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Reproductive and Recruitment Dynamics of Invasive Hybrid Cordgrasses (*S. alterniflora*
x S. foliosa) in San Francisco Bay Tidal Flats

By

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DISSERTATION

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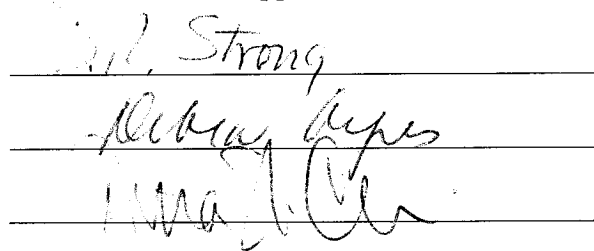
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Abstract

Maritime cordgrasses in the genus *Spartina* (*S. alterniflora* and *S. anglica*) are potent invaders of naturally open intertidal mudflats worldwide. Successful colonization appears to be due to large open tidal mudflats providing regeneration sites for seedlings, and the ability of large cordgrasses to survive in this harsh environment. In Willapa Bay, WA, for instance, the rate of *S. alterniflora* spread is exponential, and an expected endpoint to this invasion, without control, would be extensive meadows of cordgrass, as are seen within the native range of this species. In San Francisco Bay (SFB), the *Spartina* invasion is different in that the introduced species, *S. alterniflora*, hybridized with a native species, *S. foliosa*. Subsequent introgressive hybridization has left only a small number of extant SFB *S. alterniflora*, and has produced a diverse array of hybrid genotypes, now spreading at a rate exceeding exponential growth.

Due to their invasive histories and extreme and accelerating spread rates, *Spartina* cordgrasses are model organisms for studying biological invasions, and are so the subjects of many contemporary ecological and evolutionary investigations, including *Spartina* population genetics and systematics, and the hybridization between *S. alterniflora* and *S. foliosa* in SFB. In order to increase the low number of 11 available microsatellite markers, I describe an additional 24 disomic microsatellite loci in *Spartina alterniflora* in Chapter 1. All new loci were polymorphic and amplified in Pacific and Atlantic *S. alterniflora*, and in a swarm of *S. alterniflora* x *S. foliosa* hybrids. All loci also amplified in *S. foliosa*, but most were monomorphic. A subset of loci successfully amplified in *S. densiflora*, and showed some polymorphism. I established several species-

specific alleles for *S. densiflora* and *S. foliosa*. Levels of allelic variation in these 24 new loci were again lower in invasive populations of *S. alterniflora* in pacific estuaries than in native east coast populations.

Hybridization has been proposed as a stimulus to invasiveness. The contemporary invasion of hybrid cordgrass, introduced *Spartina alterniflora* x native *S. foliosa* in SFB, serves as a model system for the role of hybridization in increasing invasiveness. In Chapter 2, I investigate the possibility that increased self-fertility has heightened the spread rate of hybrid *Spartina* in this system, characterized by salt marsh estuaries and open, unvegetated tidal flats. My results showed both parent species to be largely self-incompatible, with pollen availability limiting seed set. Hybrids *in situ* set on average 13% (range: 0% to 76%) self-fertilized seed, while neither parent did so in SFB. As well, some of the most recent descendents of this ca 30 year-old hybrid swarm have colonized and reproduced successfully in slightly deeper, open, unoccupied mud in tidal flats; they were more self-fertile than their progenitors in a nearby cordgrass meadow along the shore. Their survival in the harsh mudflat environment indicates that selfing interacts with other transgressive survival traits in these colonizers. Allee effects can limit invasion rates, and pollen limitation at the low-density leading edge has reduced the rate of invasive *S. alterniflora* in Willapa Bay, WA. In contrast, self-fertilization ensures sexual reproduction at the leading edge of hybrid *Spartina*. In the absence of inbreeding depression, colonizing plants can so contribute vast numbers of seeds, establishing as robust transgressive seedlings in the open mud or float elsewhere. Hybrid cordgrasses in SFB have spread at greater than exponential rate, and I propose that increasing frequency

of a few hybrids possessing transgressive traits, including overcoming pollen limitation, have allowed colonization and reproduction at low density and are keys to this invasion success.

Hybrid cordgrasses (*Spartina alterniflora* x *S. foliosa*) have invaded SFB and are not only spreading within native *Spartina* marshes; isolated individuals, capable of self-pollination, have also established in the vast, un-vegetated SFB tidal flats. This establishment, if unchecked, will cover these tidal flats, and result in the loss of valuable shorebird foraging habitat, seriously impacting migrating birds on the Pacific Flyway route. As local recruitment intensity is one factor underlying the rate and direction of spatial spread of invasive organisms, in Chapter 3, I examine SFB hybrid genetic structure, hybrid colonization history, potential source populations for regional tidal flat recruitment, and the local fine-scale hybrid seedling recruitment dynamics at three hybrid cordgrass populations along the eastern SFB shoreline. These tidal flat populations are linked by tidal creeks to a nearby *Spartina* hybrid meadow in the south, and directly adjacent to one in the north. I combined molecular microsatellite marker and geographical (GPS/GIS) data, via multivariate ordination, Bayesian clustering, parentage/sib-ship studies, and spatial genetic analysis. I found the geographically most distant northern population to be genetically distinct from two southern, sympatric and closely related populations. At each site, meadow and tidal flat adult plants were genetically related. In the northern population, the majority of tidal flat seedlings were a product of adults from the adjacent meadow, while at both southern locations, tidal flat hybrids produced most seedlings. At both tidal flat populations, a single isolated

individual contributed up to 55% of all surveyed seedlings, the majority of which were inbred.

Tidal flat seed dispersal follows the stepping stone model and most recruitment occurs non-randomly and locally. Positive spatial genetic structure up to ~ 200 meters and negative structure above ~440 or 540 meters showed seedling recruitment to be spatially non-random, and suggests a large number of seedlings to establish close to their mothers in sheltered 'safe sites.' All genotyped seedlings were hybrid and seedling recruitment increased on average 6-fold from 2003 to 2004. Seedling size and shoot number surveys show 9 %, 12 %, and 45 % seedling survival per site to one-year or greater. Rapid evolutionary changes in this hybrid *Spartina* swarm have resulted in a few extremely fit individuals responsible for the majority of locally recruiting seedlings at the forefront of the tidal flats invasion. By producing large numbers of inbred, highly fit propagules in isolation, these transgressive plants ensure further colonization of the tidal flats habitat they have adapted to, and heighten the likelihood of long-distance dispersal. This successful recipe for invasion, coupled with pollen swamping close to native marshes, may explain greater than exponential hybrid spread, and if unchecked, will assure the continuing invasion of SFB by hybrid *Spartina*.

Acknowledgements

This work was supported by the California Coastal Conservancy (CalFed grant #99-110), California Sea Grant (Project R/CZ-176), and the National Science Foundation Biocomplexity program (DEB #0083583). I would like to thank my mentors Debra Ayres, Don Strong, Dina St.Clair, and Bernie May for their guidance and encouragement throughout. I further greatly acknowledge and thank Heather McGray, Heather Davis, Jun Bando, Janie Civile, Richard Hall, Caz Taylor, John Lambrinos, Michael Blum, David Lowry, Alex Lee, and Patty Bongiovanni for their assiduous help in the field and the laboratory. I also sincerely thank my family, my husband Marc Sloop, and my sons Ian and Nico, for standing by me throughout.

Chapter 1

Characterization of 24 additional microsatellite loci in *Spartina* species (Poaceae)

Abstract

As model organisms for studying biological invasions *Spartina* cordgrasses are the subjects of many current ecological and evolutionary investigations. Here I describe an additional 24 disomic loci in *Spartina alterniflora* important for investigations of *Spartina* population genetics and systematics, and the hybridization between *S. alterniflora* and *S. foliosa* in San Francisco Bay. Levels of allelic variation in these 24 new loci are again lower in invasive populations of *S. alterniflora* in pacific estuaries than in native east coast populations. All loci amplified in Pacific and Atlantic *S. alterniflora* and a swarm of *S. alterniflora* x *foliosa* hybrids. Only a subset of loci successfully amplified in *S. densiflora*. I establish several species-specific alleles for *S. densiflora* and *S. foliosa*. The majority of loci are monomorphic in *S. foliosa*.

