

ORIGINAL ARTICLE

Spatial and temporal genetic structure in a hybrid cordgrass invasion

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Invasive hybrids and their spread dynamics pose unique opportunities to study evolutionary processes. Invasive hybrids of native *Spartina foliosa* and introduced *S. alterniflora* have expanded throughout San Francisco Bay intertidal habitats within the past 35 years by deliberate plantation and seeds floating on the tide. Our goals were to assess spatial and temporal scales of genetic structure in *Spartina* hybrid populations within the context of colonization history. We genotyped adult and seedling *Spartina* using 17 microsatellite loci and mapped their locations in three populations. All sampled seedlings were hybrids. Bayesian ordination analysis distinguished hybrid populations from parent species, clearly separated the population that originated by plantation from populations that originated naturally by seed and aligned most seedlings within each population. Population genetic structure estimated by analysis of molecular variance was

substantial ($F_{ST} = 0.21$). Temporal genetic structure among age classes varied highly between populations. At one population, the divergence between adults and 2004 seedlings was low ($F_{ST} = 0.02$) whereas at another population this divergence was high ($F_{ST} = 0.26$). This latter result was consistent with local recruitment of self-fertilized seed produced by only a few parental plants. We found fine-scale spatial genetic structure at distances less than ~ 200 m, further supporting local seed and/or pollen dispersal. We posit a few self-fertile plants dominating local recruitment created substantial spatial genetic structure despite initial long-distance, human dispersal of hybrid *Spartina* through San Francisco Bay. Fine-scale genetic structure may more strongly develop when local recruits are dominated by the offspring of a few self-fertile plants.

Heredity (2011) **106**, 547–556; doi:10.1038/hdy.2010.63; published online 26 May 2010

Keywords: invasive species; hybridization; population structure; seed dispersal; microsatellites; *Spartina*

Introduction

The contemporary origin and spread of invasive hybrids pose unique opportunities to study evolutionary processes. Recently arisen invasive hybrid populations behave as 'natural laboratories' for studying the processes underlying the expanding margins of a species' range (Parisod and Bonvin, 2008). Natural hybrid zones can move in space and time, and have profound consequences for both evolutionary and conservation biology (Buggs, 2007). Hybrid swarms that have recently arisen from the union of closely related native and nonnative congeners, after the latter has been introduced in the formers range, can result in extremely fit (exceeding parental species) and invasive hybrids by transgressive segregation (Rieseberg *et al.*, 1999). To better understand the processes of invasive spread of an expanding, highly genetically diverse hybrid swarm, the underlying population genetic context and the combined structuring dynamics of hybridization history, varying trajectories of hybrid development, predominant breeding system, dispersal mechanisms and selection intensities have to be evaluated.

Hybridization admixture of two parent species can initially increase allele frequencies at all loci more than

any other known type of invasion (Long, 1991). Hybridization can erode spatial genetic structure if hybrids are viable and backcross to parent species or cross with other hybrids. However, over time structure can form in hybrid populations, depending on the genetic endowment of founding populations, the rate of spatial spread, the frequency of cross-hybrid matings and backcrosses, and spatial differences in selection (Abbott, 1992; Ellstrand and Schierenbeck, 2000). Differences in female success in hybrid classes or even single plants can lead to stronger local structure (Schnabel *et al.*, 1998; Valbuena-Carabaña *et al.*, 2007). The amount and spatial pattern of hybrid seedling recruitment can also affect fine-scale spatial genetic structure (Schnabel *et al.*, 1998).

Generally, spatial genetic structure created during colonization can persist on several geographical scales (Austerlitz and Garnier-Géré, 2003; Epperson *et al.*, 2003; Williams *et al.*, 2007). Greater spatial structure will result from an ancestry of colonists that includes few adults, resulting in genetic distinction among populations even if within-population variation is low (Levin, 1981; Slatkin, 1987). Genetic drift, where changes in allele frequencies result from chance mating events in very small populations (Primack and Kang, 1989; Husband and Schemske, 1996), and the effect of distinct environmental optima affecting fitness can also cause structure as populations diverge. Founding events are accompanied by a change from the original population allele frequency (founder effect), as the members of the new colonizing population contain a random allelic subsam-

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Received 14 November 2009; revised 15 April 2010; accepted 23 April 2010; published online 26 May 2010

ple that may deviate from the overall allele frequencies in its native range. By and large, the ancestry of local introductions is a biased sample of genotypes from the native range; however, multiple separate introductions and long-distance dispersal can erode the spatial structure arising early in an introduction (Lambrinos, 2004).

Density, dispersion and breeding system of reproducing adults as well as dispersal mechanisms have central roles in generating the spatial genetic structure of populations (Vekemans and Hardy, 2004). For plants, the spatial scale and patterns of seed and pollen dispersal combine in this central role. In many plant species, seed dispersal yields a long-tailed, leptokurtic, distribution, in which most seeds land near the parental plant, with only a few going far away (LeCorre *et al.*, 1997; Donoghue, 1998; Higgins and Richardson, 1999). Species with long-distance seed dispersal, through either biotic or abiotic means, are expected to show significantly less within-population structure than species with limited dispersal (Hamrick and Nason, 1996). So, populations with common long-distance dispersal of either pollen or seeds should tend to be genetically more similar and display less spatial structure, whereas limited dispersal should result in genetically structured populations and eventually lead to pronounced population divergence.

Intertidal *Spartina* species and their hybrids have invaded worldwide, and their spread after human introduction to new regions shows high potential for long-distance dispersal (Strong and Ayres, 2009). *Spartina* seed floats for several weeks and can disperse widely on intertidal tides and currents. Seeds remain viable for 6–8 months, and germinate in the spring following late summer or autumn seed set (Huiskes *et al.*, 1995). The spread of *S. alterniflora* to ca. 6000 hectares and ca. 27% of intertidal lands in Willapa Bay, WA during the ca. 110 years since its introduction was the result of floating seed carried on currents, rather than dispersion of rhizome fragments (Civille *et al.*, 2005). Anemophilous *Spartina* pollination can be surprisingly local. Field experiments showed dense pollen clouds to be so diluted as to be ineffectual within 100 m, and plants isolated by even a few tens of meters produced no seed in obligately outcrossing *S. alterniflora* (Davis *et al.*, 2004).

