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RESEARCH ARTICLE

Limited pollen dispersal, small genetic neighborhoods, and biparental inbreeding in *Vallisneria americana*

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PREMISE OF THE STUDY: Pollen dispersal is a key process that influences ecological and evolutionary dynamics of plant populations by facilitating sexual reproduction and gene flow. Habitat loss and fragmentation have the potential to reduce pollen dispersal within and among habitat patches. We assessed aquatic pollen dispersal and mating system characteristics in *Vallisneria americana*—a water-pollinated plant with a distribution that has been reduced from historic levels.

METHODS: We examined pollen neighborhood size, biparental inbreeding, and pollen dispersal, based on seed paternity using the indirect paternity method KinDist, from samples of 18–39 mothers and 14–20 progeny per mother from three sites across 2 years.

KEY RESULTS: On average, fruits contained seeds sired by seven fathers. We found significant biparental inbreeding and limited pollen dispersal distances (0.8–4.34 m). However, in a number of cases, correlated paternity did not decline with distance, and dispersal could not be reliably estimated.

CONCLUSIONS: Frequent pollen dispersal is not expected among patches, and even within patches, gene flow via pollen will be limited. Limited pollen dispersal establishes genetic neighborhoods, which, unless overcome by seed and propagule dispersal, will lead to genetic differentiation even in a continuous population. Unless loss and fragmentation drive populations to extreme sex bias, local pollen dispersal is likely to be unaffected by habitat loss and fragmentation per se because the spatial scale of patch isolation already exceeds pollen dispersal distances. Therefore, managing specifically for pollen connectivity is only relevant over very short distances.

KEY WORDS Chesapeake Bay; dispersal; fragmentation; gene flow; Hydrocharitaceae; KinDist; pollination; wild celery.

The ecological process of dispersal underlies the demographic and evolutionary dynamics of plant populations (Austerlitz et al., 2000; Austerlitz and Garnier-Gere, 2003; Robledo-Arnuncio et al., 2014). Evolutionary dynamics are driven by genes, which in plants are dispersed via pollen, seed, and vegetative propagules. This dispersal is critical because it largely determines the size of genetic neighborhoods (Linhart and Grant, 1996; Van Tussenbroek et al., 2016) and the distribution of genetic diversity across a landscape (Slatkin, 1985; Broquet and Petit, 2009; Ellstrand, 2014). Understanding the scale at which each dispersal mode acts can bring insight into potential for genetic connectivity within and among populations (Manel et al., 2003; Ashley, 2010; Storfer et al., 2010). It also highlights the risk of losing connectivity if the amount and distribution of habitat changes. The potential for habitat loss and fragmentation to disrupt genetic connectivity and reduce genetic diversity through genetic drift and inbreeding has long been recognized and remains a major concern in conservation (Ellstrand and Elam, 1993; Young et al., 1996; Luque et al., 2012). Here, we investigated the spatial scale of pollen dispersal, pollen neighborhood size, and biparental inbreeding in the dioecious, submersed aquatic plant species *Vallisneria americana* Michx. (Hydrocharitaceae) in the Chesapeake Bay of eastern North America. As in most parts of its range, the extent of the species in the Bay has declined (Orth and Moore, 1983; Dennison et al., 1993). The species was always distributed patchily within suitable environments, and it is likely that the patches varied in how long they persisted in a given place (Lloyd et al., 2016). However, reductions have yielded fewer and likely smaller beds than occurred historically (Orth et al., 2010). These beds exhibit high turnover across years, which results in variation in isolation distances (Lloyd et al., 2016). We sought to understand how pollen dispersal might be affected by and

interact with these habitat changes by determining the scale of dispersal relative to the scale of patch size and isolation.

The leptokurtic nature of pollen dispersal curves (Austerlitz et al., 2004; Ellstrand, 2014; García and Borda-de-Água, 2017) dictates that even in continuous habitats the number and quality of pollen grains dispersing declines with increasing distance (Ghazoul, 2005), creating local genetic neighborhoods. In discontinuous (i.e., patchy) habitats, pollen can move only among patches that lie within distances that pollen regularly disperses. If increased isolation limits or precludes pollen movement, the likelihood of mating among close relatives and loss of genetic diversity within remaining patches will increase (Frankham, 1995, 1996), both of which are known to reduce plant fitness (Frankham, 2005). The distance at which isolation affects pollen flow depends on the scales at which a species perceives and interacts with the landscape (Levin, 1992; Holland et al., 2004; Crooks and Sanjayan, 2006; Taylor et al., 2006). This scale of interaction in turn depends on the mode of pollination of the species and its mating system.

Mode of pollination strongly affects the distances at which neighborhoods develop and at which patches in a landscape will become functionally fragmented for pollen movement (Breed et al., 2015). Mating system will determine the specific genetic effects of changes in gene flow. For example, self-compatible species will experience higher rates of selfing and biparental inbreeding, but they may have already purged their genetic load (Lande and Schemske, 1985; Winn et al., 2011). Outcrossing plant species can be more susceptible to inbreeding depression than are species that regularly self if biparental inbreeding increases (Charlesworth and Charlesworth, 1987; Barrett and Charlesworth, 1991). Dioecious and self-incompatible species also may experience Alleé effects if population size declines and isolation increases are accompanied by reduction in number of compatible mates (Elam et al., 2007). Experimental evidence indicates potential for such a lack in small *V. americana* populations (Engelhardt et al., 2014). Specifically, when the number of male and female genotypes were varied in experimental populations, microcosms with smaller numbers of genotypes had biased sex-ratios and higher proportions of single-sex populations (Engelhardt et al., 2014). Field observations of clones that extend hundreds of kilometers and sites dominated by single genotypes (Lloyd et al. 2011) also raise concerns of a potential lack of compatible mate diversity in wild patches.

For animal-mediated pollination, which dominates terrestrial landscapes, pollinator mobility determines the functional connectivity of a landscape (Breed et al., 2015). In insect-pollinated species, the number of pollinator visits, quality of pollen, and seed set are negatively affected at isolation distances of 100–1000 m (Jennersten, 1988; Steffan-Dewenter and Tscharntke, 1999; Wolf and Harrison, 2001). By contrast, bird-pollinated species often show no or dampened impacts of patch isolation because birds can fly between distant patches (Breed et al., 2015). In wind-pollinated tree species, the scale of pollen dispersal (250 m to >3 km) also often surpasses distances among isolated habitat patches, and thus, connectivity is maintained in discontinuous habitat (Ashley, 2010). In many such species, pollen contributes more to gene flow at longer distances than does seed dispersal.

Dispersal distances for pollen transferred to stigmas in water (hydrophilous pollination), just above the water surface (hydrophilous mimics), or on its surface (epihydrophilous pollination), as is characteristic of submersed aquatic vegetation (SAV) species (Cook, 1982; Du and Wang, 2014), are less well known. The few existing accounts indicate aquatic pollen is far more locally limited than wind-dispersed pollen (Les, 1988; Laushman, 1993; Kendrick et al., 2012), typically on the order of several to <100 m (Ruckelshaus, 1996; Zipperle et al., 2011; McMahon et al., 2014; Sinclair et al., 2014a; Van Tussenbroek et al., 2016). Such limited dispersal would be more sensitive to changes in patch distribution and isolation. Aquatic pollen movement is controlled by a number of abiotic factors including the size of the water-body in which an individual is located (Cox, 1988; Laushman, 1993; Waycott and Sampson, 1997), prevailing wind direction and water flow (Sinclair et al., 2014b), and tidal height at the time of pollen release (Ruckelshaus, 1996). Biological factors that are similar to those found in terrestrial species include flowering shoot density (Reusch, 2003; van Tussenbroek et al., 2010) and the extent of incompatible clones through which pollen must move to reach a compatible stigma (Ruckelshaus, 1995).