Primer Note

In San Francisco Bay (SFB), CA, an invasive hybrid swarm between the Atlantic and Gulf coast native cordgrass *Spartina alterniflora* and the California native *S. foliosa*, is spreading at a rate exceeding exponential growth (Ayres *et al* 2004). *Spartina alterniflora* is also spreading at an extraordinary rate in Willapa Bay (WB), WA (Civille *et al* in press), where *S. foliosa* does not occur. My colleagues and I initially developed 11 microsatellite loci to elucidate how *Spartina* invasions proceed (Blum *et al* 2004). Here I characterize an additional 24 microsatellite loci to enable more thorough molecular investigations of ecological and evolutionary processes driving *Spartina* invasions. All new loci were developed from four enriched genomic libraries targeting (GA)_n, (CA)_n, (AAG)_n and (CTA)_n repeat motifs constructed by Genetic Identification Services (GIS) (<http://www.genetic-id-services.com>) from *S. alterniflora* genomic DNA (>100 µg) isolated from a plant sampled from North Carolina. Following GIS polymerase chain reaction (PCR) library screening guidelines, I found that approximately 40% (196 of 500) of the *E. coli* clones had incorporated *S. alterniflora* DNA fragments. A forward universal *pUC19* primer and ABI PRISM® BigDye™ Terminator chemistry on an ABI 3100 sequencer (PE Applied Biosystems) were used to sequence the PCR products. Locus specific primers were designed for 152 clone sequences using PRIMER 3 (Rozen & Skaletsky 1998) and <http://www.gramene.org> (Ware *et al* 2002). The reaction volume for each PCR amplification trial per microsatellite locus was 14 µL, consisting of 5 µL of DNA (20 ng), 0.15 µL (1U/µL) *taq* DNA polymerase (Promega, buffer A), 0.5 µL of dNTPs (10 µM), 0.375 µL of each primer (40 µM), 2 µL of 10x Promega PCR buffer, and 3.25 to 4.35 µL of *ddH*₂O; relative to the individually optimized amount of MgCl₂ used

per locus listed in Table 1.1. PCRs were performed on two Hybaid PCR Express gradient thermal cyclers (Hybaid US, Franklin, MA) and underwent denaturation for 2 min at 94 °C, followed by 29 cycles at 94 °C for 45 s, T_a (Table 1.1) for 45s, 72 °C for 1 min, followed by an extension phase at 72 °C for 6 min. Using fluorescently labeled forward primers, PCR products were then sized using an ABI 3730xl 96-capillary DNA analyzer and ABI GeneMapper 3.0 software (Applied Biosystems, Cupertino, CA) (Table 1.2). PCR amplifications were successful for 145 loci. I optimized PCR conditions on a per locus basis and chose 60 clone sequences yielding disomic loci for characterization with panels of individuals including: 24 Atlantic and Gulf Coast (AGC) *S. alterniflora*; 11 WB *S. alterniflora*; 34 northern California *S. foliosa* and 6 southern California/northern Mexico *S. foliosa*; 35 SFB *S. alterniflora* x *S. foliosa* hybrids; and 3 to 12 SFB *S. densiflora*. Of these 60 loci 11 were published in Blum *et al* (2004) and 24 are presented here. Of the remaining 25 unpublished loci, 16 loci were not characterized due to genotyping difficulties and the remaining 9 loci were monomorphic across all test individuals. For the 24 loci reported here, expected and observed heterozygosities and the presence of linkage disequilibrium among loci (LD) were calculated using ARLEQUIN 2.001 software (Schneider *et al* 2000) (Table 1.2).

I report the sequences for 24 primer pairs in Table 1.1. All 24 of the *S. alterniflora* loci amplified in *S. foliosa* and *S. alterniflora* x *S. foliosa* hybrids (Table 1.2), while only 18 loci amplified in *S. densiflora* (Table 1.1). Only 2 loci (SPAR.17, SPAR. 36) were at Hardy-Weinberg equilibrium in all sample groups (Table 1.2). All remaining loci exhibited heterozygote deficiency, monomorphism, or heterozygote excess in the various

test populations (Table 1.2). There was also some evidence of LD among loci in all groups. These results likely reflect mixed sexual and asexual reproductive modes, non-equilibrium population dynamics, and/or the presence of null alleles. All but 2 loci (SPAR.28, SPAR. 36) showed considerable allelic variation, ranging between 3 and 14 alleles per locus for AGC *S. alterniflora* (Table 1.2). Lower allelic variation at all loci but SPAR.17 and the observed monomorphism at 3 loci (SPAR.20, SPAR.28, SPAR.32) in WB *S. alterniflora* likely reflect the recent founding event and non-equilibrium invasion dynamics in Willapa Bay (Blum *et al* 2004). However, these monomorphic loci (alongside 13 other loci) also exhibit heterozygote deficiency in the AGC test population. Nine loci (SPAR.13, SPAR.16, SPAR.17, SPAR.20, SPAR.24, SPAR.26, SPAR.27, SPAR.28, SPAR.34) were monomorphic in *S. foliosa*, and the average of 2.2 alleles exhibited at the 15 other loci is lower than previously reported levels of polymorphism (Blum *et al* 2004).

Some overlap in *S. alterniflora*, *S. foliosa* and *S. densiflora* allele size distributions occurred for most loci, however 8 varying loci display species-specific alleles in both *S. densiflora* (Table 1.1) and *S. foliosa* (Table 1.2). I further observed unique alleles at 9 loci in WB compared to AGC *S. alterniflora*, and at 9 loci in SFB hybrids relative to both parent species (Table 1.2).

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Table 1.1. Primer sequences of 24 additional disomic microsatellite loci in *Spartina*; and *S. densiflora* cross-amplification results.

GenBank ID	Locus	Primer sequence (5'-3')	Core repeat*	T _a (°C)	MgCl ₂ (μM)	<i>S. densiflora</i> (alleles/ind.)
AY758209	SPAR.13	F: CCGTGTA CTCTAGCCTCTTG R: AGTCGAGTTGCTGTT CAGAT	(CGC) ₂ GCG(CGC) ₁	53	20.00	+ (1/12)
AY758210	SPAR.14	F: CATGTGGTATCTCCCCATC R: GTGACCATAGTTGGCTCTTG	(CTC) ₃ (CT) ₇	51	18.33	+ (1/8)
AY758211	SPAR.15	F: ATTTGCTGCTTTTGGTAGAC R: GTAGAACAATGGAAGAATGC	(TC) ₁₂	51	28.33	+ (1/3)*
AY758212	SPAR.16	F: CTTCTGCTTGAATTGGTAG R: ACATCGGTGGCAGTAGTAAC	(CT) ₁₁ AT(CT) ₃	55	30.00	- (0/3)
AY758213	SPAR.17	F: TACTTTGGTGTGTTGCTTTATC R: GAGTTAGAGGAGTTATTGCTG	(AAG) ₈	60	25.00	+ (2/3)*
AY758214	SPAR.18	F: AACTTCTGTCTCTGGGATTG R: TAGGGAGATAGGACTGGACTG	(CTT) ₈	64	25.00	+ (2/3)
AY758215	SPAR.19	F: CTAATTCCTCACCTACGC R: CTACGAGACTTCCTCACTGC	(CT) ₁₃	51	20.00	+ (1/2)
AY758216	SPAR.20	F: ACCGTGCCTCAGCTACTG R: GGTGTTTCCTCGCATAGATC	(GA) ₁₀	52	21.67	+ (2/2)
AY758217	SPAR.21	F: TGATGCTGTTTCTACCACCTTTAC R: CCTCGTCCTCCGTTTTTG	(GA) ₁₆	53	21.67	+ (1/9)
AY758218	SPAR.22	F: ACTGGTCGGTATGGATGC R: ATGAGGTCGGTCGTTGTAGC	(CT) ₁₁	60	28.33	- (0/10)
AY758219	SPAR.23	F: GGGAAGTGAAATCTGGTTGC R: GCTTGCTTGTCTCAGTCC	(CT) ₁₈	55	21.67	+ (1/10)*
AY758220	SPAR.24	F: TTACACTTGACCTTCTCATC R: GAAACGACTACAGCAATAAG	(CTT) ₈	64	28.33	+ (1/3)
AY758221	SPAR.25	F: CGGTAGAGACGGAGTTGTGG R: GCTTGGGAGATGAGACTGGAC	(CTT) ₁₀	69	30.00	+ (1/3)
AY758222	SPAR.26	F: TTCAACTGGCGTAGTGATTCC R: AACATTTCCGACTGGTAGAGC	(TC) ₁₂	58	31.67	+ (2/3)*
AY758223	SPAR.27	F: CATCAAAAGCAAGAGGA R: GACACCAACGGAACCTG	(GA) ₂₃	50	21.67	+ (1/3)*
AY758224	SPAR.28	F: CACCGTTCAATCACAGTT R: GGAAGCAGGAGGGGTTGG	(TC) ₂ ...(TC) ₂ ...(TC) ₅	59	20.00	+ (1/3)
AY758225	SPAR.29	F: GAACGGTGCATTCTCGATTT R: AGCTTACATGGCGGTGTGAT	(AG) ₈ ...(AG) ₄	55	23.33	- (0/3)
AY758226	SPAR.30	F: CTATGAGTAGTTGGCCGTTT R: TGTGTACTTCTTCTGGATGC	(AT) ₃ ...(GA) ₄ ...(GA) ₃	53	21.67	+ (1/12)*
AY758227	SPAR.31	F: GATCGGACAACCTCTATGGAC R: CCAGAAGAAAGTACACAAAG	(CTT) ₇	55	16.67	+ (1/3)
AY758228	SPAR.32	F: TGGGAACACTTATCAACAATGG R: AGGTGGAGACAACGGAGCAG	(CT) ₄ ...(CT) ₅ ...(CT) ₄ ...(CT) ₄	59	25.00	- (0/3)
AY758229	SPAR.33	F: ACCGTAACAACCTGAACCTCTG R: TAGACGACGACCACTGCTTG	(TC) ₈	58	21.67	+ (2/3)*
AY758230	SPAR.34	F: TCATCATCGACCGAAAAC R: TCACCAAGTGTCAAGCAGAG	(CT) ₁₂ C(CT) ₂	55	33.33	- (0/3)
AY758231	SPAR.35	F: TGGAACCTGTAGTCAGAAGC R: GAGGAAGATGATGAAAGTAACG	(CTT) ₁₀	59	20.00	+ (2/3)*
AY758232	SPAR.36	F: CTTCTATCCAATGTTCGTAG R: TTAGGTACTGCTGGGATTC	(CTT) ₁₀	58	31.67	- (0/3)