In San Francisco Bay, a rapid invasion of hybrid *Spartina* occurred after *S. alterniflora* from the Atlantic and Gulf coast of North America hybridized with the California native species, *S. foliosa*. *S. alterniflora* was deliberately planted at a former salt pond at Alameda Creek, Fremont in the 1970s (Figure 1; Faber, 2000); it is inferred that the first hybridizations occurred there (Daehler and Strong, 1997; Ayres *et al.*, 1999). Introgressive hybridization, potentially occurring in parallel at more than one geographic location over time, threatens the native *S. foliosa* with local extinction, and has left only a few nonhybrid *S. alterniflora* in San Francisco Bay. Whereas backcrossing produced a diverse array of advanced generation hybrid genotypes within a period of 30 years (Ayres *et al.*, 1999). Hybrids were planted in a few sites and spread by floating seed to the shores of much of South San Francisco Bay and a few spots north of the Golden Gate (Ayres *et al.*, 2004). The subset of genotypes that grew faster, attained larger size, greater



Figure 1 Map of San Francisco Bay. Study sites: Elsie Roemer Marsh, Alameda; Robert's Landing and Hayward. (The site of the first *Spartina alterniflora* introduction to San Francisco Bay at Alameda Creek, Fremont is also shown.)

culm density, higher pollen production and/or much higher self-fertility than either parental species have driven the invasion of San Francisco Bay (Sloop *et al.*, 2009). *Spartina* hybrids have further expanded into San Francisco Bay tidal mudflats (lower in elevation than shoreline marshes), where neither parent species can persist.

This study explores spatial and temporal genetic structure of expanding hybrid populations early in the invasion. We assessed allelic diversity, and spatial and temporal genetic structure of descendants of founding hybrids, and their progeny in this area. We posited that the high potential for long-distance dispersal of floating seed might preclude local structure among these hybrid populations. Conversely, locally restricted seed and pollen dispersal and plant establishment could generate spatial genetic structure. Microevolutionary development of distinct hybrid populations, in which natural selection acts upon a subset of fit hybrids, may proceed more rapidly when those populations are genetically and spatially isolated. Here we sought to determine the spatial and temporal genetic structure of hybrid populations by examining the genetic relationships between putative hybrid founders along shore and their alleged descendants in adjacent mudflats, and mudflat seedling populations across 2 successive years.

Materials and methods

Study sites

Hybrid *Spartina* grows from high in the salt marsh, among other terrestrial vegetation, down to open mud in the low intertidal, whereas the parental species grow only in the upper part of the gradient in San Francisco Bay (Ayres *et al.*, 2004, 2008). We studied invading hybrid *Spartina* and parent species populations at three distinct geographic locations along 18 km of San Francisco Bay shoreline south of Oakland, CA during 2003 and 2004. From north to south the populations were: Elsie Roemer Marsh at Alameda (Figure 1); Robert's Landing (Figure 1) 16 km south of Alameda and Hayward (Figure 1) another 2 km further south (Sloop *et al.*, 2009). The remainder of this 18 km of shoreline was virtually un-invaded by hybrid *Spartina* and without parent species at that time. We used aerial photographs and visual assessments during field surveys to locate all *Spartina* plants along 20 km of this shoreline, between Elsie Roemer Marsh to the north and the Cogswell Marsh in Hayward to the south. The site of the original hybridization was New Alameda Creek (Figure 1), 8 km further south of Cogswell Marsh.

The Alameda *Spartina* population at Elsie Roemer Marsh began in the 1970s when *S. alterniflora* and *Spartina* hybrids (*S. alterniflora* × *S. foliosa*), which were unknown at that time, were planted by the US Army Corps of Engineers; native *S. foliosa* also grew at Alameda at this time (US Army Corps of Engineers, 1978; Faber, 2000). These hybrids have since formed a continuous meadow along the shore at Elsie Roemer Marsh, Alameda (Figure 1; Ayres *et al.*, 1999; Sloop *et al.*, 2009). Seven hybrid plants colonized the tidal flats to form isolated, island-like patches in the center of the Alameda estuary.

The Robert's Landing population consisted of scattered plants on a tidal flat that was connected to an inland salt marsh meadow by a narrow tidal channel.

Aerial photographs taken in 1986 and 2003 (Supplementary Figure 1) indicated that the tidal flat was colonized before 1986, whereas the inland meadow was opened to tidal action in 1994 (M Taylor, Hayward Regional Shoreline, personal communication). Hybrid *Spartina* was present in both of these habitats in our earliest surveys (Ayres *et al.*, 1999).

Hayward, the third population, consisted of a few *S. foliosa* and hybrids along shore and some hybrid plants scattered on the mudflat. Photographic evidence indicates that this shoreline was colonized before 1986.

Genetic analysis

We studied cordgrass spatial genetic structure by assaying all hybrid *Spartina* adults growing on tidal flats at Alameda, Robert's Landing and Hayward (Table 1) at 17 microsatellite loci: SPAR.01, SPAR.08, SPAR.09, SPAR.10, SPAR.11, SPAR.15, SPAR.16, SPAR.17, SPAR.18, SPAR.20, SPAR.21, SPAR.23, SPAR.26, SPAR.27, SPAR.28, SPAR.29 and SPAR.33 (Blum *et al.*, 2004; Sloop *et al.*, 2005). We extracted DNA from leaf samples using Qiagen DNeasy Plant Mini Kits (Qiagen, Valencia, CA, USA). PCR volumes and genotyping procedures are outlined by Sloop *et al.* (2005).

