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Genotypic diversity at multiple spatial scales in the foundation marsh species, *Spartina alterniflora*

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ABSTRACT: Fine-scale variation in both the number and relatedness of genetic individuals can influence a range of ecological and evolutionary processes. However, we often have little information from natural populations regarding the fine-scale distribution of genetic diversity. We quantified multiple metrics of genetic diversity in the widespread, dominant marsh plant *Spartina alterniflora* across 3 spatial scales in 16 natural marshes in the Florida Panhandle. We also examined correlations between genetic diversity and marsh plant species diversity, along with key environmental variables that may influence both. Most of the variance in *S. alterniflora* genetic structure was explained by variation within sampling areas across sites. *S. alterniflora* genetic diversity and marsh plant species diversity increased with spatial scale within sites. In addition, diversity was generally higher on mainland marsh sites compared to discrete marsh islands, consistent with theoretical predictions from island biogeography and population genetics. Multiple metrics of genetic diversity increased with island area and with distance to the mainland. Despite significant correlations between diversity and tidal height, sediment organic content, and sediment porewater salinity, site type (mainland or island) and spatial scale were the best predictors of *S. alterniflora* genetic and marsh plant species diversity. We show that natural populations of *S. alterniflora* exhibit significant fine-scale spatial genetic structure, and thus continued loss of habitat could lead to a substantial loss of genetic diversity.

KEY WORDS: Genetic diversity · Isolation-by-distance · Relatedness · Genetic structure · Salt marsh · Island biogeography

INTRODUCTION

Genetic diversity within a species is predicted to be distributed unevenly across a landscape (Rauch & Bar-Yam 2004), with both evolutionary and ecological implications (Slatkin 1987, Whitham et al. 2006, Hughes et al. 2008). For instance, fine-scale variation in both the number and relatedness of genetic individuals can influence a range of ecological processes at the population and community level, including productivity, species abundance and diversity, and resilience to disturbance (Hughes et al. 2008, Biernaskie 2011, Aguirre et al. 2013, Stachowicz et al. 2013). Evolutionarily, the spatial scale of genetic structure will interact with the processes of migration, selection, and drift to determine how organisms will adapt and evolve in response to environmental change (e.g. Glanville fritillary butterflies; Hanski 2011). Despite the documented importance of genetic variation in theory and from experimental manipulations, we have little information regarding the distribution of genetic diversity at fine spatial scales in natural populations (i.e. local structure) and what factors may influence it.

Understanding patterns of genetic variation in natural populations may be particularly important for

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widespread, dominant species. Genetic variation in dominant species is more likely to be linked to community and ecosystem processes, as illustrated by correlations between genetic diversity and species diversity across a range of systems (Vellend 2003, Vellend & Geber 2005). These linkages can occur causally; for instance, experimental manipulations of plant genetic diversity commonly affect the diversity, abundance, and distribution of species associated with those plants (Hughes & Stachowicz 2004, Crutsinger et al. 2006, Johnson et al. 2006, Crawford et al. 2007, Johnson & Stinchcombe 2007). However, correlations between genetic diversity and species diversity can also occur as a parallel response to the same process (i.e. island area; Vellend 2003, Vellend & Geber 2005). Thus, understanding the distribution of genetic diversity in natural populations could provide broader insights into the forces creating and maintaining biodiversity more generally (Vellend 2003).

In this study, we examined spatial variation in genetic diversity and structure in the dominant marsh plant species *Spartina alterniflora*. *S. alterniflora* ranges from the Gulf of Mexico to the North Atlantic, and it provides ecologically and economically important ecosystem services including shoreline stabilization, habitat provision, and water filtration (Pennings & Bertness 2001, Barbier et al. 2011). *S. alterniflora* can spread vegetatively via underground rhizome expansion of individual clones, or it can reproduce sexually via wind-dispersed pollen. *S. alterniflora* pollen has a relatively limited range (1 to 3 m), and the reproductive stems produce small seeds (2 to 5 mm) that are dispersed via the tides (Davis et al. 2004, Travis et al. 2004). Long-distance dispersal via rafting reproductive stems, seeds, or clonal fragments has also been documented (Taylor & Hastings 2004, Travis et al. 2004). Vegetative expansion is considered highly prevalent within marshes, supported by evidence that individual clones dominate isolated patches (Daehler & Strong 1994, Travis et al. 2004). Seedlings resulting from sexual reproduction are most often observed in open space, leading to the assumption that sexual reproduction is key for the colonization of new habitats. However, the relative frequency of sexual versus vegetative reproduction within marshes and the resulting effects on spatial genetic structure are largely unknown.

As with other foundation plant species (Hughes et al. 2008), fine-scale diversity in *Spartina alterniflora* can be important for plant productivity and colonization success (Wang et al. 2012). However, we have relatively little information regarding the distribution of diversity at these spatial scales in natural marshes. Although there are numerous analyses of the genetic diversity and structure of *S. alterniflora* (Ayres et al. 1999, O'Brien & Freshwater 1999, Anttila et al. 2000, Richards et al. 2004, Travis et al. 2004, Travis & Hester 2005, Blum et al. 2007), most have focused on variation across sites or geographic regions, intentionally sampling at intervals large enough to minimize collecting multiple samples from the same clone. Only a handful of surveys (Travis et al. 2004, Edwards et al. 2005) have compared patch diversity at multiple spatial scales (plots of 1, 6, and 12 m diameter) within sites: it is these within-site spatial scales that are likely most relevant to plant-plant and plant-animal ecological interactions. These few studies show that *S. alterniflora* genotypic diversity is relatively high even at small spatial scales (50 to 80% of the stems sampled representing different genotypes) and that it generally increases with increasing spatial scale within sites (Travis et al. 2004, Edwards et al. 2005).

To further our understanding of fine-scale genetic diversity and structure in *Spartina alterniflora*, we quantified genetic diversity, relatedness, and structure across 16 spatially separated marshes at 3 spatial scales (plots of 1, 3, and 6 m diameter) within each marsh, producing the most comprehensive dataset regarding fine-scale genetic diversity in this ecologically important species. We focused on the following questions: (1) What is the distribution of genetic variation in *S. alterniflora* within and across marsh sites? (2) Do *S. alterniflora* marshes exhibit a pattern of isolation-by-distance, and what can that tell us about the scale of realized sexual dispersal in this species? (3) Is genetic diversity within *S. alterniflora* correlated with species diversity of the surrounding marsh plant community? (4) Do environmental variables predict *S. alterniflora* genetic diversity or marsh plant species diversity?