* Core repeat from original clone sequence

T_a, optimized annealing temperature; MgCl₂, optimized concentration per locus (μM);

+ indicates successful amplification; - indicates no amplification; * indicates presence of species-specific allele.

Table 1.2. Characteristics of 24 additional disomic microsatellite loci in *Spartina alterniflora*, *S. foliosa* and *S. alterniflora* x *S. foliosa* hybrids

Locus	<i>S. alterniflora</i> (Atlantic/Gulf coast)			<i>S. alterniflora</i> (Willapa Bay)			<i>S. foliosa</i> (California & Mexico)			<i>S. alterniflora</i> x <i>S. foliosa</i>		
	Size range (bp)	No. alleles/ no. individuals	H_o	H_E	Size range (bp)	No. alleles/ no. individuals	H_o	H_E	Size range (bp)	No. alleles/ no. individuals	H_o	H_E
SPAR_13	292-313	3/24	0.29	0.26	292-295	2/11	0.18	0.5	295	1/40	0.0‡	NA
SPAR_14	226-232	4/21	0.28†	0.65	226-228	2/10	0.10	0.1	226-228	2/35	0.2	0.21
SPAR_15	265-289	12/23	0.48†	0.86	271-279	2/11	0.36	0.48	266-268	2/39	0.08†	0.31
SPAR_16	368-384	8/19	0.53†	0.83	372-384	3/10	0.40	0.62	380	1/30	0.0‡	NA
SPAR_17	372-376	3/8	0.63	0.68	374-384	4/8**	1.00	0.81	374	1/18	0.0‡	NA
SPAR_18	178-207	6/19	0.47†	0.7	180-186	2/7	0.29	0.38	181-196	2/8*	1.0	0.53
SPAR_19	328-342	8/10	0.5†	0.86	317-338	2/5**	0.27	0.35	317-332	3/25	0.04†	0.4
SPAR_20	167-179	6/13	0.46†	0.73	177	1/6	0.0‡	NA	171	1/40*	0.0‡	NA
SPAR_21	201-244	7/11	0.09†	0.88	236-244	3/10**	0.20	0.2	226-239	3/32*	0.0†	0.40
SPAR_22	367-419	6/11	0.64†	0.84	371-419	3/10	0.10	0.28	369-416	3/20*	0.2†	0.35
SPAR_23	248-276	6/15	0.4†	0.81	248-278	5/9**	0.44	0.56	262-268	4/30*	0.27†	0.40
SPAR_24	173-200	6/20	0.85	0.69	173-191	2/9	1.00	0.53	173	1/39	0.0‡	NA
SPAR_25	245-264	3/20	0.90	0.54	245-258	3/11**	1.00	0.65	245-249	2/38*	1.00	0.51
SPAR_26	261-297	14/22	0.73†	0.93	271-285	4/8	0.38	0.74	263	1/38	0.0‡	NA
SPAR_27	309-337	8/17	0.53†	0.9	306-341	3/9**	0.55†	0.71	314	1/30	0.0‡	NA
SPAR_28	419-476	2/11	0.09†	0.44	419	1/8	0.0‡	NA	416	1/30*	0.0‡	NA
SPAR_29	347-364	8/22	0.36†	0.89	353-362	3/7	0.14	0.4	353-360	3/31	0.07†	0.13
SPAR_30	285-298	3/16	1.00	0.6	283-298	3/6**	1.00	0.67	283-298	3/38	0.95	0.53
SPAR_31	191-206	5/8	0.25†	0.83	191-209	4/7**	0.57	0.77	222-240	7/14*	0.29†	0.92
SPAR_32	443-507	3/13	0.08†	0.54	445	1/10	0.0‡	NA	443-507	2/29	0.04†	0.32
SPAR_33	243-258	8/12	0.92	0.9	245-252	2/9	1.00	0.53	247-258	2/25	1.00	0.51
SPAR_34	368-380	5/14	0.36†	0.79	368-376	2/4	0.50	0.75	368	1/24	0.0‡	NA
SPAR_35	261-294	4/21	0.38	0.38	261-288	3/10**	0.70	0.51	261-285	3/28	1.00	0.54
SPAR_36	245-248	2/24	0.46	0.36	245-248	2/11	0.55	0.5	245-248	2/40	0.08	0.14

† Heterozygote deficiency ($P < 0.05$).

‡ Monomorphic in test population.

H_o , observed heterozygosity; H_E , expected heterozygosity.

* Species-specific allele present; ** Unique alleles present in WB *S. alterniflora*, and SFB hybrids.

Chapter 2

Self-fertility overcomes pollen limitation in invasive cordgrass hybrids

(*Spartina alterniflora* x *S. foliosa*)

Abstract

Hybridization has been proposed to stimulate invasiveness. I investigated whether increased self-fertility underlies the accelerating spread rate of hybrid cordgrass, introduced *Spartina alterniflora* x native *S. foliosa*, in San Francisco Bay (SFB). Both parent species were largely self-incompatible, with pollen availability limiting seed set. I found that *in situ* hybrids set on average 13% (range: 0% to 76%) self-fertilized seed, while neither parent did so in SFB. As well, some of the most recent descendents of this ca 30 year-old hybrid swarm have colonized and reproduced successfully in the unoccupied SFB tidal flats; they were more self-fertile than their progenitors in a nearby cordgrass meadow along the shore. Their survival in the harsh tidal flat environment indicates that selfing interacts with other transgressive survival traits in these colonizers. Allee effects can restrain invasion rates, and pollen limitation at the low-density leading edge has reduced the rate of invasive *S. alterniflora* in Willapa Bay, WA. In contrast, self-fertilization ensures sexual reproduction at this leading edge in SFB. In the absence of inbreeding depression, colonizing plants thus produce much seed, which establish as robust, equally transgressive seedlings in the open mud, or float elsewhere. I propose that increasing the frequency of transgressive hybrid traits, such as self-compatibility, which allows colonization and reproduction at low density, is key to the success of this invasion.

Introduction

Exotic species can evolve rapidly (Darwin 1859, Mack 1996, Barrett and Richardson 1982), especially after hybridization (Vila *et al* 2000). While the well known Dobzhansky-Muller incompatibilities render many hybrids sterile and even inviable (Orr and Turelli 2001), some hybrids are extremely fit invaders (Lambrinos 2004). The genetic and phenotypic variability of hybrids can be huge, and transgressive segregation can lead to individuals with fitness that exceeds both parents (Arnold 1997), geographical zones of high hybrid fitness (Lexer *et al* 2003a, b), and accelerated adaptation of invasive hybrids to new environments (Ellstrand & Schierenbeck 2000). Transgressive quantitative trait loci (QTL) can drive invasiveness (Rieseberg *et al* 2003, 1999).