We also collected samples from 25 hybrid adults from the adjacent marsh population at Elsie Roemer marsh at Alameda, and 30 adults from the inland *Spartina* hybrid marsh at Robert's Landing. In addition, we assayed seedlings (distinguished from adults by their size of <1–10 cm, by having only one or a few stems, and occasionally by the presence of a seed coat) growing on tidal flats at these sites. In 2003, we sampled by haphazardly collecting leaf samples from about 11% of all seedlings ($n = 109$) over the full spatial extent of the seedling cloud per tidal flat site (Table 1), whereas in 2004, we collected seedling DNA samples by walking two parallel transects along the length of the shoreline

Table 1 Population mean observed and expected heterozygosities over all 17 loci

Population	n	H_e (s.d.)	H_e^* (s.d.)	H_o (s.d.)	F_{IS}	N alleles
<i>Parent species:</i>						
<i>S. foliosa</i> (range wide)	50	0.11 (0.18)	0.12 (0.19)	0.09 (0.25)	NA	1.6
<i>S. alterniflora</i> (range wide)	35	0.75 (0.12)	0.78 (0.13)	0.46 (0.20)	NA	8.67
Alameda <i>S. alterniflora</i>	5	0.64 (0.12)	0.71 (0.13)	0.61 (0.32)	−0.15	4.21
Alameda <i>S. foliosa</i>	extinct	—	—	—	—	—
<i>Hybrids</i>						
<i>Alameda</i>						
Meadow	25	0.65 (0.13)	0.66 (0.12)	0.59 (0.22)	0	4.76
Tidal flat	9	0.64 (0.12)	0.69 (0.13)	0.49 (0.22)	0.21*	4.29
Seedlings 2003	33	0.69 (0.10)	0.71 (0.10)	0.54 (0.22)	0.07*	5.24
Seedlings 2004	67	0.69 (0.09)	0.70 (0.09)	0.52 (0.20)	0.09*	5.65
<i>Robert's Landing</i>						
Meadow	30	0.60 (0.17)	0.61 (0.18)	0.52 (0.23)	0.03	5.06
Tidal flat	17	0.66 (0.12)	0.68 (0.13)	0.40 (0.22)	0.39*	4.41
Seedlings 2003	42	0.60 (0.13)	0.61 (0.14)	0.41 (0.18)	0.11*	4.59
Seedlings 2004	54	0.50 (0.14)	0.51 (0.15)	0.30 (0.15)	0.31*	5.47
<i>Hayward</i>						
Tidal flat	24	0.61 (0.11)	0.62 (0.11)	0.46 (0.20)	−0.05	5.18
Seedlings 2003	32	0.62 (0.09)	0.65 (0.10)	0.36 (0.16)	0.17*	3.65
Seedlings 2004	69	0.54 (0.13)	0.54 (0.13)	0.43 (0.19)	0.12*	5.12

H_e^* calculated without bias (Nei, 1978); n = number of plants sampled, s.d. = standard deviation.

*Significant population specific F_{IS} indices (1023 permutations) at $P < 0.05$; N alleles = mean number of alleles per locus.

tidal flats, collecting a sample every 20 paces, resulting in 4% of all seedlings ($n = 190$) (Table 1). We used Global Positioning System loggers (Trimble GeoExplorer 3.0, Sunnyvale, CA, USA) to map the geographic locations of all genetically surveyed adults and seedlings.

We further examined the parental species throughout their range: *S. foliosa*, northern California: 10 plants from the Hayward shoreline (Figure 1) and 29 plants from six sites in San Francisco Bay (other than Alameda, Robert's Landing and Hayward), Bolinas and Tomales Bay; *S. foliosa*, southern California: Chula Vista, and northern Mexico (Tijuana); *S. alterniflora*: 27 plants from six Atlantic Coast/Gulf Coast locations (New York, Georgia, Florida, Alabama and Mississippi), and three sites in Willapa Bay, WA (Palix River, Tower Slough and Peninsula), and 5 plants growing on the shoreline at Elsie Roemer Marsh at Alameda (Table 1). *S. alterniflora* is rare in San Francisco Bay and occurs only at a few sites. A previous analysis using randomly amplified polymorphic DNA (D Ayres, unpublished data) helped to identify *S. alterniflora* plants in San Francisco Bay. Species-specific loci were developed from genetic surveys of 50 individuals of *S. alterniflora* and 50 individuals of *S. foliosa* obtained from throughout their ranges. Species-specific alleles had to be found in at least 10% or greater of sampled individuals per species, and had to be entirely absent in all sampled individuals of the other species. Of 17 microsatellite loci, 9 contained 15 *S. foliosa*-specific alleles (not found in *S. alterniflora*, yet introgressed into San Francisco Bay hybrid *Spartina*), and for 15 loci, 1–7 alleles per locus were found only in hybrid individuals. We found 39 *S. alterniflora* species-specific alleles at 15 loci. We calculated allelic frequencies, and observed (H_o) and expected (H_e) heterozygosity using GENETIX (Belkhir *et al.*, 1996–2004). We carried out Hardy–Weinberg (H-W) exact tests (using Markov chain default settings) for all eight adult populations using GENEPOP (Raymond and Rousset, 1995, <http://wbimed.curtin.edu.au/genepop/genepop/>).