MATERIALS AND METHODS

Study system

This study was conducted in St. Joseph Bay, Florida, a shallow protected coastal embayment with little freshwater input along the northeastern Gulf of Mexico (Fig. 1). The shoreline of the bay is bordered by salt marsh habitat that is dominated by the marsh plant species *Spartina alterniflora*. Other marsh plant species present at our survey sites were *Juncus roe-*

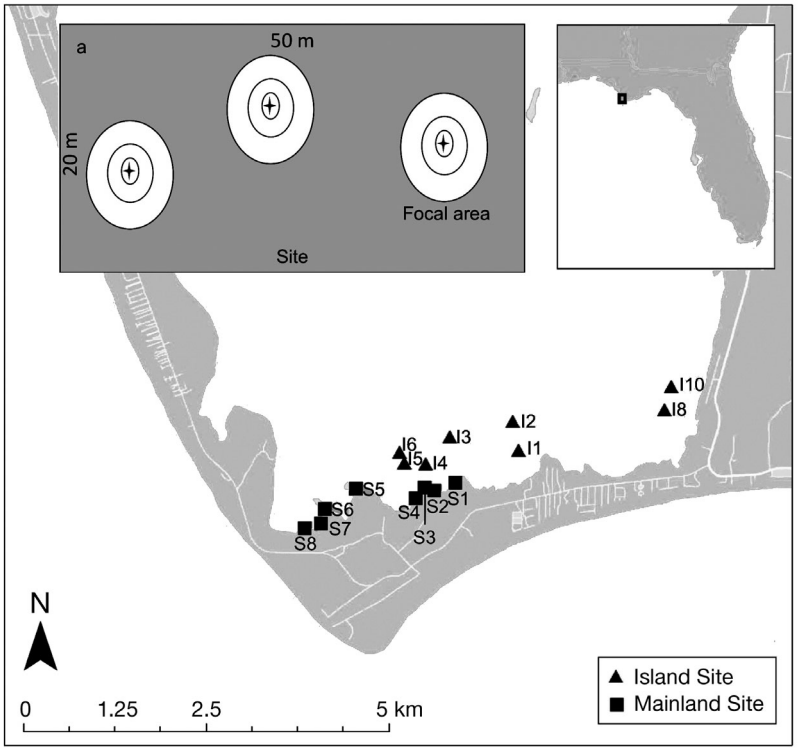

Fig. 1. Study sites in St. Joseph Bay, FL, USA. Sites included discrete marsh islands (triangles) and mainland marsh sites (squares) selected from continuous fringing marsh along the shoreline. Mainland marsh sites were separated by at least 200 m. Inset (a) shows sampling design within a site. We established a 50 × 20 m plot, with the long axis parallel to the tide line. Three focal areas (stars) were randomly selected, and samples were collected from subplots of 1, 3, and 6 m diameter

merianus, *Salicornia virginica*, *Limonium carolineum*, *Distichlis spicata*, *Batis maritima*, and *Borrichia frutescens*. We focused on the southern portion of St. Joseph Bay, where there is substantial marsh habitat both along the shoreline (hereafter referred to as 'mainland' marsh) as well as on small marsh 'islands' that are separated from each other and the mainland by submerged sand flat and seagrass habitat.

Field survey of *Spartina* genetic diversity, plant species diversity, and environmental variables

We quantified *Spartina alterniflora* genetic diversity and plant species diversity at multiple spatial scales within 8 mainland marshes (S1 to S8) and 8 island marshes (I1 to I6, I8, I10) in St. Joseph Bay (Fig. 1). At each site, we established a 50 × 20 m plot with the long axis oriented parallel to the tide line. Within each site, we randomly selected 3 non-overlapping focal points containing *S. alterniflora* around which we centered nested circular subplots of 3 sizes (1, 3, and 6 m diameter focal areas; Fig. 1a). We then stratified each subplot into 12 'slices' and haphazardly selected one tissue sample from each of the slices in each subplot (N = 36 per focal area: 12 within 1 m, 12 between 1 m and 3 m, and 12 between 3 m and 6 m). Thus, diversity was estimated based on 12 samples per 1 m diameter area, 24 samples per 3 m diameter area, and 36 samples per 6 m diameter area, with 3 replicates of each spatial scale per site. At 1 mainland site, we sampled 4 focal areas rather than 3. All samples were stored on ice for transport to the laboratory and frozen at –80°C.

Simultaneously with *Spartina alterniflora* tissue collection, we also surveyed marsh plant species diversity in each focal area by haphazardly selecting ten 0.25 m^2 quadrats divided among the 3 spatial scales (1 within 1 m, 3 between 1 m and 3 m, and 6 between 3 m and 6 m; min. spacing between quadrats = 1 m) and then quantifying plant species identity and percent cover. Species richness values were calculated as the cumulative number of plant species found within a given spatial scale (i.e. the 3 m plant species richness values include any unique species found within the nested 1 m and 3 m diameter areas).

We also sampled 3 key environmental variables: mean low tidal height, sediment organic content, and sediment porewater salinity. Tidal height was quantified in each subplot relative to a tidal logger installed at 1 site; because of a logger malfunction, these data are only available for 72 of the 147 subplots (18 mainland, 54 island). Sediment organic content was quantified as percent mass loss on ignition from a 2 cm wide and 5 cm deep sediment core taken in each focal area. Sediment porewater salinity was collected from a depth of 5 cm in each focal area and quantified using a refractometer.

Identifying *Spartina* genotypes

Prior to DNA extraction, we ground approximately 0.25 g of frozen leaf tissue with liquid nitrogen. DNA was then extracted with 150 µl of Sarkosyl-

urea extraction buffer (1% Sarkosyl, 8 M Urea, 20 mM sodium phosphate, 1 mM EDTA, and dH_20). Digestions took place in a 65°C water bath for 1 to 2 h. DNA extractions were purified with Agencourt® SprintPrep® magnetic beads (Beckman Coulter Genomics #A29178) and the resulting DNA concentration of each sample was estimated using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific).