Quantifying pollen movement in aquatic environments is challenging, and the spatial scale of sampling can bias dispersal estimates to the short end of the dispersal curve. Methods based on assigning paternity to known individuals can miss the long tail of the distribution altogether due to practical limitations of the size of area in which all potential fathers can be identified. For example, pollen of the monoecious *Zostera noltii* Hornem dispersed a median distance of ≤3.22 m (Zipperle et al., 2011). However, sampling was done in a 100-m^2 plot, and paternity could only be assigned for 34% of seeds. Fathers of the remaining ~66% of seeds came from unknown distances, many of which could have been beyond the plot area. Mean pollen dispersal distance estimates of <30.9 m in the monoecious species *Posidonia australis* Hook. f. were based on paternity assignment of <3% of seedlings (Sinclair et al., 2014a). Although there was no way to determine the locations of unassigned fathers, dispersal distances from the few known fathers were twice the mean clone size and were smaller than the sampled areas (250 × 25 m and 300 × 120 m). In the dioecious *Thalassia testudinum* Banks ex. Konig, average pollen dispersed from 0.3 to 1.6 m, and genetic neighborhood sizes were restricted to a 1–17-m^2 area (Van Tussenbroek et al., 2016), but both were estimated from samples collected within a 4.8-m diameter around a focal release point.

We contribute to the emerging picture of aquatic pollen dispersal and mating system characteristics in that *V. americana* is a dioecious species and is considered to have water-facilitated contact pollination (hydrophilous mimic) (Cook, 1982; Les, 1988), an unsampled life history combination. Pollination in *V. americana* occurs when free-floating staminate flowers or pollen grains that are moved on the water surface by currents, winds, animals, and tides contact pistils of female flowers that are borne at the water surface (Cook, 1982; Korschgen and Green, 1988). Pollinated flowers ripen in late summer to early fall (Catling et al., 1994) into fruits that have an average of 150–200 seeds (Marsden et al., 2013). We sought to use the KinDist method (Robledo-Arnuncio et al., 2006) to understand correlated paternity of seedlings within these multi-seeded fruits and among mothers at three sites in different environments and to estimate the distance over which pollen is dispersed in *V. americana*. We chose KinDist because it is designed to estimate pollen neighborhoods and dispersal distances without complete sampling of potential fathers. This feature allows the possibility of capturing more of the long tail of the dispersal curve. We also sought to understand the number of sires contributing to seeds within fruits because pollination by different fathers can yield lower genetic relatedness among offspring relative to full siblings (Ritland,

1989). This relatedness is important to us because management agencies collect whole fruits for use in restoration plantings. Such plantings comprising large numbers of highly related seeds from few fathers could have reduced fitness and thus lower chances of long-term persistence. Even seeds sired by different fathers could, however, share common ancestry if genetic neighborhood size (e.g., effective number of pollen donors) is smaller than the size of genets (Les, 1988). In such cases, biparental inbreeding can occur through sibling mating. To determine this potential, we also estimated pollen neighborhood size and biparental inbreeding at each site.

Finally, we used knowledge of *V. americana* pollen dispersal, pollen neighborhood size, and biparental inbreeding to assess the potential that patterns of patch size and isolation previously documented (Lloyd et al., 2016) disrupt pollen flow. Assessing this potential is of critical importance in the Chesapeake Bay where SAV habitat loss and patch isolation are due to human-influenced eutrophication and increased sedimentation (Orth and Moore, 1983; Dennison et al., 1993). In any 1 year between 1984 and 2010, total potential *V. americana* coverage ranged from 7064 ha to 14,938 ha, patches averaged between 5.65 ha and 8.51 ha in size, and patch number ranged from 1079 to 2164 ha. High patch turnover between years yields variation in distances among patches and contributes to a range of distances required to connect all patches on the landscape (235.8–245.7 km; Lloyd et al., 2016). Understanding the nature of genetic connectivity within and among sites can inform conservation and restoration efforts. Pollen dispersal distances indicate the potential scales at which inbreeding and local adaptation can impact potential for population persistence (Weeks et al., 2011). In dioecious species pollen dispersal distance can indicate the isolation distances that will reduce or preclude reproduction if population reductions lead to biased sex ratios or increased matings among close relatives. This information can be used to establish objectives for patch distributions and to set priorities for restoration that will both decrease SAV patch isolation and increase overall area.

MATERIALS AND METHODS

Sampling and protocol

In October 2008 and 2009, we sampled plant material from each of three sites supporting *Vallisneria americana* within the Chesapeake Bay (Fig. 1). Sites were selected to represent different conditions that have potential to affect pollen movement including one open-water (Open Bay, OB) and two shoreline sites (Elk Neck, EN; Mariner Point, MP; Fig. 1). The open-water site was part of a large, relatively continuous *V. americana* bed known as Susquehanna Flats. The area extending 1 km around all the sampled mothers at that site was dominated by a single continuous patch totaling ~380 m^2 in 2008 and 785 m^2 in 2009. The mothers at EN were also within a single patch, but the area occupied within 1 km of the mothers during the sampling years was only 38–50 m^2. *Vallisneria* habitat within a 1-km

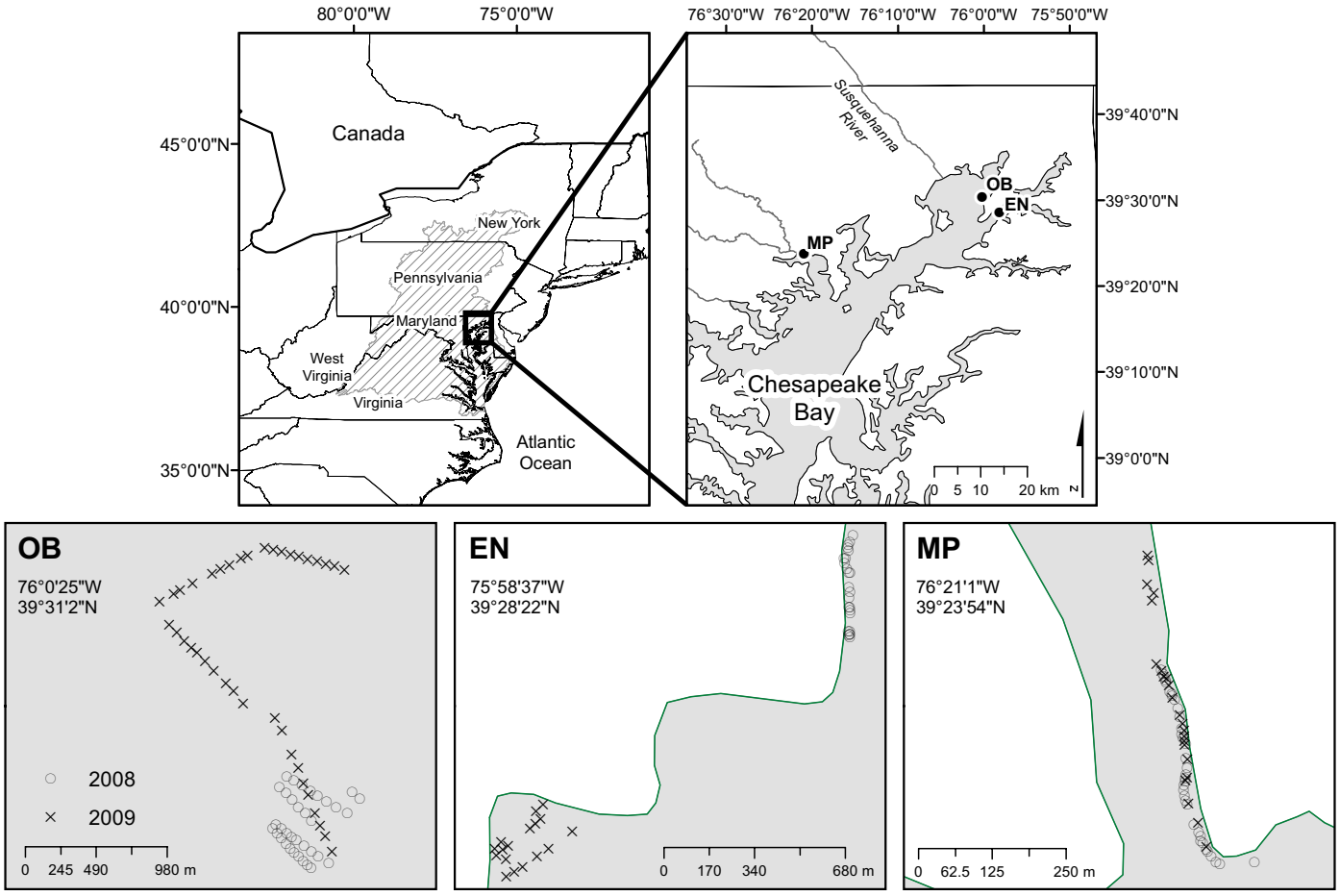

FIGURE 1. Overview of *Vallisneria americana* sites sampled in 2008 and 2009 and the distribution of ramets analyzed at each site. EN, Elk Neck; MP, Mariner Point; OB, Open Bay.

radius of the MP mothers was distributed in 26 patches with a total area of ~117 m^2 in 2008 and 112 m^2 in 2009. The largest patch in this area was ~79 m^2, and the mean patch size was ~4 m^2. Patches at MP were isolated from one another by an average of 98 m in 2008 and by an average of 67 m in 2009.