Grasses include the most common, aggressive, and harmful invasive plants; the lion's share are pollinated by wind and are self incompatible (Koenig and Ashley 2003, Bauman *et al* 2000). The large-scale invasion of the intertidal habitat of Willapa Bay, WA (WB) by *Spartina alterniflora*, introduced from the Atlantic and Gulf coasts of North America (Bando, in review) was slowed by self-incompatibility (Davis *et al* 2004a). The scattered plants at the leading edge of the invasion set little seed (Davis *et al* 2004b). Pollen on the wind and on stigmas was sparse at the leading edge, and it increased only after decades of rhizomatous growth and repeated recruitment filled in these areas to create dense meadows, where seed set was high. Low seed set at the leading edge created an invasion in slow motion (Taylor *et al* 2004). The weak Allee effect set the rate of spread to about half of that among dense plants in meadows. This

invasion is probably about 100 years old, and its constant 12% rate of spread led to ca 8% occupancy of suitable habitat by the year 2000 (Civille *et al* in press). Without the Allee effect, the intertidal habitat would have been virtually covered by *S. alterniflora* decades ago (Strong, in press). WB has no native *Spartina* and no potential for hybridization of the invader. The nearest native, *S. foliosa*, California cordgrass is 900 km to the south in San Francisco Bay, CA (SFB).

In contrast, a hybrid swarm of *Spartina* has swept in fast-forward over tidal marshes and intertidal lands of SFB. The hybrids, with native California cordgrass, *S. foliosa*, appeared soon after introduction of *S. alterniflora* ca 30 years ago (Ayres *et al* 2004, 1999, Daehler and Strong 1997, Callaway & Josselyn 1992). Unlike the constant, long term spread of *S. alterniflora* in WB, rate of hybrid spread in SFB has accelerated from a doubling time of about 8 years in the period of 1975 -1993 to a doubling time of about 2 years in the period from 1993- 2003 (Ayres *et al* 2004). Hybrid traits crucial to fitness are transgressive: culm height, rhizomatous expansion, intertidal amplitude, salinity tolerance, and pollen production of some hybrids exceed the maxima of both parental species (Ayres *et al* 2003, Pakenham-Walsh 2003, Zaremba 2001). These traits allow hybrids to invade native marshes and displace *S. foliosa*, and they lead to export of large amounts of seed from hybrid crosses and backcrosses with *S. foliosa* (Ayres *et al* 2003, Huxel 1999). Exported seed floats to invade other native marshes. Native salt marsh vegetation and California cordgrass, *S. foliosa* are restricted to elevations above mean sea level (MSL) (Hinde 1954), and I have speculated that the transgressive traits are what allow hybrids to invade the vast unvegetated mud flats below MSL (Ayres *et al* 2003,

Anttila *et al* 1998, Daehler 1998, Pakenham-Walsh 2003, Zaremba 2001, Schweitzer *et al* 2002).

In this study, I focused upon selfing as a trait key to plant invasion (Hurka *et al* 2003). Apropos, I asked about pollen limitation and selfing in hybrids and parents in SFB. As well, I asked about inbreeding depression in *Spartina* hybrids. Finally, I studied hybrid colonization of open mud flats below MSL, which is one of the leading edges of the hybrid invasion (colonization of native marshes above MSL is the other leading edge). Open mud is colonized by scattered plants (Fig. 2.1a) that are isolated from the pollen produced in meadows at the edges of the estuary. Calculations suggest that selfing by isolated hybrids could create positive feedback, increasing the fractions of hybrids in the overall *Spartina* population and accelerating the invasion rate (Hall *et al*, in review).

Materials and Methods

Study system

"Hybrid" denotes *Spartina alterniflora* x *S. foliosa* in SFB (Daehler and Strong 1997, Ayres *et al* 2004). *Spartina* grow by rhizomatous expansion beneath the surface of the mud. Culms grow upward from rhizomes each spring and set seed in summer. Culms die, break off, and float away bearing inflorescences and many seeds in winter. Some seeds disperse singly; others disperse on mats of wrack comprised of dead stems (K. Sayce, unpublished data). Both parental species are protogynous, largely self-incompatible, and wind-pollinated (Davis *et al* 2004a, b, Anttila *et al* 1998, Somers and Grant 1981).

Spartina are ecosystem engineers (Crooks 2002) and can change open intertidal mudflats into dense cordgrass meadows (Daehler and Strong 1997). *Spartina* plants grow into large circular clones that increase in diameter by vegetative, rhizome growth (Fig. 2.1a, right, and Fig. 2.1c). Isolated plants grow from seeds germinating on open mud at low density. Meadows of continuous plants develop as isolates grow together (Fig. 2.1a, left). Aggregations of *Spartina* plants accrete sediment and elevate the level of the marsh (Morris *et al* 2002, Crooks 2002). Hybrid spread was predicted to profoundly alter the SFB ecosystem (Ayres *et al* 2003).

Pollen manipulations

To test for self-fertilization, I manipulated pollen (Fig. 2b, c; Daehler and Strong 1994, Davis *et al* 2004b). My pollen manipulations were: **Ambient** (not manipulated) with open inflorescences that could receive pollen carried on the wind from other inflorescences and plants; **Excluded**, with inflorescence enclosed inside a polypropylene tube mounted on a pole; **Added**, with fresh pollen from several other plants applied by brush to the stigmas of the open inflorescence. The rarity of *S. alterniflora* meant that I had to use hybrid pollen for the pollen addition treatment level for both *S. alterniflora* and hybrids. I used *S. foliosa* pollen for the **added** treatment level in experiments with *S. foliosa*.

I conducted experiments in Aug. and Sept., 1999 at Cogswell marsh (37° 39' 00" N, 122° 07' 12" N) on the Hayward Regional Shoreline (Fig. 2.2); in July 2002 at Blackie's Pasture marsh (37° 53' 44" N, 122° 28' 18" N) in northern SFB (BP) (Fig. 2.2), in July

and Aug. 2004 at Elsie Roemer marsh (37° 45' 35" N, 122° 28' 48" N) in Alameda (ALA) (Fig. 2.1a, Fig. 2.2); Robert's Landing (RL) (37° 40' 13" N, 122° 09' 42" N) on the San Leandro shoreline; and on the Hayward shoreline (HAY) (Fig. 2.2). In 1999 at Cogswell, I selected 16 distinct hybrid *Spartina* individuals, determined by RAPD markers (Ayres *et al* 1999), and known from previous work to produce seed (Zaremba, 2001). In 2004, I determined with RAPD and microsatellite markers (Blum *et al* 2004, Sloop *et al*, in press, Chapter 1) that 17 isolated plants growing in open mudflats at ALA, RL, and HAY were hybrids; I found no isolated *S. alterniflora* or *S. foliosa*; 5 plants growing in continuous meadows on the shoreline, amidst hybrids at Elsie Roemer marsh were *S. alterniflora* (Fig. 2.1a, left), but *Spartina foliosa* does not now grow at Elsie Roemer marsh. I could not use previously transplanted *S. foliosa* in this study because they died or failed to produce inflorescences in 2004. *S. foliosa* data were from July 2002 for 9 *S. foliosa* individuals at Blackie's pasture marsh (Fig. 2.2). For native *S. foliosa* at BP, and *S. alterniflora* plants in the AL meadow, the three treatments I imposed on three inflorescences per treatment were: **excluded**, **ambient**, and **added**. I assigned **excluded** and **ambient** treatments to 5 inflorescences per treatment per haphazardly selected meadow plant at Cogswell marsh. On the isolated hybrid plants I imposed treatments on three inflorescences per treatment: **excluded** (at upwind edge), **upwind ambient** (at edge of plant directly facing into the wind), **downwind ambient** (at edge of plant away from wind, towards which the wind is blowing across the plant), and **added** (at upwind edge). Tubes were removed once flowering ceased, and inflorescences were collected four weeks thereafter, before seed shattering. I determined seed set per inflorescence by counting all seeds (fertile and infertile) from 5 branches per inflorescence and

multiplying average seed set per branch by total number of branches. I again assessed seed set for all treatments as described above.

Germination, seedling growth and survival

Seed collected from experiments were stored in the refrigerator in individual plastic bags until germination. Seeds were pre-treated by bathing in cold (4° C), dilute seawater (ca 8 ppt salinity) in the refrigerator for approximately six weeks, changing the solution frequently to minimize microbial growth. Immediately before germination, seeds were washed in 25% liquid bleach for five minutes and subsequently rinsed four times in fresh distilled water. Seeds were then exposed to sunlight inside sealed Petri dishes, atop filter paper, saturated with autoclaved distilled water. Seed germination was recorded on a weekly basis for two to three weeks. Germinated seeds were then planted in 14 cm² pots in 3 parts potting soil and 1 part vermiculite in March 2000. Seedlings were grown in the greenhouse at Bodega Marine Laboratory (BML) inside light penetrating cloth cages, which excluded insects. Additionally, the cages were periodically sprayed with insecticidal soap. Seedling height, shoot production and survival were assessed every two weeks until mid-May and at monthly intervals until September 25, 2000.