We used 8 DNA microsatellite markers developed specifically for *Spartina alterniflora* to differentiate among genotypes (Blum et al. 2004, Sloop et al. 2005): Spar02, Spar05, Spar07, Spar08, Spar16, Spar20, Spar22, and Spar34. PCRs for all primers took place in an 8 µl volume with the following reagents: 5 µl Qiagen (Venlo) Multiplex PRC Master Mix, 0.25 µM forward primer for each of 3 loci, 0.25 µM reverse primer for each of 3 loci, 1.5 µL nanopure water, and 20 ng template DNA. Forward primers were fluorescently labeled with NED, HEX, 6-FAM dyes. Loci were multiplexed for fragment length analysis in the following combinations: (1) Spar02, Spar07, Spar22; (2) Spar05, Spar16; (3) Spar08, Spar20, Spar34. Amplification conditions were identical for all loci except for the annealing temperature. PCR was performed on a Veriti Thermocycler (Applied Biosystems). PCR began with a 1 min, 95°C denaturation step, followed by 16 cycles of 94°C for 30 s, first annealing temperature for 1.5 min, and 72°C for 1.5 min, followed by another 16 cycles of 94°C for 30 s, second annealing temperature for 1.5 min, and 72°C for 1.5 min. The program ended with an extension at 72°C for 10 min. The annealing temperatures were 58/56°C for loci Spar02, Spar07, Spar08, Spar20, Spar22, and Spar34 and 56/54°C for loci Spar05 and Spar16.

PCR products were visualized on a 3130xl genetic analyzer with capillary electrophoresis (Applied Biosystems), and alleles were grouped into 2-base pair bins (because all loci contained di-nucleotide repeats) that corresponded to peaks in allele size frequency.

Genetic analyses

Three of our 8 markers (Spar05, Spar20, Spar22) exhibited polyploidy. Thus, clonal identity was assessed in the program GENODIVE, which can accommodate data of mixed ploidy levels (v2.0b22, Meirmans & Van Tienderen 2004). Clonal identity was computed on the entire dataset with a stepwise mutation model and threshold = 2. The threshold is the maximum genetic distance allowed between genotypes for them to be considered clones. This threshold was chosen based on the distribution of genetic distance between pairs of individuals (Meirmans & Van Tienderen 2004, Arnaud-Haond et al. 2007). Any individuals missing data were removed from the analysis, resulting in 9 to 12 individuals per 1 m diameter area, 19 to 24 individuals per 3 m diameter area, and 31 to 36 individuals per 6 m diameter area.

Clonal diversity was also calculated in GENODIVE for a range of commonly used metrics to facilitate comparison across studies (Arnaud-Haond et al. 2007): number of genotypes (i.e. genotypic richness; num), effective number of genotypes (corrected for sample size; eff), evenness corresponding to Nei's index (eve), and corrected Shannon's diversity index (i.e. genotypic diversity; shc; Meirmans & Van Tienderen 2004). Allelic richness for each focal area was estimated with FSTAT (Goudet 1995). We used a bootstrap procedure in GENODIVE with 1000 permutations to test for differences in clonal diversity between island and mainland populations; the individuals were resampled from the populations and the diversity indices were compared after every replicate (Manly 1991).

For all analyses that follow, we excluded repeatedly sampled ramets, thereby limiting the dataset to unique genets. We also excluded the 3 loci that exhibited polyploidy, because they did not meet the assumptions of these analyses. Analysis of molecular variance (AMOVA) was performed using the HIERFSTAT package in R (Goudet 2005). We tested for the effect of marsh type (island/mainland), sites within islands or mainlands, and focal areas within sites. Within-group relatedness (r) for each spatial scale was calculated using the program STORM 2.0 (Frasier 2008).

Tests for Hardy-Weinberg equilibrium (HWE), the estimation of pairwise and average *F*-statistics, and tests for linkage disequilibrium were implemented in Genepop version 4.0.10 (web version, Raymond & Rousset 1995, Rousset 2008) using the unique genet dataset. F_{ST} (the proportion of genetic variation in the total population that is attributable to differentiation among subpopulations, i.e. sites) and F_{IS} (the proportion of genetic differentiation within sites that is due to differences among individuals, which increases with increased homozygosity) were estimated using the method of Weir & Cockerham (1984). Significance of overall F_{ST} and pairwise F_{ST} values between sites was determined by an exact G test in Genepop with the default Markov chain parameters. Geno-

typic linkage disequilibrium was estimated using the log likelihood ratio statistic to test whether the microsatellite loci are independent. This metric is significant when loci are linked in the genome or when individuals are closely related. We used the Bonferroni method to correct for multiple tests.

Isolation by distance (IBD) was calculated from the correlation between site divergences and physical distances. The significance of the IBD slope was assessed with a Mantel test using 999 permutations in the ade4 package in R (version 2.15.2, R Development Core Team 2012). Pair-wise genetic distance between sampling sites was measured as F_{ST} / (1 − F_{ST}) and pair-wise geographic distance was measured as Euclidean distance between sites.

We used the IBD slope to estimate the standard deviation of the dispersal distribution or dispersal kernel, which is a function that describes the probability of dispersal at different distances from the source. The dispersal estimate from IBD reflects the rate of seed dispersal, because the analysis was restricted to unique genets at the site scale (only 3 genets were shared across more than 1 site; Fig 2). For 2-dimensional habitats such as salt marshes, the standard deviation of the dispersal kernel (σ, also known as the axial parent-offspring distance) is estimated from the relationship $\sigma = 1 / \sqrt{(4\pi D_e m)}$, where D_e is the effective density and m is the slope of the relationship between F_{ST} / (1 − F_{ST}) and geographic distance (Rousset 1997). We estimated D_e (with confidence intervals) as the effective number of genotypes per square meter, averaged across all sites. We used reduced major axis (RMA) regression to estimate confidence intervals on m. RMA accounts for uncertainty in the measurement of distance between sites. RMA was implemented with the lmodel2 package in R, with 999 permutations to assess confidence intervals on the slope. Confidence intervals on D_e and m were used to calculate the confidence intervals on σ.