Bayesian cluster analysis

To infer population structure and assign individuals to populations, we applied a model-based Bayesian clustering method to all multilocus genotypes using STRUCTURE (Pritchard *et al.*, 2000; Falush *et al.*, 2003; Pritchard and Wen, 2004). In this analysis, if there is genetic admixture due to hybridization, individuals are probabilistically assigned to either a single cluster (the population of origin), or more than one cluster (the parental populations, Falush *et al.*, 2003). The program assumes the neutral unlinked markers to be in Hardy–Weinberg equilibrium (HWE) and linkage equilibrium and that recent hybridization or migration would likely produce departures from these equilibria. STRUCTURE identifies the K unknown populations (genetic clusters) of individuals' origin and concurrently allocates all individuals to populations, giving their 90% confidence intervals. STRUCTURE was run using the 'admixture model' and correlated allele frequencies, with a burn-in period of 10 000, followed by 100 000 iterations. To detect the true number of clusters (K), we followed the graphical methods and algorithms outlined by Evanno *et al.* (2005). Under the assumption that the sampled

plants belong to an unknown number of K genetically distinct clusters, we used priors from 2 to 14 to estimate the average posterior probability values for K (log-likelihood; $\ln L$) for 20 runs each. This method established $K = 4$ as the best value of K for our data, distinguishing two parent species clusters and two *Spartina* hybrid groups.

Analysis of molecular variance

To quantify hybrid population structure, we used analysis of molecular variance using Arlequin 3.11 (Excoffier *et al.*, 2005). To determine the spatial scale of genetic structure, we compared hybrid populations by geographic location, and to ascertain the temporal variation in genetic structure, we evaluated differences between adult descendents and two consecutive generations of seedlings in the tidal flats at each site. Using Arlequin, we further calculated population pairwise F_{ST} values and population specific F_{IS} indices across all loci.

Temporal genetic structure

Hybrid 'descendents' from previously established hybrid 'founders' were determined using aerial photographs (Supplementary Figure 1; digital orthophoto quadrangles dating from 1993 obtained from the USGS; and historical (1986) and contemporary aerial photos (2003), obtained from the Invasive *Spartina* Project), historical documents (USACE, 1978) and the personal observation of managers of the East Bay Regional Parks District (M Taylor, Park Ranger). According to these aerial photographs and historical accounts the primary hybrid founders were meadow plants at Alameda and specific tidal flat plants at both Hayward and Robert's Landing. The descendents are those plants that established subsequent to these initial founders; at Alameda these are the plants growing on the tidal flats; and at Robert's Landing these are the plants growing in the adjacent meadow, opened to tidal action in 1994. Seedlings are those we surveyed on tidal flats during 2003 and 2004. Because in wind pollinated *Spartina*, pollination distances are relatively short (Davis *et al.*, 2004), and our previous work in this system has shown that seed dispersal is local (Sloop *et al.*, 2009), we feel confident that many of our hypothesized descendents really are descended from the early founders.

Spatial genetic analysis

To determine fine-scale spatial genetic structure within populations, we used spatial genetic analysis. This analysis shows the relationship between geographical distance and genetic relatedness between individuals. This type of analysis is based on the mean genetic distance between pairs of individuals of the same distance class (Degen *et al.*, 2001). Within-population distograms were produced using Spatial Genetic Software v.1.0d (Degen, 2000; Degen *et al.*, 2001), comparing genetic distances with geographic distances, using the number of alleles/haplotypes in common over all loci, between pairs of individuals belonging to a given spatial distance class (adapted from Surlles *et al.* (1990) and Hamrick *et al.* (1993)). Values exceeding the reference value indicate positive spatial genetic structure (that is, individuals are more related than by random chance), and values below the reference value show negative

spatial structure (that is, individuals are less related than by random chance) (Degen, 2000). Permutation tests were used to evaluate the statistical significance of all measures, to assess deviations from a spatially random distribution. A total of 1000 permutations were performed to obtain 95% confidence intervals under the hypothesis of no spatial correlation. The data set consisted of all genetically surveyed adults and seedlings (Table 1).

Results

We found no native California cordgrass, *S. foliosa*, at either Alameda in the north or Robert's Landing in the south, based on the molecular determinations in this study. Introduced *S. alterniflora* was extremely rare at Alameda and occurred only at the upper end of the tidal gradient among hybrid *Spartina* and other species of plants. No *S. alterniflora* was detected at the other two sites. The nine large plants that established upon the tidal flat at Alameda during the decade preceding this study were all hybrids; all of the seedlings recruiting around these tidal flat plants were hybrids. At Robert's Landing all adults and seedlings were hybrid *Spartina*; we found no plants of the parental species. The Hayward *Spartina* included 10 native *S. foliosa* plants growing along shore and 24 large adult *Spartina* hybrids growing on the tidal flats well below other vegetation. All of the tested 299 seedlings at all sites were hybrid. We found neither native *S. foliosa* nor *S. alterniflora* growing on tidal flats at any of the three sites.

Genetic variation

S. foliosa was genetically depauperate throughout its range with an average of only 1.6 (± 0.9) alleles per locus. Only 5 of 17 loci were polymorphic in *S. foliosa*, mainly indicating distinctions between San Francisco Bay and the southern California/northern Mexico group. The Hayward *S. foliosa* population had only two polymorphic loci. *S. alterniflora* had genetic diversity range roughly five times more than *S. foliosa*, with an average of 8.7 (± 3.0) alleles per locus. San Francisco Bay *S. alterniflora* at Alameda had lower average allelic diversity with 4.2 (± 1.4) alleles per locus, compared to the range wide *S. alterniflora* value. This is consistent with the introduction history of *S. alterniflora* into San Francisco Bay from a single Virginia *S. alterniflora* source (Faber, 2000). We found hybrid *Spartina* allelic diversity to range from 3.65 to 5.65 alleles per locus.

All adult hybrid and *S. alterniflora* individuals had unique genetic fingerprints (differing at >3 alleles) confirming that we did not sample clones more than once. We found 21 unique *S. foliosa* multilocus genotypes (in 43 samples, differing at only one or two alleles); some *S. foliosa* plants with the same genotypes were present at more than one sampling site, whereas genetically indistinguishable plants were spatially discrete within sites, indicating that the low genetic variation detected in this species could not resolve all individuals' genotypes. All seedlings assayed in this study were hybrids. We found no *S. alterniflora* during sampling at either Robert's Landing or Hayward.