Genetic survey

I determined genetic marker profiles of 15 hybrid mothers at Cogswell marsh and their offspring by using RAPDs (Randomly Amplified Polymorphic DNA). I extracted DNA from leaf samples of all mothers and 307 surviving seedlings from 15 families according to the molecular methods in Ayres *et al* (1999). In addition to the seven primers reported

by Ayres *et al* (1999), I used 3 additional primers (University of British Columbia: UBC 212, UBC 239, UBC 243), resulting in 11 polymorphic bands. I performed genetic analysis as detailed in Ayres *et al* (1999) and scored the dominance, or presence, of each species-specific band per individual. I also determined genetic marker profiles of 17 isolated SFB mudflat hybrid plants and some of their progeny using 7 *Spartina* specific microsatellite (SSR) markers: SPAR.08, SPAR.09, SPAR.23, SPAR.26, SPAR.27, SPAR.28, and SPAR.29 (Blum *et al* 2004, Sloop *et al*, in press, Chapter 1). I extracted leaf DNA from all 17 mothers and 96 offspring from 5 mudflat families that were germinated according to the above protocol and grown in a greenhouse at UC Davis for 6 months. PCR (polymerase chain reaction) volumes for all microsatellite genotyping procedures are reported in Sloop *et al* (in press, Chapter 1). These RAPD and SSR marker profiles were used to identify hybrids and determine single- and multi-locus out-crossing rates of individuals in both habitats.

Statistical methods

To test whether SFB meadow *Spartina* hybrids at Cogswell marsh set self-fertilized seed I used one-way nested ANOVA on treatment (**excluded** vs. **ambient**), with individual plant ID as a random variable, and replications nested within ID. In this and all subsequent analyses seed set was weighted by an estimation of the total number of florets of each inflorescence. This estimate was calculated as follows: average number of florets per branch (= total number of assessed florets from five branches divided by five) multiplied by the number of branches per inflorescence.

Self-fertilization was tested in two separate one-way ANOVAs for the effects of treatment (**excluded**, **added**, **ambient**) on the proportion of filled seeds in either species, as data were obtained from different sites and years. In order to achieve normal data distributions in both cases it was necessary to arcsine-transform, and rank-transform weighted seed set data for *S. alterniflora* and *S. foliosa*, respectively.

To determine differences in hybrid self-fertilization ability due to plant density/habitat type (meadow vs. isolated) I compared treatment effects (**excluded** vs. **ambient**; combining all replications of **ambient upwind** and *downwind* here) on weighted seed set in 16 Cogswell marsh (meadow) and 17 mudflat (isolated) plants, using two-way nested split-plot analysis. Replications (rep) were nested within the random variable ID, and the interaction of ID and habitat was nested within rep. I assessed pair-wise differences in the interaction of treatment by habitat via post hoc Tukey multiple comparisons. To gauge whether seed set in the isolated plants is influenced by within clone inflorescence position relative to ambient pollen flow (wind direction), I further compared weighted seed set by treatment (exclusion, control upwind, control leeward, addition) using a one-way nested split-plot analysis. Replications were nested within the random variable ID. I again assessed pair-wise treatment differences by using post hoc Tukey multiple comparisons.

To investigate inbreeding depression I compared the performance of seed made by selfing with that made by outcrossing for the traits of germination rate and survival to six months. I used one-way nested ANOVA to compare the proportions of germinated seeds

from Cogswell marsh, and seedling greenhouse survival to 6 months, by treatment (**excluded** vs. **ambient**). In both analyses replications were nested within the random variable individual plant, ID. I performed multivariate analysis of variance (MANOVA) to assess treatment differences (**excluded** vs. **ambient**) in seedling height and shoot production in the greenhouse at 6 months seedling growth. All above analyses were performed with SAS 8.02 (SAS Institute, Cary, NC).

I assessed the single- and multi-locus out-crossing rates of 11 meadow and 5 mudflat families with 11 dominant RAPD and 7 co-dominant microsatellite markers respectively, using MLTR 3.0 software (Ritland 2002; <http://genetics.forestry.ubc.ca/ritland/programs>). I further examined the Mendelian segregation for inbreeding by comparing observed allele frequencies of mudflat families to expected Hardy-Weinberg inbreeding ratios by performing Chi-square tests in Excel X.

Results

Hybrids set seed in the absence of pollen from other inflorescences while neither parental species did so (Fig. 2.3). On average, 13% of hybrid florets set viable seed inside the experimental, pollen-excluding tubes; in this treatment no florets of either *S. alterniflora* or *S. foliosa* set any seed. This implies that the hybrids, but neither parent, are autogamous and self-pollinating. Pollen addition increased seed set of hybrids and *S. alterniflora*, and very few *S. foliosa* seeds were set on **ambient** (mean $\pm$ stdev: $0.001 \pm$

0.003; $n = 9$ inflorescences) and **added** (*S. foliosa* pollen: mean $\pm$ stdev: 0.036 ± 0.066 , $n = 9$ inflorescences; hybrid pollen: mean $\pm$ stdev: 0.013 ± 0.024 , $n = 9$ inflorescences; $p = 0.34$) inflorescences. In detail for hybrids, pollen exclusion reduced seed set of hybrids to ca. half (overall mean $\pm$ SE; 0.13 ± 0.02 , $n = 68$ inflorescences) of that on the open inflorescences (0.24 ± 0.02 , $n = 67$ inflorescences, $p < 0.001$) (Table 2.1). For hybrids there was no difference in germination ($p = 0.57$), seedling survival ($p = 0.96$), or height and shoot production (Wilks' Lambda = 0.98, $p = 0.10$) between seed set inside the pollen exclusion tubes and seed set in open inflorescences, which is consistent with a lack of inbreeding depression. Different plants within treatment levels varied in seed set ($p < 0.0001$), in seed germination ($P < 0.0001$) and in seedling growth (Wilks' Lambda = 0.38, $p < 0.0001$) but not in seedling survival ($p = 0.19$) (Table 2.1). In detail for *S. alterniflora*, pollen exclusion (**excluded**) reduced seed set to zero ($n = 14$), while on the open inflorescences (**ambient**) the pollen on the wind led to about half of the seed set (0.06 ± 0.02 , $n = 14$) of inflorescences to which pollen was added (0.12 ± 0.03 , $n = 15$), $p = 0.012$ (Fig. 2.3); pollen added vs. excluded = $p < 0.0001$ (Table 2.1). Stigmas of florets on open inflorescences (0-5 grains per stigma) bore less than 25% of the pollen of inflorescences to which I added pollen (>20 grains per stigma) for *S. alterniflora*. In detail for *S. foliosa*, pollen exclusion reduced seed set to zero, while on the open inflorescences (0.0014 ± 0.0009 , $n = 9$) the ambient pollen on the wind led to about a tenth of the mean seed set of inflorescences with pollen added (0.013 ± 0.008 , $n = 9$). Mean seed set did not differ between open and pollen-addition inflorescences ($p = 0.10$), or between open and closed inflorescences ($p = 0.42$) (Fig. 2.3).

Consistent with the hypothesis of hybrid evolution of autogamy and self-compatibility, isolated hybrids that had recently colonized open mud set seed at ca twice the rate (0.28 ± 0.06 , $n = 20$) on inflorescences from which pollen had been **excluded** as their putative progenitors on the adjacent shoreline (0.13 ± 0.02 , $n = 68$, $P = 0.015$) (Table 2.1, Fig. 2.4) Open inflorescences, exposed to ambient pollen, set seed at about twice the rate of meadow inflorescences from which pollen had been excluded, with no difference between the habitats (isolated or meadow, 0.23 ± 0.03 , Fig. 2.4). Seed set of open, ambient inflorescences did not differ between habitats/years ($P = 0.46$).

Geitonogamy increases seed set. I found that downwind inflorescences set more seed than those at the upwind edge of isolated hybrid plants that have enlarged rhizomatously. Ambient, open inflorescences at the upwind edges of 17 isolated hybrid plants set only 35% of the seed (0.13 ± 0.03 , $n = 40$) of ambient, open inflorescences at the downwind edges (0.35 ± 0.05 , $n = 26$, $P < 0.0001$) (Fig. 2.5). This is to say that a plant with only a single inflorescence can set some seed, and that this rate will increase as the plant grows more inflorescences. Ambient downwind inflorescences set seed at about twice the rates as upwind pollen **added** inflorescences (0.18 ± 0.04 , $n = 35$), probably because the added pollen was blown away by the consistent winds of SFB. Inflorescences at the upwind edge produced ample pollen because the seed set rate in tubes ($n = 20$) was as high as in the downwind ambient (downwind and enclosed: 0.31 ± 0.055 , $P = 0.16$) (Fig. 2.5) inflorescences. Enclosed seed set (0.28 ± 0.06) was about twice that of upwind **ambient** inflorescences ($P = 0.056$) (Fig. 2.5).