Relationships among marsh environment, spatial scale, and diversity

We used a linear mixed model-selection approach to examine relationships among marsh environmental variables, spatial scale, and diversity. Model selection offers an alternative approach to traditional null hypothesis testing, whereby several competing hypotheses are simultaneously confronted with data to allow identification of the model that best explains the data (Burnham & Anderson 1998, Johnson & Omland 2004, Bolker 2008). This information-theoretic approach focuses on providing strength of evidence for an *a priori* set of alternative hypotheses, rather than a statistical test of a null hypothesis (Anderson et al. 2000). For each analysis, we fitted the data to a series of nested models to determine which best explained the observed results. Nested models are models that can be obtained by restricting a parameter in a more complex model to be zero. These models included a null model with an intercept and only the random effects, models that included the random effects with each fixed factor independently, and models that included random effects and all possible additive and interactive effects among fixed factors. We then performed model selection using Akaike's Information Criterion corrected for small sample sizes (AIC_c; Burnham & Anderson 1998). The identification of the best candidate model was based on Akaike weight (w_i), which was calculated as the model likelihood normalized by the sum of all model likelihoods. Weights close to 1.0 indicate greater confidence in the selection of the best model (Burnham & Anderson 1998). We also calculated the difference between the AIC_c of a particular model and the AIC_c of the most likely model (i.e. model with w_i closest to 1.0). If this observed difference (or ΔAIC score) between the top candidate model and the alternative model under consideration was greater than 2.0, then we considered the top candidate model significantly stronger (Richards 2005). Candidate models and their ΔAIC scores and AIC weights are provided in tables. Analyses were conducted with R statistical software (version 2.11.1) using the lmer function in the lme4 package and the AICctab function in the bblme package.

We first examined whether marsh type (fixed effect: mainland or island), spatial scale (fixed continuous effect: 1, 3, or 6 m diameter plots), or their interaction explained significant variation in num, shc, r, or plant species richness. We included random effects of focal area nested within site and site nested within marsh type in all models. For the analysis of plant species richness, we also tested models including independent, additive, and interactive effects of genotypic richness to examine whether genotypic richness was a significant predictor of plant species richness.

To better understand the environmental variables that may underlie relationships between marsh type and genotypic richness or plant species richness, we conducted separate linear regression analyses for each of the single-variable relationships between diversity (genetic or species) and tidal height, sediment organic content, and salinity. We hypothesized

that some of these relationships may be nonlinear, so we included a quadratic term for each environmental variable in the analysis. We then repeated the model selection procedure from above, adding models that included the significant environmental variables as covariates to determine whether they increased our explanatory power beyond simply knowing marsh type and spatial scale.

For the island sites only, we conducted a separate model selection analyses to test the independent and interactive effects of island area and distance to the mainland on num, shc, r, and plant species richness.

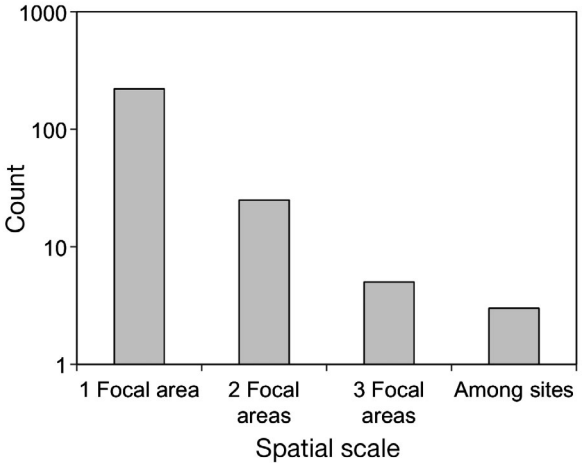

Fig. 2. Distribution of genets across sites. Frequency of *Spartina alterniflora* genotypes found across multiple focal areas and sites

RESULTS

Genetic analyses

We genotyped a total of 1692 individuals that were grouped into 345 distinct genets. Most *Spartina alterniflora* genets were found within a single focal area, although a few were more spatially widespread (e.g. 5 genotypes were found across all 3 focal areas within a site, and 3 genotypes found at more than one site; Fig. 2). Island sites tended to have lower genetic diversity than mainland marshes, although the significance of this difference varied depending on the metric of clonal diversity (Table 1, Table S1 in the Supplement at www.int-res.com/articles/suppl/m497p105_supp.pdf). Within island and mainland groups, diversity was significantly different among sites (Table S1). The AMOVA results using the unique genet dataset were largely consistent with these patterns: there were significant effects sites within marsh type (mainland/island) and focal areas within sites on the genetic structure of *S. alterniflora* (Table 2). Most of the variance was explained within focal areas.

After correction for multiple tests, a few loci exhibited linkage disequilibrium (LD) within populations (Table S2 in the Supplement). Of the 10 possible pairings between loci, significant LD was observed at Sites I1 (1 pair), I10 (1 pair), I3 (4 pairs), S2 (2

Table 1. Clonal diversity in *Spartina alterniflora*. Metrics are sample size (N), number of genotypes (num), effective number of genotypes (eff), evenness corresponding to Nei's index (eve), corrected Shannon's diversity index (shc), relatedness (r), allelic richness (ar), and F_{IS}. *F_{IS} values indicate significant deviations from Hardy-Weinberg equilibrium, after correction for multiple tests

Site	N	num	eff	eve	shc	r	ar	F_{IS}	Island size (m^2)	Distance to mainland (m)
I1	104	8	3.99	0.50	0.70	0.064	3.95	0.115	2594.50	165
I2	105	10	4.34	0.43	0.76	0.074	4.24	–0.005	3897.38	378
I3	102	18	7.75	0.43	1.09	0.249	3.63	–0.005	7695.67	343
I4	103	15	3.51	0.23	0.83	0.058	3.83	–0.014	5863.04	172
I5	106	6	3.27	0.54	0.60	0.354	3.09	–0.106	744.55	350
I6	106	12	4.72	0.39	0.84	0.059	4.63	–0.044	951.96	472
I8	96	6	2.36	0.39	0.50	0.023	3.80	0.023	2104.69	150
I10	102	8	4.37	0.55	0.74	0.100	3.51	0.107	1509.60	276
S1	105	7	1.43	0.20	0.34	0.324	3.73	–0.285	NA	NA
S2	106	20	6.89	0.35	1.09	0.103	4.12	0.157	NA	NA
S3	107	29	14.81	0.51	1.37	0.041	4.60	0.036	NA	NA
S4	103	24	12.53	0.52	1.27	0.257	3.84	0.087	NA	NA
S5	106	19	10.83	0.57	1.16	0.256	3.64	0.087	NA	NA
S6	104	37	9.09	0.25	1.44	–0.008	4.58	0.193*	NA	NA
S7	102	27	10.70	0.40	1.31	0.001	4.41	0.101	NA	NA
S8	135	12	2.04	0.17	0.59	0.141	3.79	0.185	NA	NA

Table 2. Analysis of molecular variance in *Spartina alterniflora*

	df	Variance components	Proportion of variation	*F*	p
Among mainland/island	1	0.001	0.041	0.0004	0.29
Among sites within mainland/island	14	0.277	7.972	0.080	0.01
Among focal areas within sites	33	0.212	6.103	0.066	0.01
Within focal areas	293	2.985	85.884		

Table 3. *Spartina alterniflora.* F_{ST} and F_{IS} by locus for non-polyploid loci. Significance of F_{ST} was determined by an exact G test in Genepop. All values are significant ($p < 0.002$)

	F_{ST}	F_{IS}
Spar2	0.091	0.360
Spar7	0.107	–0.095
Spar16	0.102	0.198
Spar34	0.094	0.109
Spar8	0.105	–0.218
ALL LOCI	0.100	0.066

pairs), S3 (4 pairs), S4 (3 pairs), S5 (2 pairs), S6 (2 pairs), and S7 (3 pairs). Significant LD within each population was not driven by any 1 locus, suggesting that individuals in these populations were closely related.