All seedling populations were inbred as assessed by the inbreeding coefficient F_{IS} (Table 1). Adult tidal flat

plants at Robert's Landing were the most inbred of established plants, followed by the tidal flat adults at Alameda. Inbreeding was not detected in *Spartina* in the shoreline meadows at Robert's Landing and Alameda, or among Hayward adults growing on open mud below the meadows. Adults of neither hybrids nor *S. foliosa* at Hayward were inbred.

H-W exact tests, examining the random union of gametes, showed more loci to be in H-W equilibrium (HWE) at Alameda (Alameda meadow = 77% of loci in HWE, Alameda tidal flat = 94% of loci in HWE, Alameda *S. alterniflora* = 88% of loci in HWE), and in the meadow at Robert's Landing (Robert's Landing = 65% of loci in HWE), than in the tidal flats at Robert's Landing (12% of loci in HWE), and Hayward (18%). Loci in *S. foliosa* populations were either monomorphic (65% of loci in HWE), or deviated from H-W (35% of loci in HWE). In a mix of *S. alterniflora* populations from the native range on the Atlantic and Gulf coasts of North America, only 12% of tested loci showed HWE, as expected for a combination of individuals from several geographically distinct source populations.

Bayesian cluster analysis

Bayesian cluster analysis clearly distinguished *S. foliosa* (blue color in Figure 2), native range *S. alterniflora* (red color in Figure 2) and the *S. alterniflora* plants at Alameda (yellow color in Figure 2). It also confirmed at least two distinct hybrid membership groups. The first group aligned Alameda hybrid plants (Figure 2, groups 4–7) with the Alameda *S. alterniflora* (Figure 2, group 3). A second group included the hybrid *Spartina* at Robert's Landing (Figure 2, groups 11–14) and Hayward (Figure 2, groups 8–10). At all tidal flat sites, seedling groups (Figure 2, groups 6, 7, 9, 10, 13 and 14) are most like adults at the same location, shown by similar color patterns (Figure 2, groups 4, 5, 8, 11 and 12).

Analysis of molecular variance

We found a large regional genetic distinction between plants at Alameda and the Hayward/Robert's Landing populations: $F_{ST} = 0.21$ (Table 2). Genetic distinction between neighboring Hayward and Robert's Landing adults was lower and ranged between $F_{ST} = 0.08$ (across mudflats at both sites, Table 3), and $F_{ST} = 0.01$ (Hayward mudflat versus Robert's Landing inland marsh, Table 3). Population structure among all three sites increased when both seedling generations from 2003 and 2004 were included: $F_{CT} = 0.08$ (Table 4) adults only among three populations, $F_{CT} = 0.11$ adults plus seedlings among three populations (Table 5). Generation differences (founders versus descendants versus seedlings) varied among populations (Table 6). There were small generational genetic differences at Alameda and Hayward ($F_{ST} < 0.03$), whereas there were more pronounced generational differences at Robert's Landing between primary founders, and their descendants and successive seedling generations; that is, the F_{ST} between founders and 2004 seedlings was 0.26. This general trend was independent of the potential influence of unequal seedling sample sizes on F_{ST} results, as determined by a randomized resampling of the 2004 seedling data to equalize sample sizes.

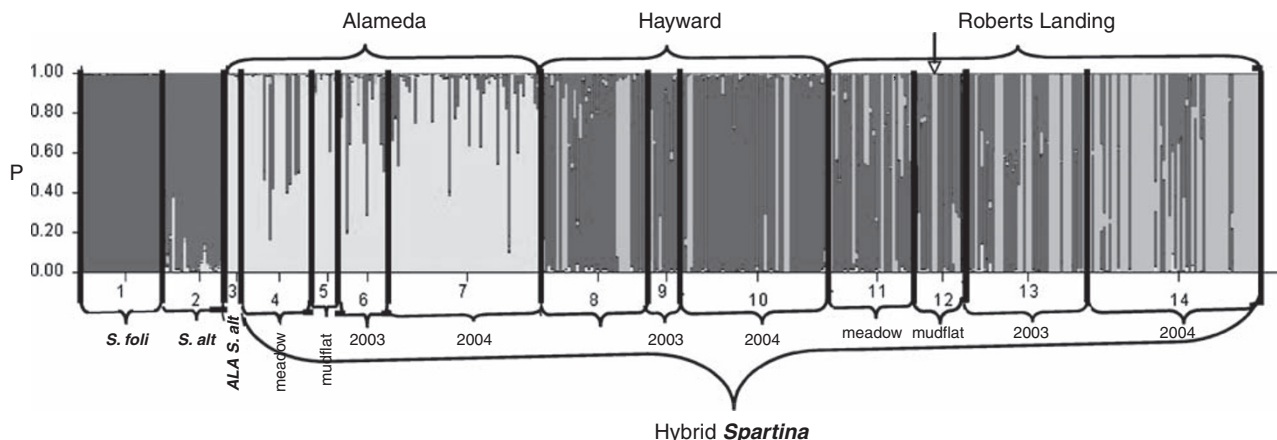


Figure 2 Bar plot estimated by STRUCTURE depicting group membership (hybrid ancestry), representing each individual as a line segment. Each segment is partitioned into $K = 4$ shaded (colored) components, representing the individual's estimated membership coefficients in the K clusters (y axis). Numbers (x axis) represent the initial geographic/demographic groupings: 1 = *S. foliosa*, 2 = *S. alterniflora*, 3 = Alameda *S. alterniflora*, 4 = Alameda meadow, 5 = Alameda mudflat, 6 = Alameda 2003 seedlings, 7 = Alameda 2004 seedlings, 8 = Hayward shoreline mudflat, 9 = Hayward 2003 seedlings, 10 = Hayward 2004 seedlings, 11 = Robert's Landing meadow, 12 = Robert's Landing mudflat, 13 = Robert's Landing 2003 seedlings, 14 = Robert's Landing 2004 seedlings. Arrow indicates Robert's Landing plant 14 (only green in group 12). A full color version of this figure is available at the *Heredity* Journal online.