Each year we collected fruits and maternal tissue from 40 mothers within each site, which is twice the minimum sample size required by KinDist (Robledo-Arnuncio et al., 2006). All pairs of sampled mothers within sites and years were separated by distances ranging from 0.003 to 2.1 km in an attempt to encompass the critical distance over which pollen is no longer transferred (Table 1; Fig. 1). At OB (2008, 2009) and EN (2009), we sampled by boat, and distances among pairs of adjacent mothers (i.e., nearest neighbors) were longer (30–80 m in 2008 and 50–120 m in 2009 (Table 1) due to lack of maneuverability. Distances among mothers at MP were dictated by where fruits were found and by accessibility from the shoreline; distances among mothers were 3–361 m in 2008 and 3–500 m in 2009 (Table 1).

For each sample, we collected a single mature fruit and the subtending peduncle, and we recorded its latitude and longitude coordinates using global positioning system technology. Fruits were harvested into individually labeled Whirl-Pak bags (Nasco, Fort Atkinson, WI, USA); peduncles were placed in separate labeled bags. All plant material was kept on ice while it was transported to University of Maryland College Park after which it was stored at -80°C until extraction. Twenty seeds from each fruit were placed into individual wells in 96-well plates and stored at -80°C until extraction.

Extraction and genotyping

DNA was extracted from maternal tissue and 20 seeds per mother using a modified Chelex extraction protocol (Walsh et al., 1991). Before extraction, the seed coat was removed, and embryonic tissue was macerated with a sterilized flame-sealed glass pipette tip and then boiled for 15 min in 200 μL of 10% Chelex solution. Using methods described by Burnett et al. (2009), we genotyped five robust microsatellite loci (AAG_X012, M13, AAG_X051, M49, ATG002). PCR products tagged with fluorescence-labeled forward primers (Applied Biosystems, Carlsbad, CA, USA) were separated and measured on an ABI 3730xl DNA Analyzer with a GeneScan 500 LIZ Size Standard (Applied Biosystems). PCR products were separated, measured, and peaks analyzed using identical methods and quality control procedures as detailed by Lloyd et al. (2011). Quality control included repeated analyses to ensure high reproducibility of PCR reactions.

Fruits appeared ripe when sampled; however, upon processing many contained either immature or rotten seeds. We attempted to amplify all loci for all individuals; however, DNA from the immature and old seeds was not consistently amplified, and we were able to analyze fewer maternal samples analyzed than we collected (N = 18–39; Table 1). The number of seeds analyzed from each site ranged between 274–780, with 14–20 seeds coming from each mother (Table 1). In total, we sampled 3153 seeds from 175 mothers. There were 2.5% missing data in the final total data set. All missing data were in offspring; maternal genotypes were complete for all loci.

Measures of genetic diversity

We used the program GenClone v2.0 (Arnaud-Haond, 2007) to check for multilocus genotype matches among mothers to account for multiple samples of the same maternal clone. In such cases, we retained both samples as different ramets can sample different pollen pools. Number of alleles (A) as well as expected (H_e) and observed (H_o) heterozygosity were separately calculated for all mothers and offspring with the program GDA v1.1 (Lewis and Zaykin, 2001). Wright's F_{is} was calculated for mothers and offspring using the estimator f (Weir and Cockerham, 1984) in GDA to test for nonrandom mating as indicated by deviations from Hardy–Weinberg equilibrium. F_{is} was considered significantly different from 0 if that value was not included within confidence limits around each estimate generated by 1000 bootstraps in GDA. We tested for differences in genetic measures in all pairwise comparisons among mothers and offspring within and among sites across years using Kruskal–Wallis tests in R v2.12.1 (R Core Team, 2010); we accounted for multiple comparisons in tests using Tukey's multiple test correction.

Paternity analysis and seed relatedness

We used KinDist as implemented in the program PolDisp v1.0c (Robledo-Arnuncio et al., 2007) to calculate correlated within-sibship paternity for each site and year. We calculated the effective number of pollen donors contributing to a fruit (i.e., neighborhood size) as the inverse of the correlated paternity (Ritland, 1989). We anticipated that our five loci would be sufficient for the KinDist method because simulations with five loci with similar levels of

TABLE 1. Sample sizes for mothers and offspring in each population as well as summary of pairwise distances among mothers.

				No. offspring/mother			Pairwise distance between mothers (m)		
Site	Year	N_m	N_o	Min	Max	Mean	Min	Max	Mean
OB	2008	35	682	17	20	19.5	31.8	665.9	307.5
	2009	39	780	20	20	20.0	47.4	2150.4	936.9
	Total	74	1462				17.2	2239.7	1007.8
EN	2008	24	380	6	20	15.8	3.2	383.2	152.7
	2009	18	274	6	20	15.2	21.2	302.8	139.5
	Total	42	654				2.0	1823.7	853.9
MP	2008	36	619	9	20	17.2	3.16	361.37	135.0
	2009	23	418	8	20	18.2	3.0	499.9	164.7
	Total	59	1037				2.0	548.7	157.6
	Grand Total	175	3153						
Mean	2008			6	20	17.7	3.2	665.9	206.6
Mean	2009			6	20	18.4	3.0	2150.4	660.2

Note: N_m = number of mothers; N_o = number of offspring.

polymorphism to ours were shown to provide adequate power (Robledo-Arnuncio et al., 2006). To confirm the power of our loci, we used the program CERVUS v3.0 (Kalinowski et al., 2007) to assess the probability of excluding a candidate parent when the other parent is known using these five loci for each site in each year.

We calculated multilocus (t_m) and single-locus (t_s) estimates of outcrossing using the program MLTr v1.1 (Ritland, 1996) and used the difference between them to estimate biparental inbreeding (i.e., inbreeding due to mating among relatives). Using the program Coancestry v1.0 (Wang, 2011), we calculated the average within- and among-sibship pairwise relatedness with the Wang (2002) estimator and noted the proportion of within- and among-sibship seed pairs that that were consistent with half-sib or full-sib relationships. Relatedness calculations were based on offspring allele frequencies from the combined 2008 and 2009 samples. We also used Coancestry to quantify relatedness among mothers using allele frequencies from the maternal samples combined across sites and years. The Wang moment-based relatedness estimator was selected because in simulations based on both our maternal and offspring allele frequencies it had relatively low bias and high precision combined with high correlation with true relatedness of simulated genotypes in known relationship classes (Taylor, 2015). Simulated populations for relatedness testing consisted of 700 dyads with 100 dyads in each of the following seven relatedness categories: parent–offspring ($r_{xy} = 0.5$), full siblings ($r_{xy} = 0.5$), half siblings/avuncular/grandparent–grandchild ($r_{xy} = 0.25$), double first cousins ($r_{xy} = 0.25$), first cousins ($r_{xy} = 0.125$), second cousins ($r_{xy} = 0.03125$), and unrelated ($r_{xy} = 0$). Correlation between the Wang's estimator at the true r_{xy} in the simulations was 0.58 for allele frequencies of the mothers and 0.62 for allele frequencies and missing data of the offspring.