Significant deviations from HWE were observed at each locus when pooled over all sites, indicating population structure (Table 3). However, only 1 site (S6) deviated significantly from HWE, suggesting low inbreeding within sites overall (F_{IS}; Table 1). F_{ST} was significant across all loci (F_{ST} = 0.100) and was also significant at each locus (Table 3). Pair-wise comparisons indicated that most sites exhibited significant differentiation from one another (109 out of 120 comparisons; Table S3 in the Supplement).

We found a positive but non-significant correlation between genetic distance and geographic distance (Mantel correlation = 0.13, p = 0.262; RMA slope = 0.027, 95% CI = –0.008, 0.077; R^2 = 0.017). Since limited dispersal can still result in a non-significant IBD slope that accurately reflects the scale of dispersal (Lotterhos 2012, Puebla et al. 2012), we proceeded with estimating the scale of dispersal assuming a minimum slope of 0.001. We found that D_e was 0.0752 (95% CI = 0.051, 0.099). The point estimate of σ = 6.29 m with 95% CI = 3.23, 39.50 m. This is an estimate of average dispersal (via seeds) per generation.

Relationships among marsh environment, spatial scale, and diversity

The best model for num included an interaction between spatial scale and marsh type (w_i = 0.99), indicating that the functional nature of the relationship differed between mainland and island marshes: richness increased significantly with spatial scale, but the slope of this relationship was higher at mainland than island marshes (Fig. 3a; Table S4 in the Supplement). Genotypic diversity (shc) was best explained by the model including spatial scale (w_i = 0.73). As with genotypic richness, genotypic diversity increased significantly with spatial scale (Fig. 3b; Table S4). Genetic relatedness did not vary predictably by site type or spatial scale and in this case the null model provided the best fit (w_i = 0.82; Fig. 3c, Table S4).

Marsh plant species richness exhibited a similar pattern to *Spartina alterniflora* genotypic diversity, with diversity increasing with spatial scale and an independent effect of marsh type (w_i = 0.59; Fig. 4a, Table S4). Genotypic richness was not included in the best model for plant species diversity.

Spartina alterniflora genotypic richness and marsh plant species richness were each significantly, but weakly, linearly correlated with tidal height (Fig. 5a,d) and nonlinearly correlated with sediment organic content (Fig. 5b,e). Genotypic richness was not significantly correlated with sediment salinity (Fig. 5c), but marsh plant species richness peaked at intermediate salinities (Fig. 5f). Despite these significant correlations, none of the environmental variables were included in the best model for *S. alterniflora* genotypic richness or marsh plant species richness ($w_i \leq 0.01$); instead, the interaction between marsh type and spatial scale best explained patterns of diversity (Table S4).

For the island sites only, we analyzed whether *Spartina alterniflora* genetic diversity or marsh plant species diversity was predicted by island size or distance from the mainland (shown in Table 1). The number of genotypes (num) found on islands was best predicted by island area (w_i = 0.90; Fig. 6a) but not distance from the mainland (Fig. 6d, Table S5 in the Supplement). Genetic relatedness showed the opposite pattern: it only increased with distance from the mainland marsh (w_i = 0.62; Fig. 6f) but was not related to island area (Table S5, Fig. 6c). In contrast, genotypic diversity was explained by 2 models that each had high weights: the first had an Akaike

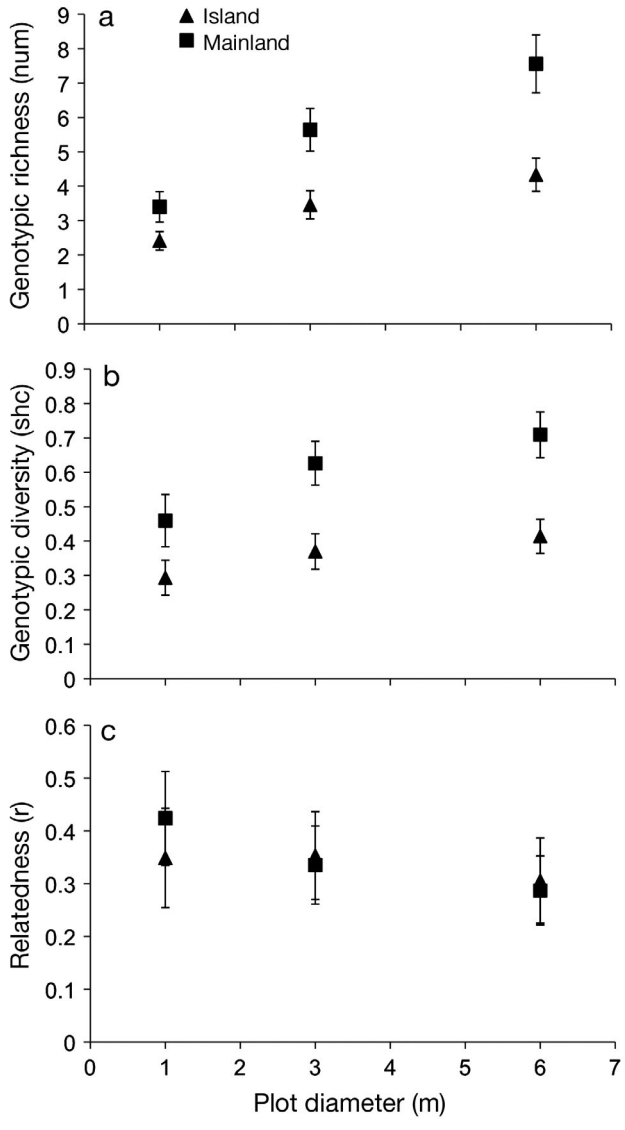

Fig. 3. *Spartina alterniflora* genetic diversity (mean ± SE) across spatial scale. (a) Genotypic richness measured as the number of unique genotypes increased with spatial scale at a faster rate for mainland ($y = 0.82x + 2.81$, $R^2 = 0.97$) than island ($y = 0.38x + 2.15$, $R^2 = 0.97$) marshes. (b) Genotypic diversity measured as the corrected Shannon's diversity index increased with spatial scale for mainland and island marshes ($y = 0.04x + 0.36$, $R^2 = 0.92$). (c) Within-group relatedness measured as r did not vary with spatial scale for mainland or island marshes