Table 2 Analysis of molecular variance (average over 17 loci) among regions 1 and 2

Source of variation	d.f.	Sum of squares	Variance components	Percentage variation
Among regions	1	131.232	0.32044	16.63**
Among individuals within regions	9	76.051	0.08898	4.62**
Within individuals	895	1358.208	1.51755	78.75**
Total	905	1565.491	1.92698	

Region 1: North, Alameda (adult *S. alterniflora* and hybrid shoreline meadow, mudflat hybrid adults and 2003 and 2004 seedlings).

Region 2: South, Robert's Landing (adult hybrid shoreline meadow and mudflat, and 2003 and 2004 mudflat hybrid seedlings) and Hayward (adult *S. foliosa* and hybrid shoreline plants, and 2003 and 2004 mudflat hybrid adults and seedlings).

Average F -statistics over all loci. Fixation indices: F_{ST} , 0.21; F_{SC} , 0.06; F_{CT} , 0.17.

**Significance at $P < 0.0001$.

Table 3 Population pairwise F_{ST} values

	Adults RL mudflat	Adults RL meadow	Seedlings RL 2003	Seedlings RL 2004	Adults HAY mudflat	Adults HAY <i>S. foliosa</i>	Seedlings HAY 2003	Seedlings HAY 2004	Adults ALA mudflat	Adults ALA meadow	Adults ALA meadow <i>S. alterniflora</i>	Seedlings ALA 2003	Seedlings ALA 2004
Adults RL mudflat	0.00												
Adults RL meadow	0.06	0.00											
Seedlings RL 2003	0.17	0.04	0.00										
Seedlings RL 2004	0.26	0.11	0.03	0.00									
Adults HAY mudflat	0.08	0.01	0.04	0.11	0.00								
Adults HAY <i>S. foliosa</i>	0.17	0.27	0.41	0.50	0.28	0.00							
Seedlings HAY 2003	0.13	0.04	0.03	0.08	0.01	0.38	0.00						
Seedlings HAY 2004	0.14	0.04	0.05	0.10	0.03	0.35	0.01	0.00					
Adults ALA mudflat	0.27	0.20	0.22	0.27	0.18	0.51	0.21	0.24	0.00				
Adults ALA meadow	0.20	0.16	0.16	0.21	0.15	0.39	0.16	0.19	0.01	0.00			
Adults ALA meadow <i>S. alterniflora</i>	0.31	0.25	0.27	0.31	0.24	0.61	0.25	0.28	0.02	0.04	0.00		
Seedlings ALA 2003	0.19	0.15	0.16	0.21	0.13	0.38	0.14	0.16	0.03	0.02	0.03	0.00	
Seedlings ALA 2004	0.22	0.18	0.20	0.24	0.16	0.38	0.18	0.21	0.02	0.02	0.02	0.02	0.00

Abbreviations: ALA, Alameda; HAY, Hayward; RL, Robert's Landing. Bold values indicate significance at $P < 0.05$.

Spatial genetic analysis

Spatial genetic analysis (Figure 3) showed significant positive spatial structure between adults and seedlings up to a distance of ca. 200 m at all three sites, and

negative spatial structure above a distance of approximately 440 m at Hayward and above ~530 m at Robert's Landing (Figure 3). At Alameda there were too few individuals in a distance class > 200 m to include in the

Table 4 AMOVA among sites: adults only

Source of variation	d.f.	Sum of squares	Variance components	Percentage variation
Among sites	2	51.500	0.16367	7.50**
Among adult populations within sites	4	29.223	0.19799	9.08*
Among individuals within populations	122	234.025	0.09865	4.52*
Within individuals	129	222.000	1.72093	78.90
Total	257	536.748	2.18124	

Site 1: Alameda, *S. alterniflora* and hybrid shoreline meadow adults, mudflat hybrid adults.

Site 2: Robert's Landing, adult hybrid shoreline meadow and mudflat.

Site 3: Hayward, adult *S. foliosa* and hybrid shoreline plants.

**Significance at $P < 0.0001$. *Significance at $P < 0.05$.

Average F-statistics over all loci. Fixation indices: F_{IS} , 0.05; F_{SC} , 0.09; F_{CT} , 0.08; F_{IT} , 0.21. F_{IS} , among individuals within populations; F_{SC} , among adult populations but within sites; F_{CT} , adult populations among sites; F_{IT} , among adult populations and among sites.

Table 5 AMOVA among three sites: across generations

Source of variation	d.f.	Sum of squares	Variance components	Percentage variation
Among sites	2	173.934	0.23677	11.12**
Among generations within sites	10	94.474	0.11990	5.63**
Among individuals within generations	452	898.340	0.21471	10.08**
Within individuals	465	724.500	1.55806	73.17
Total	929	1891.248	2.12944	

Site 1: Alameda, *S. alterniflora* and hybrid shoreline meadow adults, mudflat hybrid adults and 2003 and 2004 seedlings.

Site 2: Robert's Landing, adult hybrid shoreline meadow and mudflat, and 2003 and 2004 mudflat hybrid seedlings.

Site 3: Hayward, adult *S. foliosa* and hybrid shoreline plants, and 2003 and 2004 mudflat hybrid adults and seedlings.

**Significance at $P < 0.0001$. *Significance at $P < 0.001$.

Average F-statistics over all loci. Fixation indices: F_{IS} , 0.12; F_{SC} , 0.06; F_{CT} , 0.11; F_{IT} , 0.27. F_{IS} , among individuals within generations; F_{SC} , among generations but within sites; F_{CT} , generations among sites; F_{IT} , among generations and among sites.