Average pollen dispersal distance and the pollen dispersal function

We sought to calculate the average pollen dispersal distance (δ) and variance of δ in each year at each site using KinDist. Distance δ is estimated using a probability density function based on the decline in a normalized measure of correlated paternity in offspring from maternal pairs with increasing geographic distance (Robledo-Arnuncio et al., 2007). The probability density function describes the probability of a pollen grain dispersing a given distance from a source plant, and δ is the first moment of the distribution. To determine whether our data met the requirement of a significant decline in correlation of normalized correlated paternity with geographic distance, we used a Mantel test as implemented by the mantel function in the R package ecodist (Goslee and Urban, 2007). Significance was based on 10,000 permutations. Only the MP site (both years) had the suggested (Robledo-Arnuncio et al., 2007) negative correlation coefficient of greater magnitude than -0.1. We also calculated the relationship between correlated paternity and geographic distance using all individuals at each site by combining years to determine whether increasing the range of interplant distances and increasing sample sizes improved the correlation. Site MP and EN had the required decline in correlation of correlated paternity with distance, but site OB did not.

For the two single-year MP samples and the combined MP and EN samples that met the requirements of the KinDist method, we explored the behavior of the dispersal parameter using a normal, exponential, exponential-power, geometric, and two-dimensional Student's *t*-distributions. The KinDist method requires the selection of a threshold distance that defines unrelated pollen pools. The estimated value of δ is not sensitive to the threshold value selected (Robledo-Arnuncio et al., 2006); however, we varied the threshold distance to explore its effect on estimates of δ and found no effect. For individual years, we used a threshold distance of 100 m. For the MP and EN samples from combined years, we used a threshold distance of 300 m based on visual inspection of the graphs of correlated paternity. As with the MP data sets for individual years, a range of threshold distances had almost no effect on δ.

To compare the potential for pollen dispersal among patches, we used the full extent of potential *V. americana* habitat within the Bay that had been occupied from 1984 to 2010 (see methods of Lloyd et al. [2016]). We consider this composite coverage the best-case scenario for the distribution and abundance of *V. americana* and a reasonable basis for assessing maximum reasonable potential connectivity. We calculated median distance among all patches on the landscape, median nearest-neighbor distances for each patch and average and median patch size. For potential pollen dispersal distances of 30 m (the lower limit of the landscape data based on 30 m cell size) to 2000 m, we calculated the number of patch clusters (i.e., components), average number of patches per cluster, and total number of connections (i.e., edges), and average area of clusters using the program LandGraphs (Urban, 2003). Using probability density functions defined by Austerlitz et al. (2004) for the normal and exponential distributions, we calculated the probability of pollen dispersal at this 30-m distance for each case for which δ was estimated.

RESULTS

Genetic diversity

Two maternal genotypes were found in both 2008 and 2009 at MP. The first shared genotype was sampled five times, three in 2008 and two in 2009, and the second was sampled once in each year. One additional maternal genotype was shared by two ramets in the 2009 MP sample. The five ramets found in 2008 and 2009 were separated by 3 to 21 m, and ramets of the second genotype shared across years was separated by 8 m. The final two identical ramets sampled in only 2009 were separated by 130 m. All other genotypes at all sites across both years were unique. It is possible that with additional loci, all genotypes would have been unique.

Across all three sites combined, we detected 52 alleles in 2008 and 53 alleles in 2009 with an average of 10.4 and 10.6 alleles per locus (A) in 2008 and 2009, respectively. Within sites and years, A ranged from 4.6 to 7.6 in mothers and 7.6 to 9.2 in offspring (Table 2). Two sites had lower observed heterozygosity in offspring than expected (2008 MP, 2009 EN) and two sites had greater observed heterozygosity in mothers than expected (2009 EN, 2009 MP; Table 2).

Paternity analysis and seed relatedness

The combined probability of excluding an unknown father using the genotype data as calculated in CERVUS (Jamieson and Taylor, 1997) was >0.99 for both 2008 and 2009; thus, the genotypic data provided sufficient resolution for capturing the spatial structure of pollen donors.

Correlated paternity in individual mothers in all samples ranged from –0.09 to 0.97 (Table 3). The mean number of effective pollen

TABLE 2. Measures of genetic diversity in sites of *Vallisneria americana* sampled from the Chesapeake Bay in 2008 and 2009 for both mothers and offspring.

Site	Year	N_m	N_o	Maternal *A*	Offspring *A*	Maternal H_o	Offspring H_o	Maternal H_e	Offspring H_e	Maternal *f*	Offspring *f*
OB	2008	35	682	6.4 (2.3)	8.8 (3.1)	0.77 (0.07)	0.72 (0.06)	0.74 (0.08)	0.74 (0.07)	−0.04 (0.08)	0.03 (0.04)
	2009	39	780	7.6 (2.5)	9.2 (3.4)	0.76 (0.08)	0.73 (0.05)	0.74 (0.08)	0.74 (0.07)	−0.02 (0.05)	0.01 (0.03)
EN	2008	24	380	6.0 (1.2)	7.6 (1.8)	0.68 (0.16)	0.71 (0.05)	0.68 (0.10)	0.71 (0.07)	0.00 (0.11)	0.00 (0.02)
	2009	18	274	4.6 (1.9)	7.6 (2.8)	0.70 (0.14)	0.63 (0.06)	0.62 (0.07)	0.67 (0.07)	**−0.14** (0.12)	**0.07** (0.05)
MP	2008	36	619	6.0 (1.7)	7.6 (2.5)	0.68 (0.17)	0.71 (0.08)	0.68 (0.09)	0.74 (0.08)	0.03 (0.14)	**0.03** (0.03)
	2009	23	418	5.4 (1.5)	8.8 (2.8)	0.70 (0.09)	0.71 (0.05)	0.62 (0.08)	0.74 (0.07)	**−0.13** (0.11)	0.04 (0.07)
Mean	2008			6.1 (0.2)	8.0 (0.7)	0.71 (0.05)	0.72 (0.00)	0.70 (0.03)	0.73 (0.02)	0.00 (0.04)	0.02 (0.02)
Mean	2009			5.9 (1.6)	8.5 (0.8)	0.72 (0.03)	0.69 (0.06)	0.66 (0.07)	0.72 (0.04)	−0.10 (0.06)	0.04 (0.03)

Notes: N_m = number of mothers analyzed; N_o = number of offspring analyzed; *A* = mean number of alleles per locus; H_o = observed heterozygosity; H_e = expected heterozygosity; *f* = inbreeding coefficient. Bold = significant difference from 0 at $P < 0.05$. Standard deviations are in parentheses.

TABLE 3. Measures of shared paternity and relatedness in sites of *Vallisneria americana* sampled from the Chesapeake Bay in 2008 and 2009.

Site	Year	Mean correlated paternity	Mean pollen neighborhood size	Mean *r* of offspring within mothers	Mean *r* of offspring among mothers
OB	2008	0.14 (0.09)	12.56 (12.47)	0.31 (0.08)	0.04 (0.26)
	2009	0.23 (0.18)	11.06 (16.40)	0.33 (0.11)	0.03 (0.25)
EN	2008	0.32 (0.17)	4.75 (5.00)	0.39 (0.13)	0.09 (0.26)
	2009	0.22 (0.21)	11.81 (26.24)	0.34 (0.10)	0.14 (0.34)
MP	2008	0.37 (0.22)	3.92 (2.76)	0.40 (0.10)	0.05 (0.26)
	2009	0.47 (0.18)	2.51 (1.13)	0.41 (0.10)	0.02 (0.26)

Notes: Means of correlated paternity and neighborhood size (1/correlated paternity) as calculated for each mother using the computer program PolDisp. The Wang (2002) relatedness estimator (*r*) was calculated using the computer program Coancestry and was used to determine proportion of within- and among-maternal offspring. Standard deviations are in parentheses.

donors per fruit for a mother (i.e., neighborhood size) ranged from 2.51 at MP in 2009 to 12.56 at OB in 2008. The largest estimated effective neighborhood size was 113 fathers for one mother in the 2009 EN sample, and the next largest was 91 in the 2009 OB sample. All other samples had effective neighborhood sizes less than 52, 83% of mothers had less than 10 effective pollen donors per fruit, and 16% of those had between 1 and 2 effective pollen donors. One mother at EN in 2009 had a negative within-sibship correlated paternity (–0.09) indicating that the paternal genotypes were less related than the average of the sample (Robledo-Arnuncio et al., 2006; Wang, 2014).