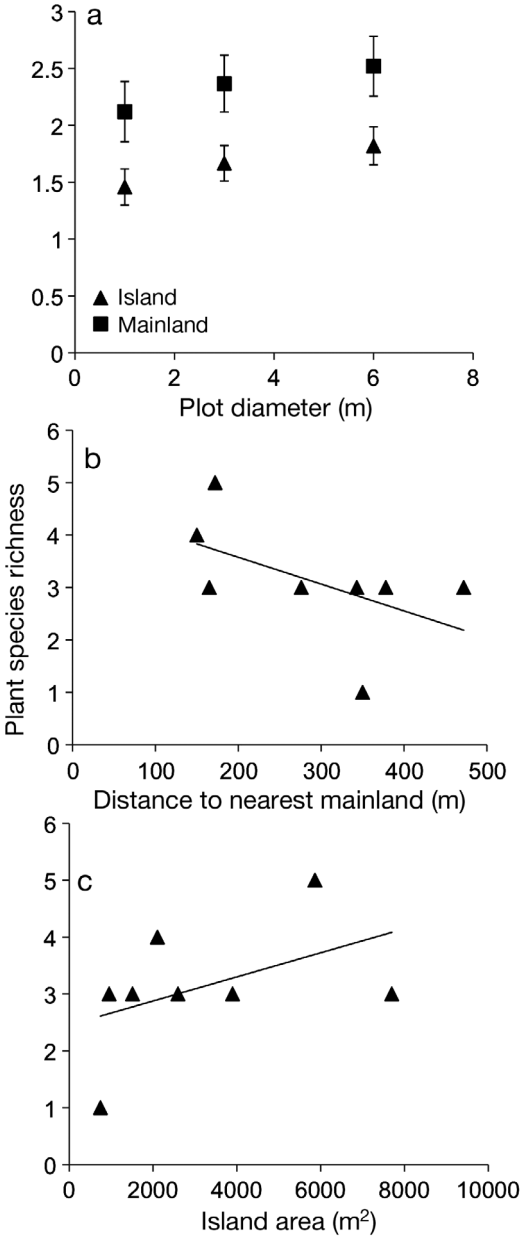

Fig. 4. Relationships between marsh plant species diversity and spatial scale. (a) Mean (±SE) number of marsh plant species increased with spatial scale within mainland ($y = 0.07x + 2.08$, $R^2 = 0.94$) and island ($y = 0.07x + 1.41$, $R^2 = 0.96$) marshes. (b) Marsh plant species diversity as a function of distance to the mainland marsh ($y = -0.005x + 4.60$, $R^2 = 0.28$). (c) Marsh plant species diversity as a function of island area ($y = 0.0002x + 2.45$, $R^2 = 0.22$)

weight of 0.64 and included distance to the mainland (Fig. 6e) and the second model had an Akaike weight of 0.30 and included island area (Fig. 6b).

Similar to genotypic diversity, plant species richness was best explained by 2 models that had high weights: the first had an Akaike weight of 0.64 and included distance to the mainland and the second had an Akaike weight of 0.41 and included island area. Plant species richness was negatively correlated with distance from the mainland (Fig. 4b), in contrast to the multiple measures of genetic diversity that were positively correlated with distance to the