Isolated hybrid plants had extremely low rates of out-crossing, reinforcing the inference that hybrid spread is driven by self-fertilization within inflorescences. Open-pollinated progeny tests showed the multi-locus out-crossing rate of 5 isolated hybrids (0.035, stdev = 0.038) to be ca 6% of that of 15 meadow hybrids (0.522, stdev = 0.099) (Table 2.2). Individual out-crossing ranged from 0.56 to 1.0 in meadow plants (Fig. 2.6), and from 0.0 to 0.1 in isolated plants (Fig. 2.7). Chi-square Mendelian segregation tests using seven presumptive disomic microsatellite loci further supported predominant self-fertilization in 5 isolated mudflat families (Table 2.3).

Discussion

Unlike their parent species, *Spartina* hybrids are highly self-compatible in nature (Fig. 2.3). I observed high seed set in enclosed hybrid inflorescences in my field manipulations, and my genetic analyses further confirmed selfing in isolated tidal flat plants (Table 2.3, Fig. 2.7). Colonizing tidal flat hybrids thus seem to have overcome pollen limitation in isolation via selfing, while cordgrass hybrids in a meadow displayed mixed mating. My genetic data illustrates that the open-pollinated seed in this meadow mainly resulted from cross-pollination, with only some self-fertilization (Fig. 2.6). Ecologically, this indicates that isolated hybrid plants in nature can have high seed set through selfing, and in addition, are capable of high seed set through mixed mating, when population size increases in the future and pollen becomes more available.

The isolated tidal flat hybrids that I examined are the most recent product of evolution of the SFB hybrid swarm (appeared about 10-15 years ago), and I further asked if they had different selfing ability than their progenitors in the meadow along the shore. In the absence of inbreeding depression, colonizing plants can so contribute vast numbers of seeds, establishing as robust transgressive seedlings in the open mud or float elsewhere. My results show that colonizing tidal flat *Spartina* hybrids self-fertilize at a greater rate than meadow plants (Fig. 2.4), and that hybrid seedlings exhibit no inbreeding depression. Selfed *Spartina* hybrid seedlings grown in the greenhouse displayed no fitness reduction, as my germination, seedling survivorship and growth data show no difference between inbred and outcrossed progeny (Table 2.1).

In the absence of inbreeding depression, self-fertilization may be especially important for colonizing individuals (Allard 1965, Allard *et al* 1968, Stebbins 1957, Price and Jain 1981, Schoen 1983, Thompson 1991) as it allows for fast local adaptation to new environments (Shields 1982, Schaal 1984). The open mudflat below Mean Sea Level in SFB, while representing an opportunity for colonizing cordgrass seed, imposes stringent environmental conditions on potential colonizers. The absence of inbreeding depression in self-fertilized hybrid progeny may allow high fitness and rapid adaptation to the extreme environmental conditions of open mudflats, especially if their seed parent is highly fit in this environment. Hybridization has created a wide range of phenotypes that have successfully invaded this habitat, some of which are positively transgressive (Rieseberg *et al* 1999, Ellstrand and Schierenbeck 2000), or superior to both parent species (Ayres *et al* 2003, Zaremba 2001).

Hybrid reproductive fitness and invasive ability (Baker 1955) are increased by self-fertilization, and this may help to explain the accelerating rate of hybrid spread in this system (Hall *et al* in review; Ayres *et al* 2004, Daehler 1998). A mixed mating system, where cordgrass hybrids are able to set self- and out-crossed seeds, (Zaremba 2001, Antilla *et al* 1998) provides a substantial fitness advantage; selfing in isolation, mainly during the colonization phase of open tidal flat habitat; out-crossing in the vicinity of congeners, to sire seeds on surrounding hybrids and native plants.

Inadequate pollination has been recognized as a major selective force leading to the evolution of self-fertilization (Rick 1950, Stebbins 1957, de Nettencourt 1977, Wyatt 1983, 1984, Richards, 1986, Bernhardt and Thien 1987, Dafni and Bernhardt 1990, Whisler and Snow 1992), suggesting an adaptive response to pollen limitation in this invasive hybrid swarm. In grasses, self-incompatibility commonly involves gametophytic control of two multiallelic genes, named S and Z, and is expressed when both S and Z alleles are matched in pollen and stigma (Baumann *et al* 2000, Charlesworth and Awadalla 1998, Lundqvist 1954, Hayman and Richter 1992). The complete breakdown of this system involves mutations at or near the S and Z loci, or at a third modifier locus, named T, (Stone 2002, Thorogood and Hayward 1991, Lundqvist 1958, 1962, 1968,). Hayman and Richter (1992) found that T-mutation carrying pollen was always compatible in *Phalaris coerulescens*, irrespective of S or Z allele. Complete breakdown of self-incompatibility in *Spartina* hybrids could readily occur as hybridization may have greatly enhanced both allelic diversity and heterozygosity at

these self-incompatibility loci. As well, both parent species probably already possess alleles conferring some self-compatibility as they are able to set self-fertilized seed when forced, and grown under highly favorable conditions (Fang *et al* 2004, Davis, in press, D. Ayres, unpubl. data).

My results suggest that *Spartina alterniflora* is pollen limited and self-incompatible in invasive populations in nature (Fig. 2.3). *S. foliosa* is self-incompatible and may be pollen limited in natural populations as well (Fig. 2.3). However, these results are based on a single population per species and need replication. Unfortunately, *S. alterniflora* is becoming increasingly rare in SFB, and seed set in *S. foliosa* is notoriously low in most years (D. Ayres unpubl. data), seriously confounding such efforts. Both *S. alterniflora* and *S. foliosa* have been shown to self when forced under favorable greenhouse conditions (Fang *et al* 2004, Somers and Grant 1981, C. Daehler, unpubl. data, D. Ayres unpubl. data), and there is evidence suggesting increased selfing-ability in invasive, pollen limited *S. alterniflora* populations in Willapa Bay, WA (Davis, in press). This ‘predisposition’ to selfing ability in both parent species may have facilitated the complete breakdown in the self-incompatibility system in their highly genetically variable hybrids, allowing certain individuals to readily produce viable self-fertilized progeny in nature.

In contrast to their predominately out-crossing parent species, SFB *Spartina* hybrids exhibit a mixed mating system. This hybrid cordgrass in San Francisco Bay has spread at greater than exponential rate (Ayres *et al* 2004), and I propose that the increasing frequency of a few transgressive hybrid traits, including overcoming pollen limitation,

has allowed colonization and reproduction at low density, and is key to this invasion success. Hybrid individuals display wide variation in their ability to set seed, and in selfing ability, ranging from plants that set very little or no seed under any conditions to those that have exceptional selfed-seed set (Figs. 6, 7). Natural selection is expected to favor transgressive phenotypes that produce large numbers of fit seedlings. The positive feedback action of natural selection, favoring ever more fit individuals, can result in growth rates that exceed exponential (Hall *et al*, in review). I propose that the continued permanent establishment and proliferation of a subset of highly adaptive transgressive hybrid cordgrass individuals will persist in driving this rampant invasion.