The average within-sibship Wang's relatedness was 0.37 (SD = 0.49) for 2008 and 0.36 (SD = 0.49) for 2009 (Table 3). The multilocus outcross rate (t_m) was not significantly different from one in all cases, which indicates plants are outcrossed, as expected for a dioecious species. Biparental inbreeding, as indicated by the difference between t_m and t_s, was significantly different from 0 in all but the EN 2008 sample. In EN 2009 and both MP samples biparental inbreeding ($t_m - t_s = 0.138 - 0.171$) was approximately twice the levels detected in the other samples ($t_m - t_s = 0.050 - 0.069$; Table 4).

Average pollen dispersal distance and the pollen dispersal function

Because the data for most sites did not meet the underlying assumption of the KinDist approach that the magnitude of correlated paternity among mothers declines with increased geographic distance, we were limited in our ability to estimate pollen dispersal functions. We found a significant decline in correlated paternity with increasing geographic distance within individual years (Fig. 2) only in the MP site (Table 5). When we combined years for each site (Fig. 3), the negative correlation was significant for EN and MP, but not for OB (Table 5).

Of the five probability density functions for pollen dispersal, only the one-parameter normal and exponential distributions were informative (Table 5). Estimates of average pollen dispersal distance (δ) based on the normal distribution were 3.47 m for the 2008 MP site, and 0.80 m for the 2009 MP site (Table 6). Estimates of δ based on the exponential distribution were 4.34 m for 2008 MP and 0.89 m for 2009 (Table 6). When samples were combined across years, estimates of δ based on the normal distribution were 2.02 m for the EN site and 1.38 for MP and based on the exponential distribution were EN = 2.70 m and MP = 1.80 m. The two-parameter functions (exponential-power, geometric, and 2-dimensional Student's *t*) provided parameters that estimated δ to include infinity, indicating a poor model fit and were therefore not used.

TABLE 4. Single-locus (t_s) and multilocus (t_m) outcrossing rates within sites calculated using the program MLTr (Ritland, 1996). Values of t_m that are < 1 for this dioecious species indicate effective selfing due to inbreeding. The difference between t_m and t_s indicates the rate of biparental inbreeding.

Site	Year	N_m	N_o	Mean t_s	Mean t_m	Mean $t_m - t_s$
OB	2008	35	682	**0.928 (0.019)**	0.997 (0.015)	**0.069 (0.022)**
	2009	39	780	**0.937 (0.024)**	0.990 (0.015)	**0.054 (0.023)**
EN	2008	24	380	**0.919 (0.038)**	0.969 (0.017)	0.050 (0.030)
	2009	18	274	**0.824 (0.044)**	0.962 (0.018)	**0.138 (0.035)**
MP	2008	36	619	**0.849 (0.030)**	0.966 (0.013)	**0.117 (0.021)**
	2009	23	418	**0.805 (0.044)**	0.977 (0.020)	**0.171 (0.037)**

Notes: Bold indicates significant difference from 1 (t_s, t_m) and 0 ($t_m - t_s$) at $P < 0.05$. Standard errors from 1000 bootstrap replicates in parentheses.

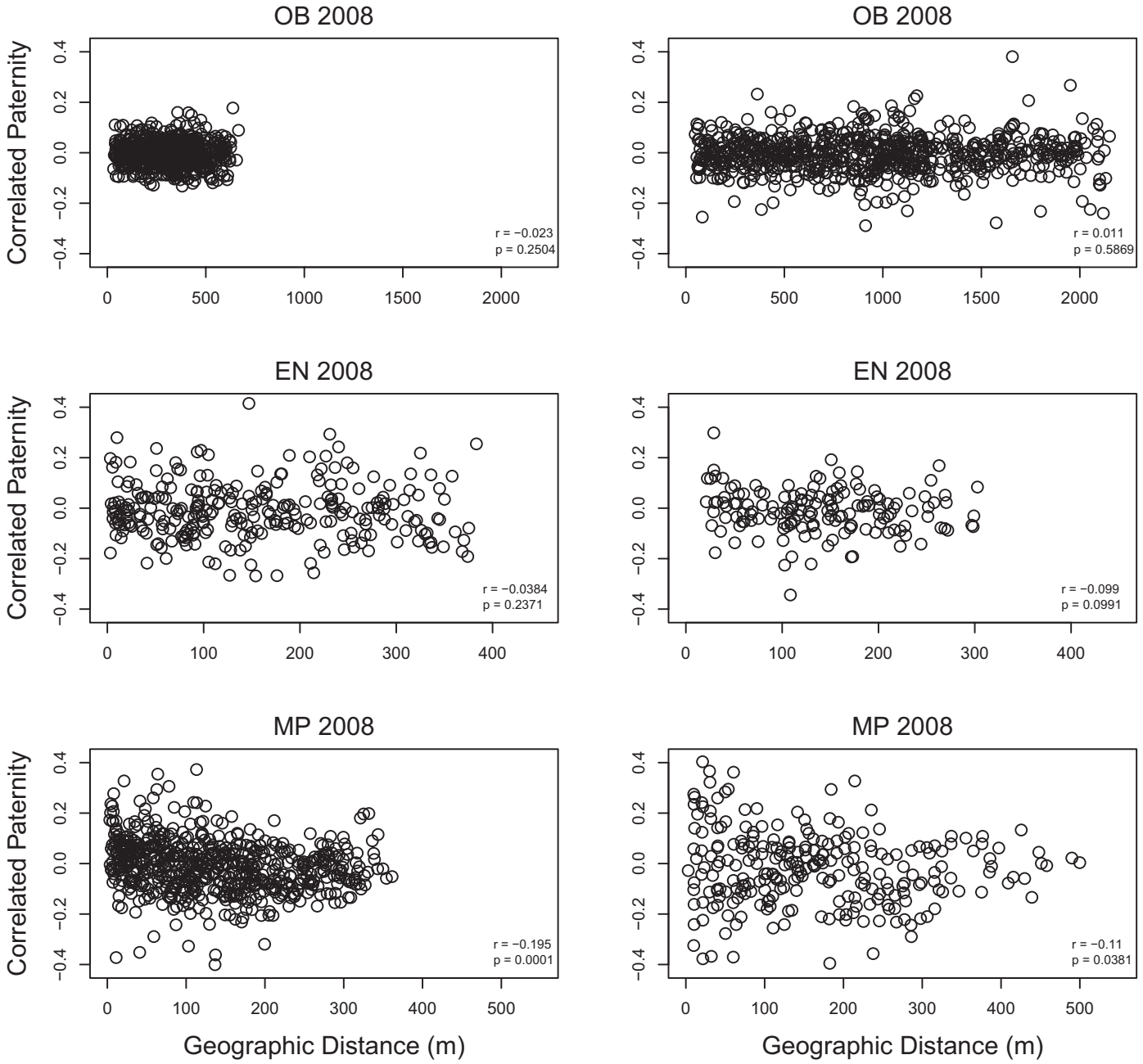

FIGURE 2. Correlated paternity among maternal pairs against geographic distance (m) for all sites and years. Correlated paternity is expected to decline with increasing geographic distance among maternal pairs. The *x*-axes are set for each site.

Pollen dispersal distances indicated were far below median distance among patches of *V. americana* in the Chesapeake Bay (261 km). The median distance among all patches exceeded the distance required to connect all patches into a single component (210.4 km; Lloyd et al., 2016). Pollen dispersal distances were also below the median nearest-neighbor distance among patches (median distance = 60 m, average distance = 773 m). Even within-patch connectivity was limited in that pollen dispersal distances are short enough that each mother would sample from neighborhoods far smaller than the size of many individual patches (average patch size = 11.4 ha, median patch size 0.27 ha). In assessing the potential connectedness of the landscape, we were limited to a minimum potential distance of 30 m due to the grain size of the SAV coverage spatial data. At the potential dispersal distance of 30 m, 1104 components formed with an average of 1.9 patches per component (SD = 2.38), and 2313 connections among patches were made across the entire landscape (Table 7). The probability for pollen dispersing 30 m ranged from >1E-324 to 3.35E-08 depending on site and probability function (Table 6).