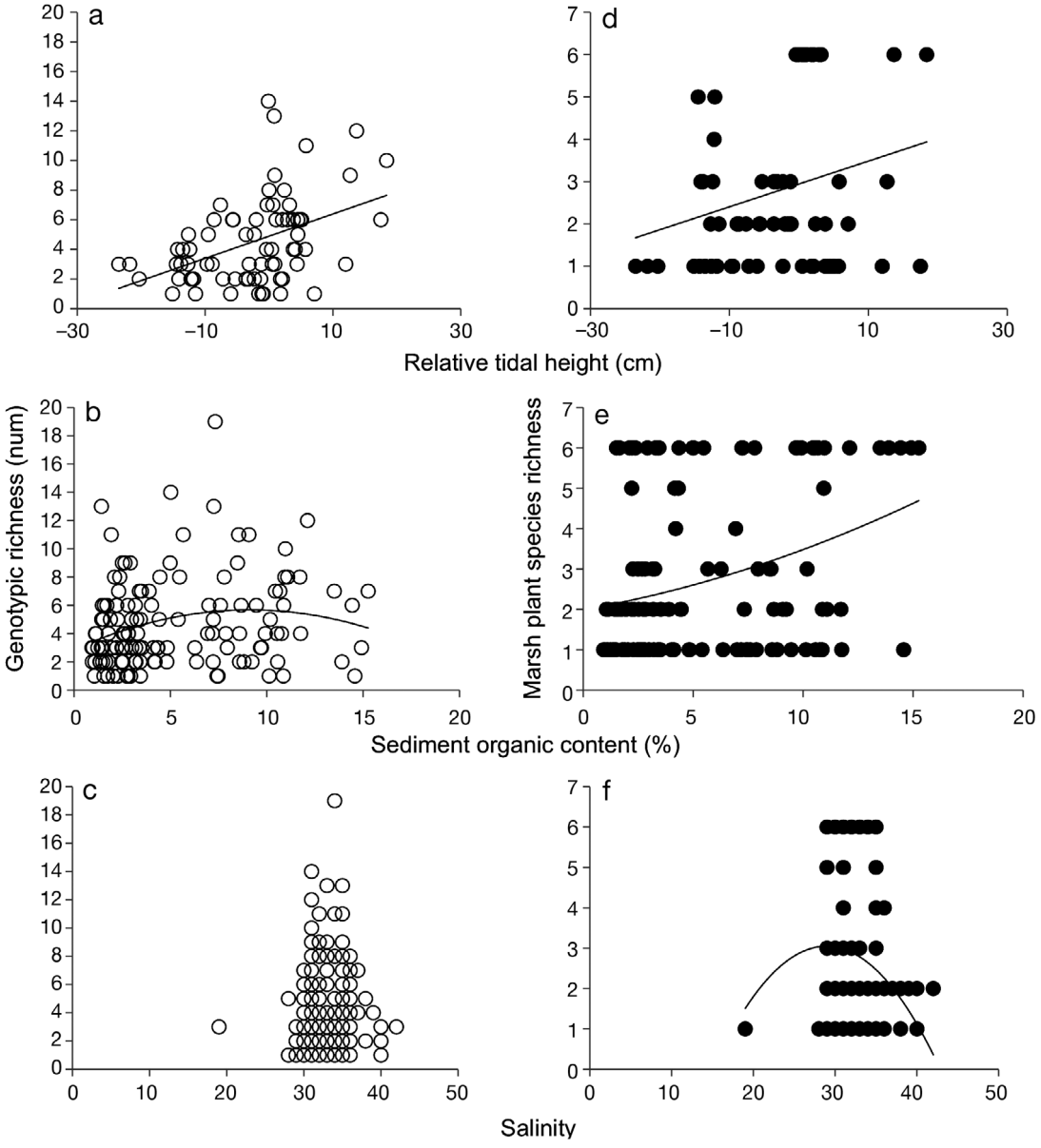

Fig. 5. Relationships between *Spartina alterniflora* genotypic richness (a–c) and marsh plant species richness (d–f) and key environmental variables. (a,d) Tidal height was correlated with (a) *S. alterniflora* genotypic richness ($R^2 = 0.19$, $p < 0.001$) and (d) marsh plant species richness ($R^2 = 0.06$, $p = 0.05$). (b,e) Sediment organic content was nonlinearly correlated with (b) *S. alterniflora* genotypic richness ($R^2 = 0.05$, $p = 0.02$) and (e) marsh plant species richness ($R^2 = 0.10$, $p = 0.03$). (c,f) Sediment porewater salinity was not correlated with (c) *S. alterniflora* genotypic richness, but it was correlated with (f) plant species richness ($R^2 = 0.06$, $p = 0.05$)

nearest mainland marsh. Plant species richness was also positively correlated with island area (Fig. 4c).

DISCUSSION

Consistent with past studies of within site genetic variation in salt marsh plants (Richards et al. 2004, Travis et al. 2004, Edwards et al. 2005), we found substantial genetic variation in *Spartina alterniflora* even at relatively fine spatial scales (Fig. 3). These results collectively provide a strong counter-argument to the prevailing wisdom that extensive vegetative propagation creates low genetic diversity in natural salt marsh populations. On islands, focal areas within sites shared on average between 0 and 5 genotypes (mean ± SE = 2.12 ± 0.69), whereas in mainland sites, focal areas within sites shared on

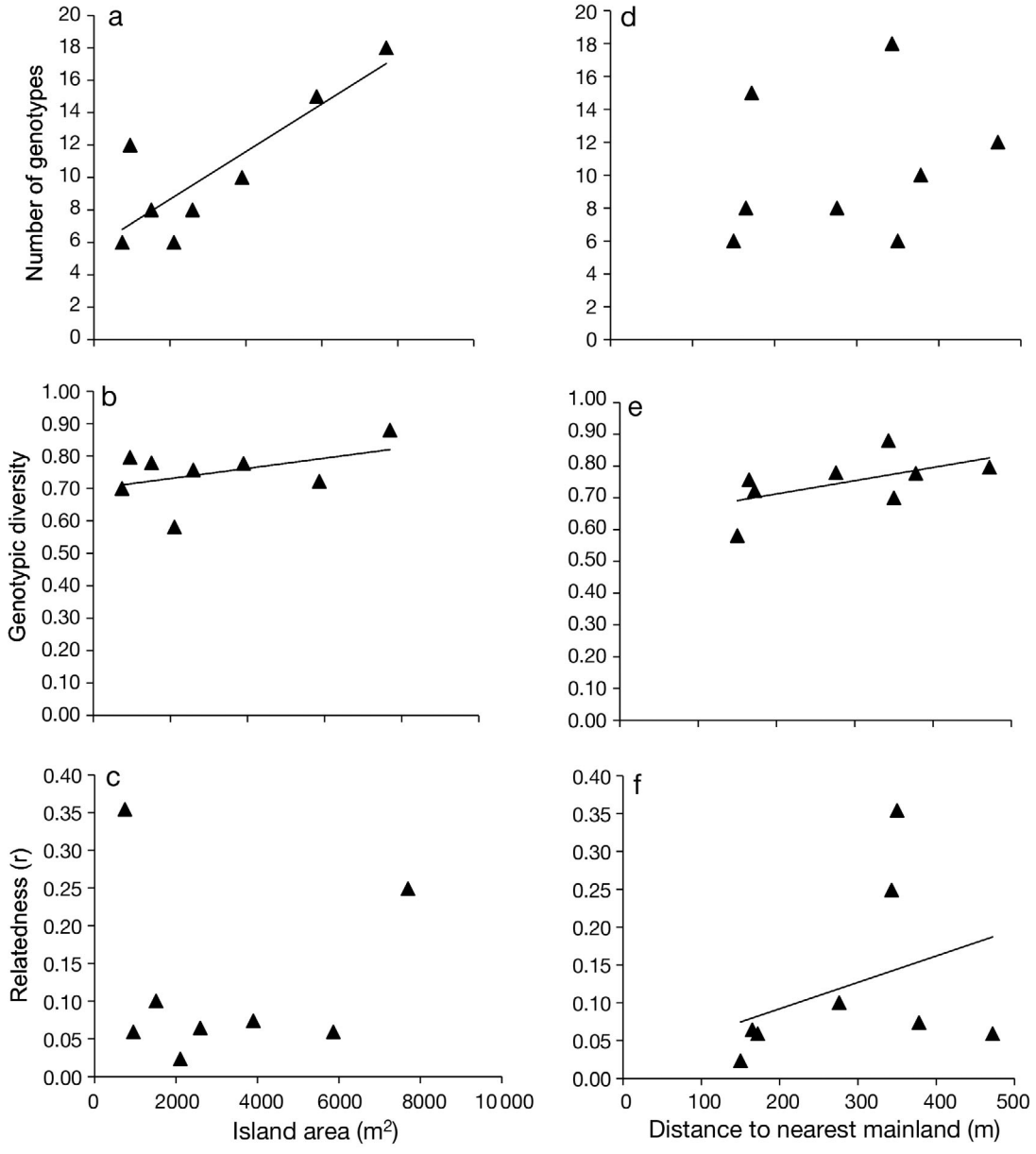

Fig. 6. For island sites only, correlations between *Spartina alterniflora* genetic diversity and (a–c) island area and (d–f) distance to mainland marsh. (a,d) The number of *S. alterniflora* genotypes is correlated with (a) island area ($y = 0.001x + 5.71$, $R^2 = 0.71$), but is not with (d) distance to the mainland marsh. (b,e) *S. alterniflora* genotypic diversity as a function of (b) island area ($y = 0.0001x + 0.70$, $R^2 = 0.20$) and (e) distance to the marsh ($y = 0.0004x + 0.63$, $R^2 = 0.32$). (c,f) *S. alterniflora* genotypic relatedness was not related to (c) island area but showed a slight overall increase with (f) distance to the marsh ($y = 0.003x + 0.02$, $R^2 = 0.13$)