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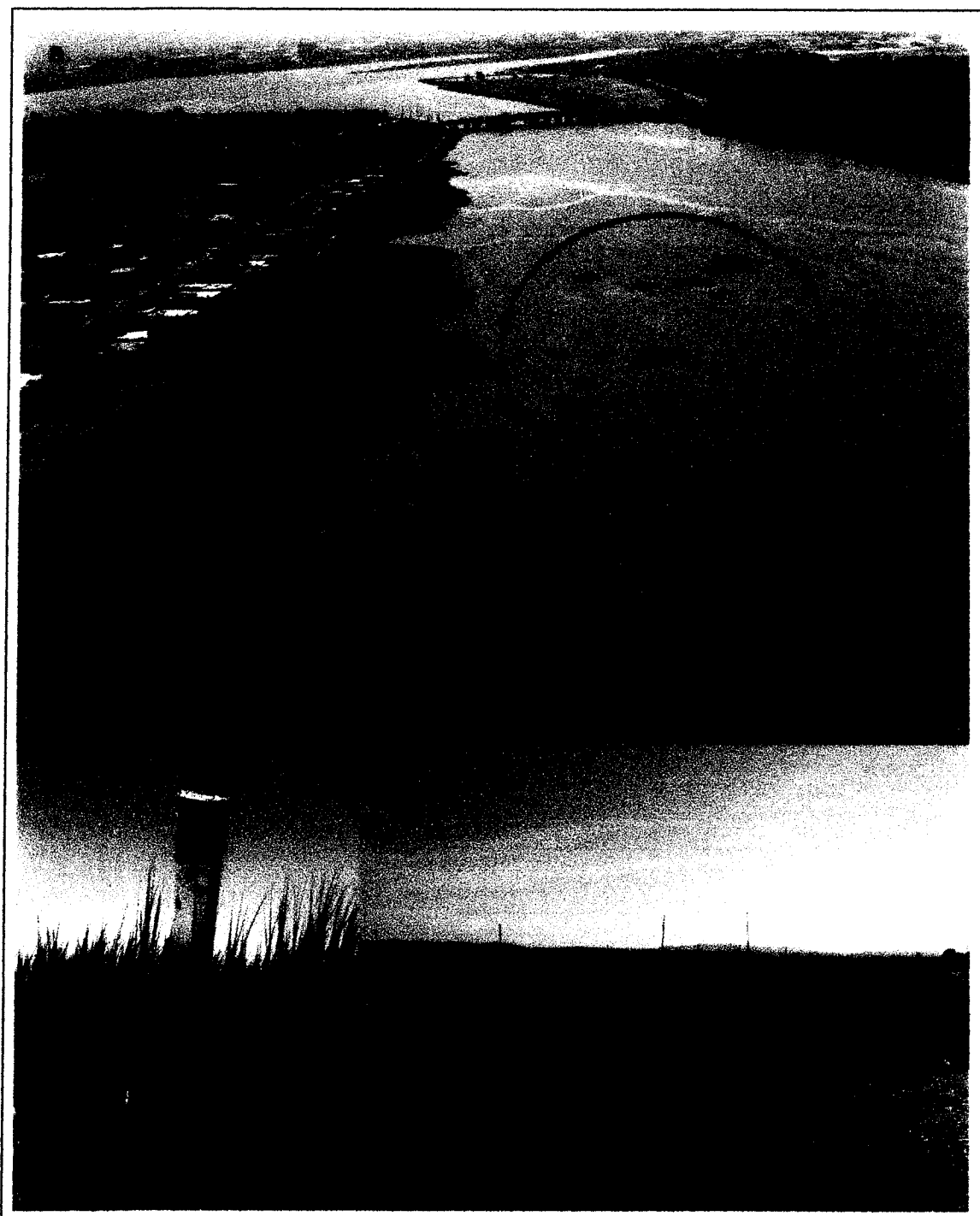


Fig. 2.1: a) Aerial view of the Alameda study site: Elsie Roemer marsh (meadow to the left, with *S. alterniflora* and hybrids), isolated hybrid *Spartina* to the right (circled). b) Close-up of inflorescence enclosure tube. c) Isolated hybrid *Spartina* with enclosure tubes at Robert's Landing. Photographs: a) E. Grijalva; b, c) C. Sloop.

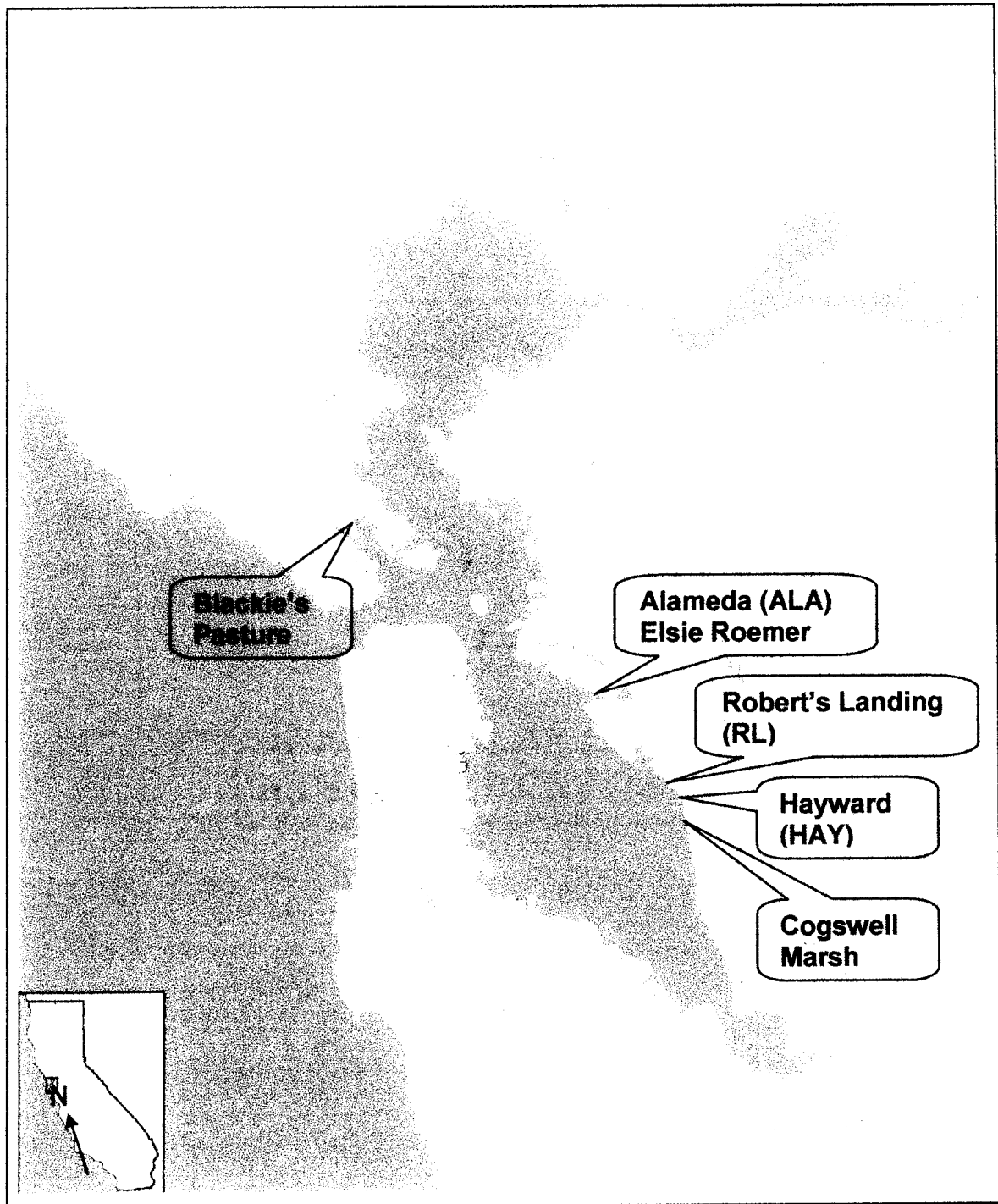


Fig. 2.2: Schematic map of San Francisco Bay. Arrows indicate study sites: *Spartina foliosa* at Blackie's Pasture, North Bay; *S. alterniflora* at Elsie Roemer marsh, Alameda; meadow *Spartina* hybrids at Cogswell marsh; and isolated mudflat hybrids along the eastern SFB shoreline at Alameda (ALA), Robert's Landing (RL), and Hayward (HAY).

