445.

Crawford, N.G., 2010. SMOGD: software for the measurement of genetic diversity. Mol. Ecol. Resour. 10, 556–557.

Crnokrak, P., Roff, D.A., 1999. Inbreeding in the wild. Heredity 83, 260–270.

Edmands, S., 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Mol. Ecol. 16, 463–475.

Ellstrand, N.C., Elam, D.R., 1993. Population genetic consequences of small population size: implications for plant conservation. Annu. Rev. Ecol. Syst. Syst. 24, 217–242.

Fenster, C.B., Dudash, M.R., 1994. Genetic considerations for plant population restoration and conservation. In: Bowles, M.L., Whelan, C.J. (Eds.), Restoration of Endangered Species: Conceptual Issues, Planning, and Implementation. Cambridge University Press, Cambridge, Great Britain, pp. 34–62.

Forrest, C.N., Ottewell, K.M., Whelan, R.J., Ayre, D.J., 2011. Tests for inbreeding and outbreeding depression and estimation of population differentiation in the bird-pollinated shrub *Grevillea mucronulata*. Ann. Bot. 108, 185–195.

Frankham, R., 2010. Where are we in conservation genetics and where do we need to go? Conserv. Genet. 11, 661–663.

Frankham, R., Ballou, J.D., Eldridge, M.D.B., Lacy, R.C., Ralls, K., Dudash, M.R., Fenster, C.B., 2011. Predicting the probability of outbreeding depression. Conserv. Biol. 25, 465–475.

Hedrick, P.W., 2005. A standardized genetic differentiation measure. Evolution 59, 1633–1638.

Hereford, J., 2009. Postmating/prezygotic isolation, heterosis, and outbreeding depression in crosses within and between populations of *Diodia teres* (Rubiaceae) walt. Int. J. Plant Sci. 170, 301–310.

Holtsford, T.P., Ellstrand, N.C., 1990. Inbreeding effects in *Clarkia tembloriensis* (Onagraceae) populations with different natural outcrossing rates. Evolution 44, 2031–2046.

Huff, D.D., Miller, L.M., Chizinski, C.J., Vondracek, B., 2011. Mixed-source reintroductions lead to outbreeding depression in second-generation descendents of a native North American fish. Mol. Ecol. 20, 4246–4258.

Hufford, K.M., Krauss, S.L., Veneklaas, E.J., 2012. Inbreeding and outbreeding depression in *Stylidium hispidum*: implications for mixing seed sources for ecological restoration. Ecol. Evol. 2, 2262–2273.

Hughes, A.R., Inouye, B.D., Johnson, M.T.J., Underwood, A., Vellend, M., 2008. Ecological consequences of genetic diversity. Ecol. Lett. 11, 609–623.

Husband, B.C., Schemske, D.W., 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50, 54–70.

Jarvis, J.C., Moore, K.A., 2007. Influence of environmental factors on *Vallisneria americana* seed germination. Aquat. Bot. 88, 283–294.

Kawecki, T.J., Ebert, D., 2004. Conceptual issues in local adaptation. Ecol. Lett. 7, 1225–1241.

Keller, L.F., 1998. Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). Evolution 52, 240–250.

Kemp, W.M., Boynton, W.R., Adolf, J.E., Boesch, D.F., Boicourt, W.C., Brush, G., Cornwell, J.C., Fisher, T.R., Glibert, P.M., Hagy, J.D., Harding, L.W., Houde, E.D., Kimmel, D.G., Miller, W.D., Newell, R.I.E., Roman, M.R., Smith, E.M., Stevenson, J.C., 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. Mar. Ecol. Prog. Ser. 303, 1–19.

Lloyd, M.W., Burnett, R.K., Engelhardt, K.A.M., Neel, M.C., 2012. Does genetic diversity of restored sites differ from natural sites? A comparison of *Vallisneria americana* (Hydrocharitaceae) populations within the Chesapeake Bay. Conserv. Genet. 13, 753–765.