average only 0 to 3 genotypes (1.22 ± 0.22). Thus, the majority of genotypes appear to occur over a spatially restricted area. In addition, most of the genetic variance in our survey was partitioned within focal areas within sites. This spatial restriction of genotypes was also reflected in pairwise F_{ST} values among sites, which was significant in 109 of 120 pairwise comparisons.

Understanding the spatial scale of dispersal in a widespread species such as *Spartina alterniflora* is important ecologically, because dispersal is critical for the colonization of disturbed or newly created marsh habitat (Proffitt et al. 2003). In addition, dispersal and gene flow (which depends both on the dispersal distribution and the density of individuals across the landscape; Kirkpatrick & Barton 1997)

interact with selection over evolutionary time scales to constrain or promote local adaptation (Lenormand 2002). The marshes in our study exhibited a non-significant pattern of isolation by distance, with the average dispersal distance per generation estimated at approximately 7 m. Although these data certainly do not rule out the importance of sexual reproduction for long-distance dispersal and colonization of new habitats, combined with the relatively high levels of fine-scale genotypic diversity, they suggest that sexual reproduction also plays a significant role in the creation and maintenance of genetic diversity at small scales within marshes.

Previous studies have shown that genetic diversity within dominant species can be causally linked to species interactions and the diversity of competing species (Booth & Grime 2003, Proffitt et al. 2005, Vellend 2006, Fridley et al. 2007, Lankau & Strauss 2008, Fridley & Grime 2010). Alternatively (or in addition), genetic diversity and plant species diversity may respond similarly to the same ecological processes (Vellend 2003). Although genotypic richness was not a significant predictor of plant species richness, the similarities in the shape and slope of the relationships among marsh type, spatial scale, and these 2 measures of richness (Figs. 3 & 4) suggest that they are responding to similar processes. In fact, both *Spartina alterniflora* genetic richness and plant species richness increased with tidal height. As with marsh plant species diversity (Pennings et al. 2005), lower *S. alterniflora* genetic diversity at lower tidal heights may result because fewer genotypes are capable of surviving in this physiologically stressful environment. In addition, vegetative propagation may be more common and/or more successful at lower tidal heights, consistent with patterns of genotypic diversity in intertidal to subtidal seagrass *Zostera marina* (Kamel et al. 2012). Because tidal height was higher on average on mainland marshes (mean ± SE = 3.75 ± 1.43 cm) than island marshes (mean ± SE = −4.73 ± 1.14 cm), it also could have contributed to the higher average diversity on mainlands.

Genetic and species diversity were also correlated with sediment organic content, although the shape of the relationships differed (Fig. 5): genetic diversity peaked at intermediate sediment organic content, whereas plant species diversity peaked at high sediment organic content. If sediment organic content is a reliable proxy for nutrient availability (Craft et al. 2003), then our finding of higher species diversity at greater nutrient availability contrasts with experimental manipulations which document a decrease in species diversity with increased resources (Eilts et al. 2011). In general, however, relationships between genetic diversity and environmental variables such as organic content and salinity were weak, and our results indicate that marsh type and spatial scale have greater predictive power for genetic and species diversity.

Both genotypic and species richness and diversity were higher on average and accumulated more quickly with spatial scale on marsh mainland sites than island sites. These differences may be due to island-biogeographic processes (i.e. island sites experience lower colonization by new genotypes and/or species than mainland sites) or to different environments between islands and mainland sites. One environmental variable that may be driving these patterns is tidal height; as noted above, tidal height was higher on average in mainland sites than on islands. However, habitat area and population size likely also contribute to greater diversity in mainland sites, as posited by the theory of island biogeography for species diversity (MacArthur & Wilson 1967) and the continent-island model of population genetics (Wright 1940) for genetic diversity (Vellend 2003, 2005). Given the relatively low dispersal distance quantified in our study, it is unlikely that islands receive many colonists (plants or genets) from either the mainland marshes or nearby islands. In contrast, gene flow and colonization rates are expected to be much higher in mainland sites that are large in area and also inter-connected.

The same theories that can be used to explain differences in mainland and island diversity can also predict the diversity of islands themselves in relation to island size and distance to the mainland (Wright 1940, MacArthur & Wilson 1967, Vellend 2003). Plant species diversity was consistent with these theoretical predictions: the number of plant species increased with island area and decreased with distance to the mainland (Fig. 4). In addition, the number of *Spartina alterniflora* genotypes and genotypic diversity (which takes into account the number as well as relative abundance of genotypes) increased with island area as predicted. Genetic relatedness increased with distance to the mainland as may be expected given limited dispersal distances, but it was not related to island area. Genotypic diversity was also significantly correlated with distance to the mainland in the opposite direction of that predicted, with higher diversity at greater distances from the mainland (Fig. 6). Island age may contribute to this discrepancy, as marsh age can be a significant predictor of *S. alterniflora* genetic diversity (Travis & Hester 2005); unfortunately, we do not have informa-

tion regarding the age of the marshes sampled in this study.

As much as 50% of salt marshes globally have been lost or degraded due to a variety of processes including marsh reclamation, physical disturbance, climate change, pollution, altered hydrological regimes, and biological invasions (Bromberg & Silliman 2009, Bromberg Gedan et al. 2009). Localized marsh loss has even occurred within St. Joseph Bay (R. A. Hughes pers. obs.). Because *Spartina alterniflora* exhibits significant spatial genetic structure, even a patchy loss of habitat can lead to a substantial loss of diversity when related individuals are clustered spatially (Rauch & Bar-Yam 2004). Given the ecosystem services provided by marsh habitats, any loss of habitat can have significant economic as well as ecological consequences (Barbier et al. 2011). By impacting the genetic structure and connectivity of marshes, habitat loss may also have significant evolutionary implications, particularly in light of increasing global change.

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