DISCUSSION

Observed pollen neighborhood sizes of 2.5–12.6 individuals and average pollen dispersal distances of <4.5 m indicate that *Vallisneria*

americana pollen dispersal is extremely restricted. Limited pollen dispersal was further indicated by high correlated paternity within mothers but not among mothers (Table 3) and biparental inbreeding (Table 4). Still, we were unable to estimate dispersal distances for half our sites and the variances in the estimates we could make were high (Table 6). Despite this uncertainty in exact pollen dispersal distances, we are confident pollen does not confer connectivity among most discrete patches of *V. americana* in the Chesapeake Bay. Even within patches, mothers most likely sample from such localized neighborhoods that pollen connectivity could emerge only in a stepping-stone fashion through small, overlapping pollen pools. Lack of correlated paternity at distances of 20–30 m indicate low potential for extensive stepping-stone connectivity in large patches. Next, we discuss the implications of our results, explore potential reasons why the dispersal estimates might have been so challenging and provide recommendations for restoration and conservation.

The mean pollen dispersal distances we observed in *Vallisneria americana* (0.8–3.9 m) similar to other submersed aquatic species: 0.3–4.8 m for the dioecious *Thalassia testudinum* Banks & Sol. ex Koenig (Hydrocharitaceae; Van Tussenbroek et al., 2016); 1.7–3.8 m in the monoecious *Zostera noltii* Hornem. (Zosteraceae; Zipperle et al., 2011), and 1.1 m in the monoecious *Zostera marina* L. (Zosteraceae; Ruckelshaus, 1996). Waterborne dispersal distances are well below distances observed in wind-pollinated trees (0.015–7.6 km) and animal-pollinated trees (0.021–88.6 km), as well as shrubs and herbaceous plants (0.113–5 km; see studies of Ashley, 2010). The random, nondirectional release of pollen into the environment during waterborne and windborne pollination yields predictions of lower fertilization efficiency than in animal-mediated pollination (Faegri and Van Der Pijl, 1979; Cox, 1983; Barrett et al., 1993). Successful pollination by water-borne and wind-borne pollen is dependent on sufficient numbers of pollen grains passively coming into proximity of female flowers through abiotic forces. Species with aquatic pollination syndromes have fluid dynamic solutions to facilitate pollination that are not needed in wind-pollinated species, which can limit pollen dispersal distances (Ackerman, 2000; McMahon et al., 2014). For example, the production of sticky pollen can cause pollen to adhere to any encountered surface, restricting pollen dispersal of submersed aquatics relative to that of wind-dispersed species (Kendrick et al., 2012). Although this limited dispersal means pollen does not regularly move over appreciable distances and is not expected to play a major role in long-distance gene flow (Kendrick et al., 2016), many SAV populations are composed of sexually produced offspring (e.g., Lloyd, 2011), and outcrossing can dominate in monoecious species (Ruckelshaus, 1996; Zipperle et al., 2011). Thus, pollen facilitates sexual reproduction and is an important aspect of submersed aquatic plant biology in general (Cox, 1988; Laushman, 1993).

Our dispersal estimates were limited by the lack of decline in correlated paternity with distance, which has several possible explanations. The first possibility is that our markers did not provide enough resolution to yield accurate estimates of paternal pollen

TABLE 5. Results for the Mantel test run with the mantel function in the R library ecodist.

Site	Mantel's *r*	*P* value	Lower 2.5%	Upper 97.5%
OB 2008	−0.023	0.2504	−0.064	0.015
OB 2009	0.011	0.5869	−0.022	0.043
OB Combined	0.002	0.5039	−0.016	0.018
EN 2008	−0.0384	0.2371	−0.101	0.01
EN 2009	−0.099	0.0991	−0.177	−0.0015
EN Combined	−0.203	0.0001	−0.261	−0.152
MP 2008	−0.195	0.0001	−0.258	−0.156
MP 2009	−0.11	0.0381	−0.196	−0.048
MP Combined	−0.15	0.0001	−0.18	−0.121

Notes: The *P*-value is the significance of a one-tailed test for the null hypothesis that the observed correlation between correlated paternity and geographic distance is not significantly less than zero based on a randomization test with 10,000 permutations.

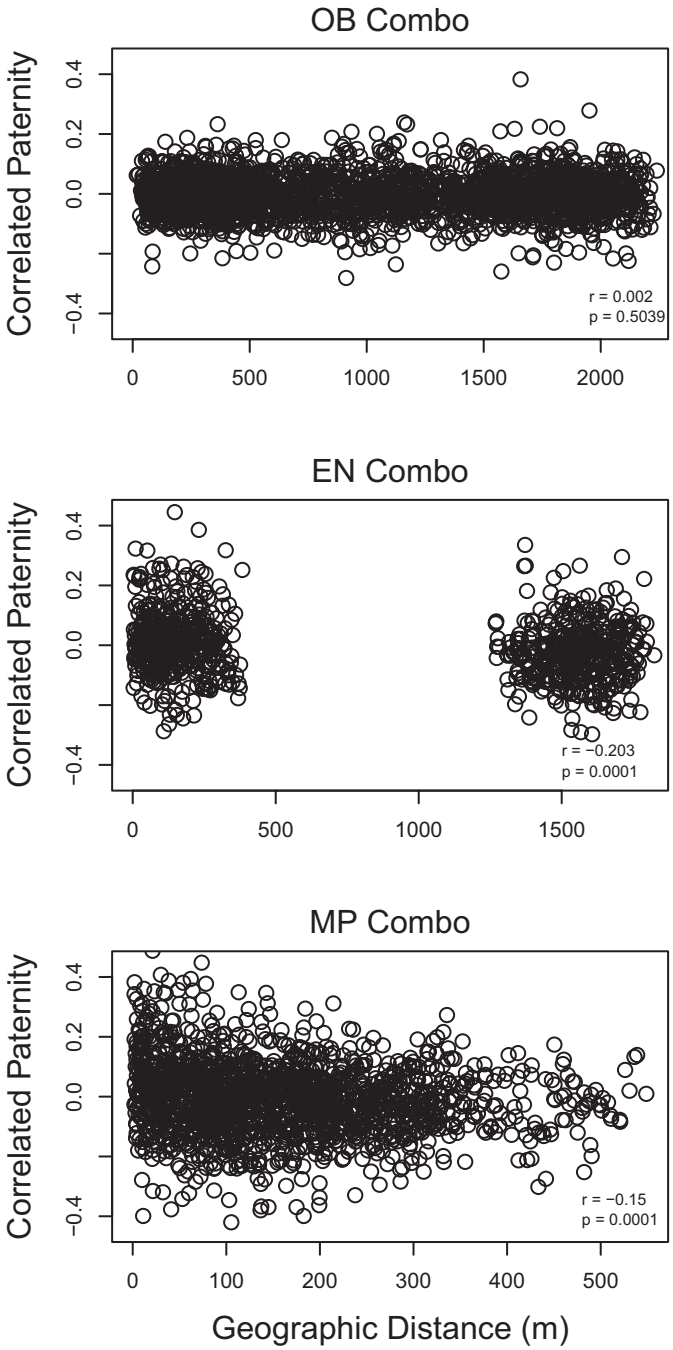

FIGURE 3. Correlated paternity among maternal pairs against geographic distance (m) for all sites and combined years. Correlated paternity is expected to decline with increasing geographic distance among maternal pairs. The *x*-axes differ by site.

TABLE 6. Best fit models for sites and years meeting the requirement of a significant decline in correlation of normalized correlated paternity with geographic distance, as calculated in PolDisp. Kurtosis for the normal distribution is 2 and for the exponential is 3.33. Probability of pollen dispersing 30 m based on the relevant probability density function is also presented.

	MP			EN
	2008	2009	Combined	Combined
Normal				
a	3.91	0.91	1.56	2.28
δ	3.47	0.80	1.38	2.02
σ^2	2.77	0.64	1.10	1.61
Residual	42.17	21.11	134.20	91.15
Prob. dispersing 30 m	5.65E-28	<1E-324	3.20E-162	3.96E-77
Exponential				
a	2.17	0.45	0.90	1.44
δ	4.34	0.89	1.80	2.70
σ^2	3.76	0.77	1.56	2.49
Residual	41.82	20.76	134.08	91.03
Prob. dispersing 30 m	3.35E-08	8.76E-30	6.56E-16	6.88E-11

Notes: a = estimated model scale parameter (see Austerlitz et al., 2004; for model details); δ = average pollen dispersal distance; σ^2 = variance in δ; residual = least-square residual.