Table 6 Temporal genetic structure among generations of individuals: primary founders, descendants and seedlings at three population sites of hybrid *Spartina*

Primary founders	Descendants	2003 Seedlings	2004 Seedlings
Alameda meadow	0.01	0.02*	0.02*
Robert's Landing mudflat	0.06*	0.17*	0.26*
Hayward mudflat	NA	0.01	0.03*

* F_{ST} difference at $P < 0.05$.

analysis (minimum value = 30 individuals per distance class; Degen, 2000).

Discussion

Spatial genetic structure existed among and temporal genetic structure existed within *Spartina* hybrid populations in San Francisco Bay. We found genetically distinct hybrid populations at Alameda and the Robert's Landing/Hayward shorelines ($F_{CT} = 0.21$) that likely resulted from different founding histories: human plantings versus natural tidal establishment. We posit that at Alameda, *S. alterniflora*, F1 and early generation hybrids were planted together, interbred and the hybrids proliferated. Our Bayesian analysis supported the view that the preponderance of introgression occurred with a set of *S. alterniflora* ancestors (yellow color in Figure 2) at Alameda that was genetically distinct from the *S. alterniflora* ancestors of the southern populations. This may have been due to a founding effect where only a small number of the original *S. alterniflora* plants were transplanted or due to a second, unreported, introduction of *S. alterniflora*, from a different East Coast source

population, at Alameda. To the detriment of both parental species, hybrids spread clonally, and interbred with neighboring hybrids (Sloop *et al.*, 2009). This resulted in a majority of loci in HWE with low linkage disequilibrium across loci. *S. foliosa* introgressive hybrids were either less frequent there, or possibly lost out in competition. Presently, there are no *S. foliosa* plants at Alameda (Ayres *et al.*, 2004).

We propose that the development of spatial genetic structure proceeded differently at Robert's Landing and Hayward. As neither hybrids nor *S. alterniflora* were planted at these sites, these shorelines were colonized by hybrid seed floating northward from invaded marshes to the south, the original plantation of *S. alterniflora* being 8 km to the south at New Alameda Creek (Faber, 2000). This colonization trajectory would have taken much more time than the human-aided hybrid establishment at Alameda allowing natural selection to act upon these later-generation hybrids. In previous work we have found that advanced generation hybrids, but not the parental species or early generation hybrids, are self-fertile (Sloop *et al.*, 2009). One outcome of self-fertility was reflected by a relatively low number of loci in HWE along the southern shoreline compared to Alameda. As the presence of native *S. foliosa* at the Hayward shoreline predates the arrival of hybrids, colonizing hybrids backcrossed with native *S. foliosa*, as suggested by high levels of *S. foliosa* alleles (blue color) in Hayward plants in Figure 2. The lower level of genetic structure between populations at Robert's Landing and Hayward ($F_{ST} = 0.08$, mudflat adults) suggests that seed movement on tidal currents may link these neighboring sites, 2 km apart. In comparison, our results imply that little if any gene flow has occurred between Alameda,

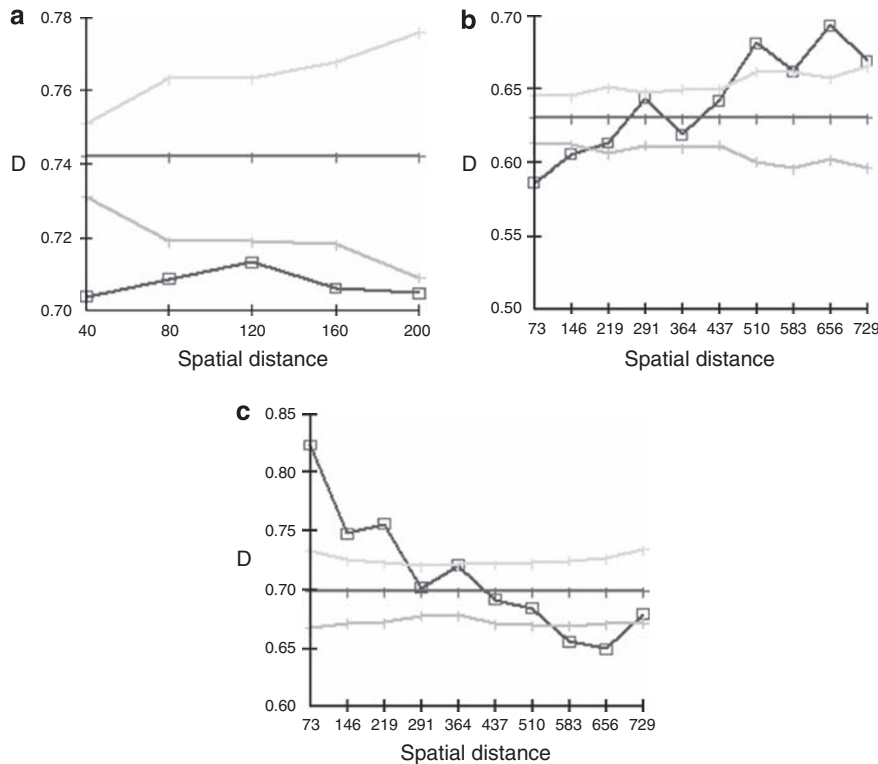


Figure 3 Spatial genetic analysis using the number of alleles/haplotypes in common over all loci for hybrid adults and seedlings at the three population sites (**a**, Alameda; **b**, Hayward; **c**, Robert's Landing) performed with SGS (Degen *et al.*, 2001). Values exceeding the reference value (95% confidence intervals—blue and green lines) indicate positive spatial genetic structure, and values below the reference line (red) show negative spatial structure (Degen, 2000). The spatial distance unit is meters. A full color version of this figure is available at the *Heredity* Journal online.