Lloyd, M.W., Burnett, R.K., Engelhardt, K.A.M., Neel, M.C., 2011. The structure of population genetic diversity in *Vallisneria americana* in the Chesapeake Bay: implications for restoration. Conserv. Genet. 12, 1269–1285.

McFarland, D.G., Shafer, D.J., 2008. Factors influencing reproduction in American Wild Celery: a synthesis. J. Aquat. Plant Manage. 46, 129–144.

McKay, J.K., Christian, C.E., Harrison, S., Rice, K.J., 2005. "How local is local?" – a review of practical and conceptual issues in the genetics of restoration. Restor. Ecol. 13, 432–440.

Merilä, J., Crnokrak, P., 2001. Comparison of genetic differentiation at marker loci and quantitative traits. J. Evol. Biol. 14, 892–903.

Montalvo, A.M., Ellstrand, N.C., 2000. Transplantation of the subshrub *Lotus scoparius*: testing the home-site advantage hypothesis. Conserv. Biol. 14, 1034–1045.

Montalvo, A.M., Ellstrand, N.C., 2001. Nonlocal transplantation and outbreeding depression in the subshrub *Lotus scoparius* (Fabaceae). Am. J. Bot. 88, 258–269.

Murren, C.J., Dudash, M.R., 2012. Variation in inbreeding depression and plasticity across native and non-native field environments. Ann. Bot. 109, 621–632.

Orth, R.J., Moore, K.A., 1983. Chesapeake Bay: an unprecedented decline in submerged aquatic vegetation. Science 222, 51–53.

Pickup, M., Field, D.L., Rowell, D.M., Young, A.G., 2013. Source population characteristics affect heterosis following genetic rescue of fragmented populations. Proc. R. Soc. B 280, 2012–2058.

Prati, D., Schmid, B., 2000. Genetic differentiation of life-history traits within populations of the clonal plant *Ranunculus reptans*. Oikos 90, 442–456.

R Development Core Team, 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (ISBN 3 900051-07-0 <http://www.R-project.org/>).

Reed, D.H., Frankham, R., 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55, 1095–1103.
Reed, D.H., Frankham, R., 2003. Correlation between fitness and genetic diversity. Conserv. Biol. 17, 230–237.
Reusch, T.B.H., Ehlers, A., Hämmerli, A., Worm, B., 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proc. Natl. Acad. Sci. 102, 2826–2831.
Reynolds, L.K., McGlathery, K.J., Waycott, M., 2012a. Genetic diversity enhances restoration success by augmenting ecosystem services. PLoS ONE 7, e38397.
Reynolds, L.B., Waycott, M., McGlathery, K.J., Orth, R.J., Zieman, J.C., 2012b. Eelgrass restoration by seed maintains genetic diversity: case study form a coastal bay system. Mar. Ecol. Prog. Ser. 448, 223–233.
Van De Casteele, T., Galbusera, P., Matthysen, E., 2001. A comparison of microsatellite-based pairwise relatedness estimators. Mol. Ecol. 10, 1539–1549.
Wang, J., 2002. An estimator for pairwise relatedness using molecular markers. Genetics 160, 1203–1215.
Waser, N.M., 1993. Population structure, optimal outcrossing, and assortative mating in angiosperms. In: Thornhill, N.W. (Ed.), The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives. University of Chicago Press, Chicago, pp. 173–199.
Weeks, A.R., Sgro, C.M., Young, A.G., Frankham, R., Mitchell, N.J., Miller, K.A., Byrne, M., Coats, D.J., Eldridge, M.D.B., Sunnucks, P., Breed, M.F., James, E.A., Hoffmann, A.A., 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. Evol. Appl. 4, 709–725.
Xie, D., Yu, D., 2011. Turion production and nutrient reserves in *Potamogeton crispus* are influenced by sediment nutrient level. Aquat. Biol. 14, 21–28.