TABLE 7. Landscape statistics for potential pollen dispersal distances ranging from 30 m to 2000 m.

Potential dispersal distance (m)	No. of patch clusters	Mean no. of patches per cluster	SD of patches per cluster	No. of links among all patches	Mean area of connected clusters (ha)
30	1104	1.9	2.4	2313.0	14.18
100	560	3.8	6.7	5943.0	23.21
200	307	7.0	14.6	11,713.0	29.11
300	210	10.2	21.5	18,045.0	26.83
400	160	13.4	25.2	23,816.0	23.24
500	127	16.8	32.0	29,406.0	27.21
600	106	20.2	35.7	34,973.0	30.19
700	94	22.8	40.9	40,289.0	33.71
800	82	26.1	49.8	45,684.0	33.32
900	72	29.7	61.7	51,265.0	15.80
1000	66	32.4	64.6	56,662.0	17.17
1500	51	41.9	72.9	78,119.0	6.01
2000	41	52.2	80.8	97,935.0	6.09

structure. In power analyses by Austerlitz and Smouse (2002) and Robledo-Arnuncio et al. (2006), five loci with 5–10 alleles per locus were sufficient to provide high exclusion probability, low bias, and mean square error for δ estimates. The five loci we used averaged 4.6 and 9.2 alleles per locus depending on site, and only one site (EN in 2009) was at the low end of the allelic diversity that is deemed necessary. However, our markers were highly heterozygous and had probabilities of exclusion (0.99987 for 2008 and 0.99985 in 2009) that were above even the highest recommended values (Austerlitz and Smouse, 2002) and thus appear sufficient.

A second potential issue is extensive clonal growth, which violates the KinDist assumption that fathers are restricted to a single point in space. Extensive male clones could preclude development of spatial genetic structure of pollen pools if distant mothers were all in close proximity to the same paternal genotype. Detailed sampling and mapping of the extent of male clones would be useful to understand the degree to which extensive male clones affected our pollen dispersal estimates. However, if distant females were mating with the same extensive male, correlated paternity among mothers would be high and number of effective pollen donors would be low. In general, we found neither of these conditions in our data. In all cases, among-mother correlated paternity was far lower than that found within mothers, with only EN having moderate correlation among mothers (Table 3).

A final factor that can influence estimates of correlated paternity is sampling distance among mothers. Intersample distances that are too short or too long relative to dispersal can both result in a lack of decline in correlation of paternity with distance. If dispersal distances exceed the longest intersample distances, the overall genetic structure of the pollen pool would be low because mothers would be sampling the same mixed pollen pool. In such a case correlated paternity would be uniformly high, and pollen dispersal distances would be underestimated (Robledo-Arnuncio et al., 2006, 2007). Given what is known about aquatic pollen dispersal in general and the magnitude of the estimates we could make, it is extremely unlikely that the same pollen pool extends across the ~500–2100 m distance ranges sampled. At the other end of the spectrum, if intersample distances exceed pollen flow distances all mothers would be pollinated from pollen pools that were equally distinct. In such a scenario, the number of fathers contributing to each fruit could be high, and among-mother correlated paternity would be uniformly low, which is precisely what we observed.

Given the estimates of relatively small neighborhood size and biparental inbreeding, combined with the estimates of pollen dispersal that we were able to make, we argue that the lack of correlation primarily resulted from sampling mothers that were too far apart at the OB site and at EN in 2009 (nearest mothers were

21–30 m apart). Although we could not estimate precise dispersal distances, we found that paternity is already uncorrelated, and the probability of dispersing these distances is infinitesimal (Table 6). Given that the correlation of paternity among mothers reaches 0 at 5δ (Austerlitz and Smouse, 2002), we can infer that δ at OB and EN in 2009 is no more than 4–6 m and thus is similar to the sites at which we could estimate pollen dispersal. The lack of correlation when the shortest distances between samples were ~21–30 m makes sense in retrospect, but we were surprised at the relatively weak decline in correlated paternity that we found, even when intersample distances spanned 3–500 m (Tables 1, 5). We suspect that although the sample sizes were acceptable, too many pairs of mothers were too far apart to be correlated, so there was no decline in correlated paternity across much of the distance range. Sampling more mothers with intersample distances <30 m would likely be fruitful.

The extremely short pollen dispersal distances are at odds with genetic structure in the Chesapeake Bay documented in a population genetic survey of *V. americana* (Lloyd et al., 2011). This bay-wide survey indicated the presence of three genetic regions within which there is genetic connectivity among discrete patches (Lloyd et al., 2011). Low genetic differentiation among sites within these regions (D_{est_Chao} = 0.01–0.02) compared to sites from different regions (D_{est_Chao} = 0.124) (Lloyd et al., 2011) implies sufficient dispersal within each region to preclude differentiation among patches. Although it is possible that rare pollen dispersal events among adjacent patches connect networks of patches in a stepping stone fashion (e.g., Van Rossum and Triest, 2012), the probability of such events (Table 6) is too low to account for the degree of genetic connectivity observed among patches within genetic regions. We suggest that this large-scale distribution and structure of genetic diversity are largely shaped by seed and vegetative propagule dispersal as for other submersed aquatic plant species (Harwell and Orth, 2002; Reusch, 2002; Les et al., 2003; Källström et al., 2008).

However, much remains to be understood about the potential for connectivity through seed dispersal. The paradigm in SAV ecology is that most seed dispersal occurs over short distances as seeds drop to the sediment from fruits that remain attached to the mother plant (Orth et al., 1994). Less-frequent longer-distance dispersal is thought to be accomplished by seed rafting on maternal plants (Korschgen and Green, 1988); floating fruits; and waterfowl, either through ingestion or seeds clinging to feathers (Santamaria and Klaassen, 2002; Figuerola et al., 2003; Higgins et al., 2003). Molecular studies are confirming that dispersal distances cover a huge range, and yet a great deal of seed dispersal is highly localized. Within-patch assessments of spatial autocorrelation indicate higher than expected relatedness at distances of 40 m for sexual reproduction and 70 m for vegetative (Migliaccio et al., 2005) in *Posidonia oceanica* (L.) Delile. Highly localized seed movement was found for the five species in which assignment tests have been used to assess origin of individuals: between 60.8% and 99% of individuals originated at the sampling location (Diekmann et al., 2005; Serra et al., 2010; Huotari et al., 2011; Nakajima et al., 2014; Oliva et al., 2014; Jahnke et al., 2015). However, sampling-scale matters, and in one study that was conducted at extremely small scale (e.g., 10 × 10 m; Zipperle et al., 2011), only 26.8–32.5% of individuals originated within the sampled area (Zipperle et al., 2011). In other studies, dispersal distances documented through assignment tests indicate movement can be on the order of kilometers to hundreds of kilometers, which would be sufficient to connect many local patch networks and beyond (Ruggiero et al., 2002; Travis and Sheridan, 2006; Tomasello et al., 2009; Zipperle et al., 2009; Serra et al., 2010; Bricker et al., 2011; Huotari et al., 2011; Nakajima et al., 2014; Oliva et al., 2014; Triest and Fenart, 2014; Jahnke et al., 2015).

Better understanding of the degree of connectivity facilitated by dispersal of seed and vegetative propagules is essential to understand the potential for maintaining gene flow and for recolonizing lost patches. Without such dispersal, allelic diversity will erode, and inbreeding will increase. Prolonged isolation of a site from seed and propagule dispersal can result in genetic differentiation (Wilkins and Wakeley, 2002), and the genetic diversity of isolated sites will be limited to what is present at the time of isolation. Certainly patches of *V. americana* were isolated for extended periods through the decades when the species was at its lowest abundance and extent (Orth and Moore, 1983; Lloyd et al., 2016). Unfortunately, we do not know what levels of diversity were present in *V. americana* before the dramatic population reductions in the last century, so we do not know how much has already eroded. Numbers of alleles per locus at the species and population levels in *V. americana* (Lloyd et al., 2011) were moderate to low compared to other SAV species in general and *Zostera marina* in the Chesapeake Bay (Neel and Engelhardt, in press). Genotypic diversity within populations based on shoots that were collected 5–10 m apart varied from 0 (1 population) to 1 (2 populations) with a median genotypic richness of 0.68. Genotypic richness was less than 0.5 for 10 of 26 sampled sites. Maintaining high-localized genetic and genotypic diversity enhances the resilience of populations to stochastic environmental effects and periodic isolation events. For example, early clonal expansion and flowering frequency of *V. americana* were greater when genotypic richness was increased (Engelhardt et al., 2014).