16 km northward, and the Robert's Landing and Hayward sites ($F_{ST} = 0.21$).

Many hybrids contained unique alleles. As most of the unique hybrid alleles were at relatively low frequencies (0.02–0.13), this may point to the allelic composition(s) of one or more *S. alterniflora* ancestors not present in our samples. Alternatively, these unique alleles may be due to genomic changes, including chromosomal rearrangements, differential gene expression, and gene silencing as has been noted to occur in hybridizing species (Baack and Rieseberg, 2007).

Despite the potential for high rates of gene flow through seed dispersal in the dynamic San Francisco Bay tidal system, parentage results confirmed that high proportions of seedlings were the progeny of nearby plants (Sloop *et al.*, 2009). Thus, predominately local seedling recruitment maintains the distinct genetic structure between Alameda and Robert's Landing/Hayward. As an extreme example of local parentage, a single plant at Robert's Landing produced, by selfing, 10% of all seedlings we genotyped there (Sloop *et al.*, 2009, see arrow Figure 2). Selfing combined with local seed dispersal can lead to the development of fine-scale genetic structure, as has been shown in hybrid oaks by Valbuena-Carabaña *et al.* (2007).

Our finding of seedling spatial autocorrelation distances of <200 m (Figure 3) indicated that within a 200 m distance, seedlings are genetically more similar to each other than expected by chance, as perhaps a plant surrounded by its seedlings. This further confirmed the local dispersal of seeds suggested by our findings of fine-scale spatial genetic structure of seedlings, and paternity

analyses in previous studies (Sloop *et al.*, 2009). Plants >440 m apart, on the other hand, are more genetically dissimilar than expected by chance (Figure 3). This dissimilarity is likely above a certain geographic distance, where seeds of genetically and geographically distant plants have settled out of the tidal waters. The Alameda site was too small to make autocorrelations at >200 m (Figure 3).

We found that genetic structure changed over time among generations within populations (Table 6). At Alameda the primary founders were meadow plants growing high in the tidal range and among other plant species. Tidal flat descendants (growing alone on open mud) were similar to the meadow founders while seedlings from successive years showed increasing genetic divergence from the founding population (Table 6). At Robert's Landing F_{ST} values between founders, descendants and 2003 and 2004 generations of seedling increased from 0.06 to 0.17 to 0.26, respectively, showing a substantially growing genetic distinction from the founders over generations (Table 6). At Hayward the temporal dynamics were similar to, but weaker than those at Robert's Landing. Increased production of self-fertilized seedlings especially at Robert's Landing and Hayward from 2003 to 2004 (Table 1, Sloop *et al.*, 2009) may explain these changes in genetic structure and are also reflected by increased inbreeding coefficients (Table 1). We found in previous work that self-seed set under experimental pollen exclusion treatments was much higher in the later-generation hybrids growing upon tidal flats than in early generation hybrids or the parental species (Sloop *et al.*, 2009).

Increased self-compatibility of isolated tidal flat colonizers and their production of inbred recruits can result in lower levels of heterozygosity than expected under HWE, and higher levels of inbreeding (Sloop *et al.*, 2009). In open San Francisco Bay tidal flats, colonizing hybrid family groups growing in isolation have set self-fertilized seeds, and have thus overcome the pollen limitation seen in invading, self-incompatible *S. alterniflora* in Willapa Bay, WA (Davis *et al.*, 2004; Taylor *et al.*, 2004). There, pollen limitation of isolated plants caused an Allee effect that greatly slowed the tidal flat invasion (Davis *et al.*, 2004; Taylor *et al.*, 2004). In San Francisco Bay, these few self-compatible hybrids, having adapted to environmentally challenging conditions, maximize their reproductive fitness by producing large numbers of self-fertilized seeds in isolation (Daehler, 1998; Sloop *et al.*, 2009). These seeds then spread on the tide and established as vigorous seedlings throughout the tidal flat, over time dominating this environmentally harsh habitat. Producing offspring in this way may greatly increase the rate of the colonization of new and challenging environments by maximizing the effects of selective forces on highly adapted genotypes, and so avoiding both inbreeding and outbreeding depression during this difficult phase of invasion (Lynch, 1991; Waser and Price, 1994; Sloop *et al.*, 2009).

The natural stepping-stone dispersal mechanism (Kimura and Weiss, 1964) and human movement of hybrid plants (for example, unintentional use of cryptic hybrid plants rather than natives in restoration efforts) have increased hybrid dispersal and influenced hybrid population genetic structure by introducing varying population founding trajectories at various sites and times around San Francisco Bay. Genetic structure in San Francisco Bay hybrid populations has been affected by a number of different founding events: site-specific environmental dynamics affecting colonization rates, and relative isolation from other populations over time. The proximity of a native or hybrid marsh, the distance between established adult populations, wind directions, tidal currents and environmental selection forces determined the rate and direction of local tidal flat seed and pollen dispersal/gene flow. Our findings therefore suggest that even though long-distance dispersal has been manifestly important in the very rapid spread of hybrid *Spartina* throughout San Francisco Bay over the past 35 years (Ayres *et al.*, 2004), local recruitment and the evolution of self-fertility (Sloop *et al.*, 2009) have also contributed, generating substantial regional, fine-scale and temporal genetic structure. Our findings provide a glimpse in the evolutionary dynamics that underlie the expansion of invasive hybrids and the colonization of new habitats as well as those occupied by the parent species.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank J Bando, H Davis, J Lambrinos, H McGray, A Lee and R Hall for their assistance. This work was supported by the California Coastal Conservancy (CalFed Grant No. 99-110), California Sea Grant (Project

R/CZ-176) and the National Science Foundation Biocomplexity program (DEB No. 0083583).

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Supplementary Information accompanies the paper on Heredity website (<http://www.nature.com/hdy>)