Clearly, pollen is not contributing to connectivity among patches of *V. americana*. Even within continuous populations, extremely localized pollen dispersal creates unequal contributions of fathers and can generate genetic neighborhoods (Wright, 1946; Koenig and Ashley, 2003; Pluess et al., 2009; Albaladejo et al., 2012). Although we discuss the potential concerns below, such neighborhoods are not automatically critical conservation issues. Rather, they can become a concern when populations become smaller or more isolated from one another so they are no longer connected to other populations by seed or propagule dispersal. Small neighborhoods can also result from increased localized pollination over consecutive generations (Turner et al., 1982; Albaladejo et al., 2012). The clonal life history of *V. americana* can exacerbate inbreeding if widespread related genets are within the short pollination distance (Van Tussenbroek et al., 2016). With such neighborhoods, substantial genetic differentiation can build up across a landscape as a function of isolation by distance (Wright, 1943), even within continuously distributed populations (Rohlf and Schnell, 1971; Neel et al. 2013). The local genetic neighborhoods can have low genetic diversity relative to the total population (Maruyama, 1972) and will yield lower effective population size estimates (N_e) than would be expected based on the census size of the whole population (Neel et al., 2013). This depressed N_e is of concern because populations will lose genetic variation at a much higher rate than the census size would indicate (Neel et al. 2013), leaving even extremely large populations at unanticipated risk of becoming genetically compromised. Depressed N_e estimates are generated by a Wahlund effect that arises when genetically divergent individuals are included in a single sample (discussed by Neel et al. [2013]). Because *V. americana* is dioecious, there is no possibility of selfing, so elevated inbreeding observed among seedlings would be due to mating among close relatives (Schierup, 1998;

Degen et al., 2004). Such inbreeding is known to reduce plant fitness in general (Frankham, 2005). In *V. americana*, low observed heterozygosity has been correlated with smaller leaves and turions in experimental settings (Engelhardt et al., 2014); however, population source and substrate also affected the performance of plants. In addition to the small departures from Hardy–Weinberg equilibrium in the offspring, we saw significant and sometimes substantial (e.g., MP 2008, 2009) evidence of biparental inbreeding (significant deviation from zero in $T_m - T_s$) in all but one site (EN 2008; Table 4). The effects of biparental inbreeding are not as severe as selfing, but it can reduce population fitness by lowering seed germination success (Richards, 2000), maternal fecundity (Ashman, 1992; Nason and Ellstrand, 1995), chance of progeny survival (Heywood, 1993), or progeny size (Waser and Price, 1994). The contrasting heterozygote excess observed in the mothers (Table 2) indicates potential selection against homozygotes before germination or as seedlings. Postzygotic mechanisms such as seed abortion following inbreeding are known to exist in other submersed aquatic species, limiting the effects of inbreeding depression at later life stages (Ruckelshaus, 1995; Balestri and Cinelli, 2003; Billingham et al., 2007; Sinclair et al., 2016).

Because these neighborhoods would have been present in *V. americana* populations even absent of anthropogenic effects, we do not consider them to be of great concern as long as populations remain large. The primary risk is decline in pollen flow that could result if population densities are reduced by extreme habitat loss and fragmentation, yielding small patches in which individuals are all more closely related than historical norms or are only one sex. This extreme risk of mate limitation due to dominance of one or a few genotypes has been noted in the Potomac (Lloyd et al., 2011). By contrast, sites within the Chesapeake Bay remain sufficiently diverse to be above levels that would cause concern.

SUMMARY AND MANAGEMENT AND RESTORATION IMPLICATIONS

The understanding of genetic connectivity we have gained from the dynamics of pollen dispersal, pollen neighborhood size, and mating patterns contribute to an emerging picture of genetic diversity in *Vallisneria americana* that informs conservation and management. From past work (Lloyd et al. 2011), we know that most natural sites in the Bay have moderate levels of genotypic and allelic diversity but are not significantly inbred. The presence of three primary genetic regions within the Bay indicates either sufficient isolation among regions for differentiation through drift or adaptation to different environments among the regions. At the same time, connectivity within each region facilitates sufficient gene flow to prevent differentiation of populations. From the data presented here, we know that pollen dispersal contributes little to maintaining this connectivity. Rather, its limited dispersal distance has the potential to establish local genetic neighborhoods. Genetic goals under such circumstances include maintaining genotypic and allelic diversity and levels of heterozygosity and maintaining the structure of genetic diversity present in different environments to retain the potential for local adaptation. These goals can mostly be met by managing to maintain or enhance occupied area and interpatch distances to reflect long-term potential for migration, colonization, and recolonization from extant patches. Managing specifically for pollen connectivity is not a priority. Frequent pollen dispersal would not be expected among patches, even in an idealized best-case scenario in which all patches of *V. americana* that have ever been mapped are concurrently occupied (as modeled by Lloyd et al. 2016). That is not to say that connectivity is not important, rather that managing for connectivity via seed and vegetative propagule dispersal is sufficient for maintaining the natural scale of pollen dispersal and the resulting local genetic neighborhoods. Populations can be enhanced passively by improving water quality (Orth et al., 2015) or through active restoration.

Active restoration has the potential to interact with natural pollen flow. The primary methods used in the Chesapeake Bay rely on collecting *V. americana* fruits from one area and broadcast seeding to other areas. Fruits have also been collected to provide seed for "Grasses in the Classes" programs in which school children grow plants and plant them in the Bay at the end of the school year. Typically, thousands of fruits are collected, and fruits have on average 137.7 seeds (Marsden et al., 2013). Thus, propagule numbers are far above levels required to be genetically secure. Assessment of eight restored *V. americana* sites indicated no difference in genotypic or allelic diversity from adjacent natural sites (Lloyd et al., 2011). However, in five of eight paired comparisons, restored populations had lower clonal richness, and three restored sites had significant inbreeding coefficients and differed in allelic composition from nearby natural sites. The apparent inbreeding could have resulted from a Wahlund effect if collected seed represented multiple genetic neighborhoods or different populations. Alternatively, it could result from biparental inbreeding in restoration plantings that were founded from many fruits collected from related mothers that sampled pollen from the same fathers. To avoid this risk, we recommend that propagules be collected from widely spaced mothers that are each separated by at least 10 m and that cover large areas.

For the most part, we see no imperative for increasing genetic diversity within *V. americana* sites in the Chesapeake Bay given the levels of genetic diversity and low levels of inbreeding. Introducing new genetic material or combining propagules from multiple populations is suggested when populations are exceedingly low in genetic diversity and are highly inbred (Fenster and Dudash 1994; Lesica and Allendorf 1999; Broadhurst et al. 2008; Hughes et al. 2008; Weeks et al. 2011). For *V. americana*, none of those conditions are realized in the tidal Chesapeake Bay. Additionally, evidence of differentiation among populations from different geographic regions or ecological regions could indicate local adaptation, especially when combined with evidence of fitness consequences of crossing individuals from different regions. Given this genetic structure, we recommend against combining genetic stock from different regions, and the risks associated with genetic pollution may be too high to warrant moving propagules among regions.

In summary, the majority of genetic connectivity in *V. americana* is generated by dispersal of seeds or vegetative fragments. Maintaining sufficient populations to support balanced sex ratios that facilitate sexual reproduction amongst unrelated mothers and fathers is sufficient to sustain pollen dispersal.

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DATA ACCESSIBILITY

Data have been deposited on the Dryad Digital Repository (https://doi.org/10.5061/dryad.53qg6).

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