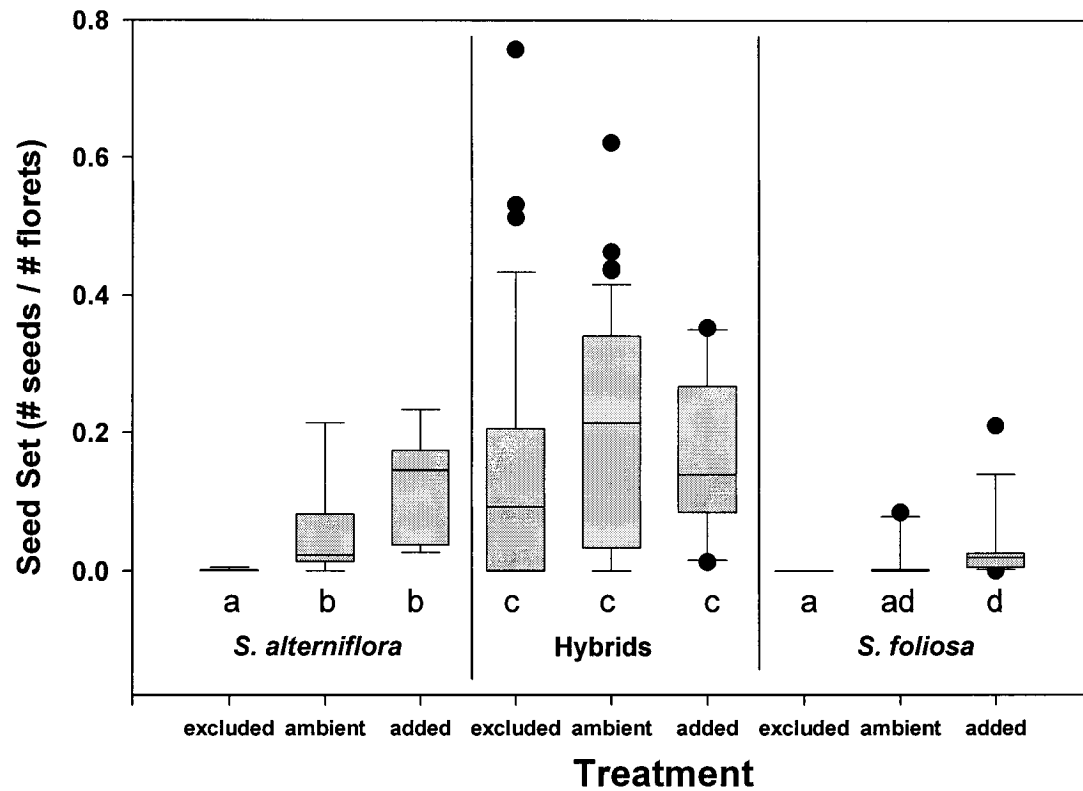


Fig. 2.3: Box plot of seed set (# seeds / # florets) of excluded, ambient, and added pollen treatments in *S. alterniflora*, *Spartina* hybrids (Hybrids) and *S. foliosa* in SFB. Solid lines show medians; bar extensions show 10th and 90th percentiles; dots show all outliers. Different letters indicate statistical differences at $P < 0.05$. There were no statistical treatment differences for added pollen at $P < 0.05$ within each species category.

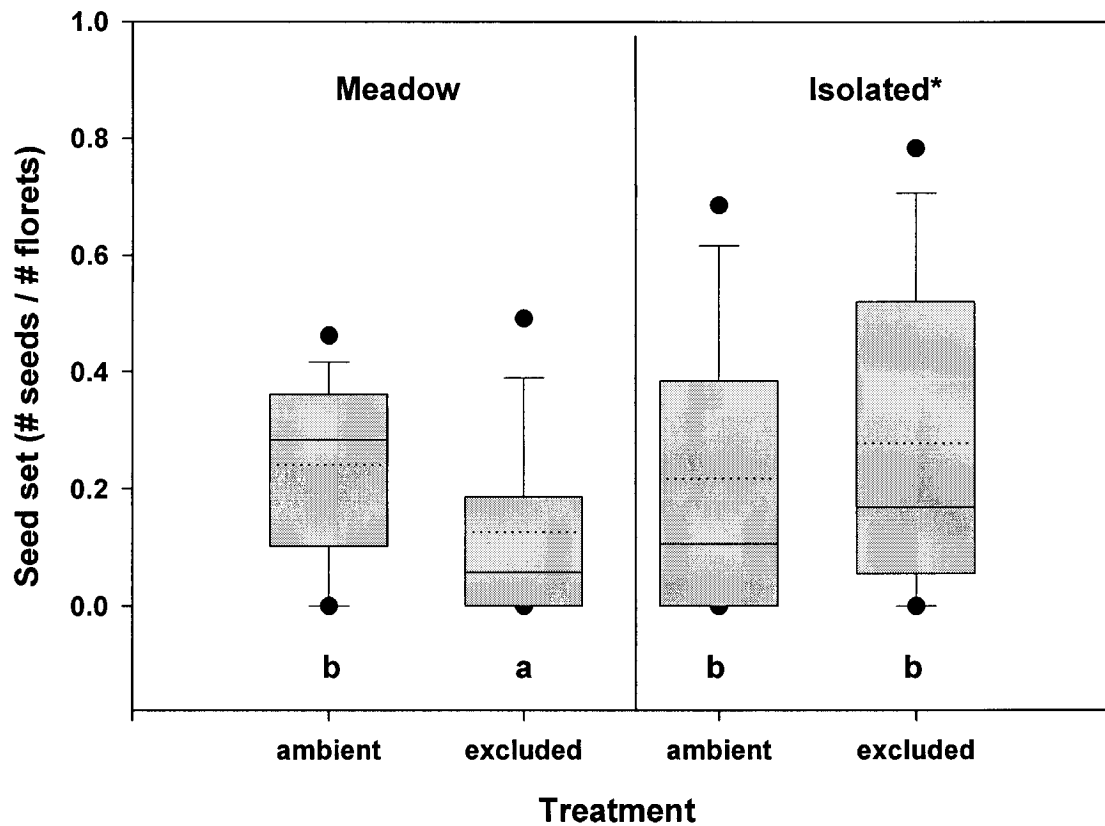


Fig. 2.4: Box plot of hybrid *Spartina* seed set (# seeds / # florets) of ambient and excluded inflorescences by habitat type: Meadow (Cogswell marsh, 1999) versus Isolated (ALA, RL, HAY tidal flats, 2004). Different letters indicate statistical differences at $P < 0.05$. Dotted and solid lines show means and medians, respectively; bar extensions show 10th and 90th percentiles; dots show 5th and 95th percentiles. *Ambient Isolated data were pooled from upwind and downwind inflorescences.

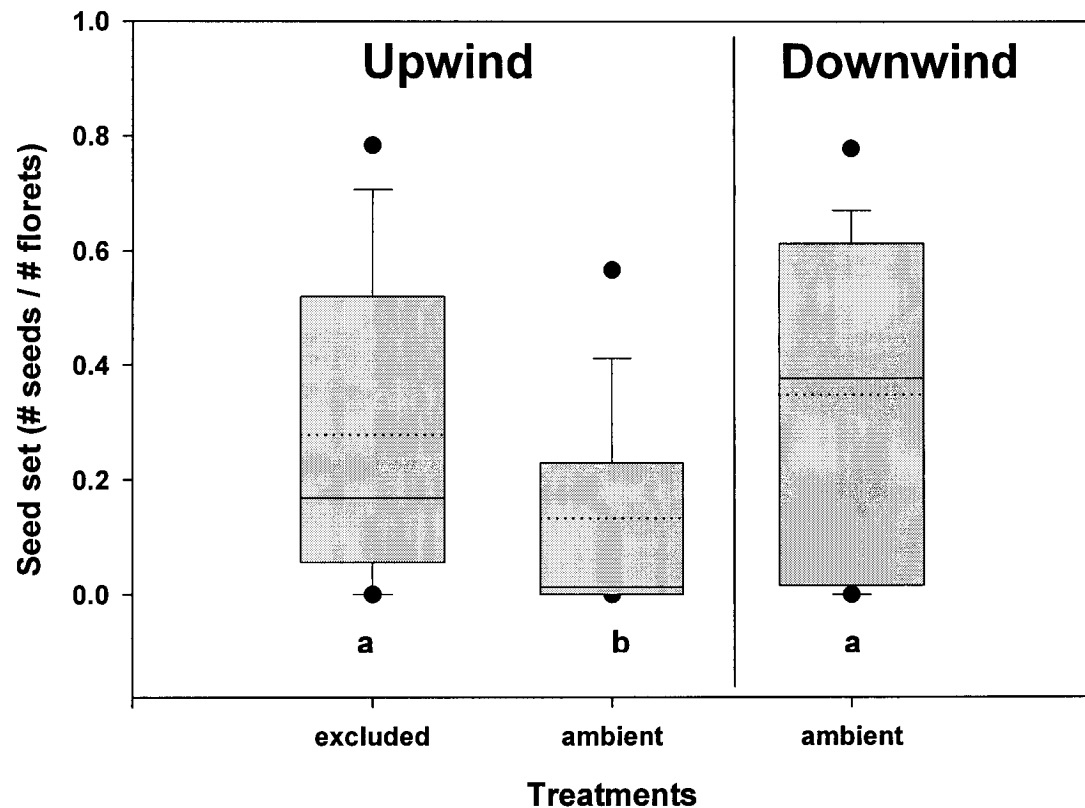


Fig. 2.5: Box plot of isolated hybrid *Spartina* (ALA, RL, HAY, 2004) seed set (# seeds / # florets) of excluded *upwind* inflorescences and ambient inflorescences: at the *upwind* and *downwind* clone edges. Different letters indicate statistical differences at $P < 0.05$. Dotted and solid lines show means and medians, respectively; bar extensions show 10th and 90th percentiles; dots show 5th and 95th percentiles.

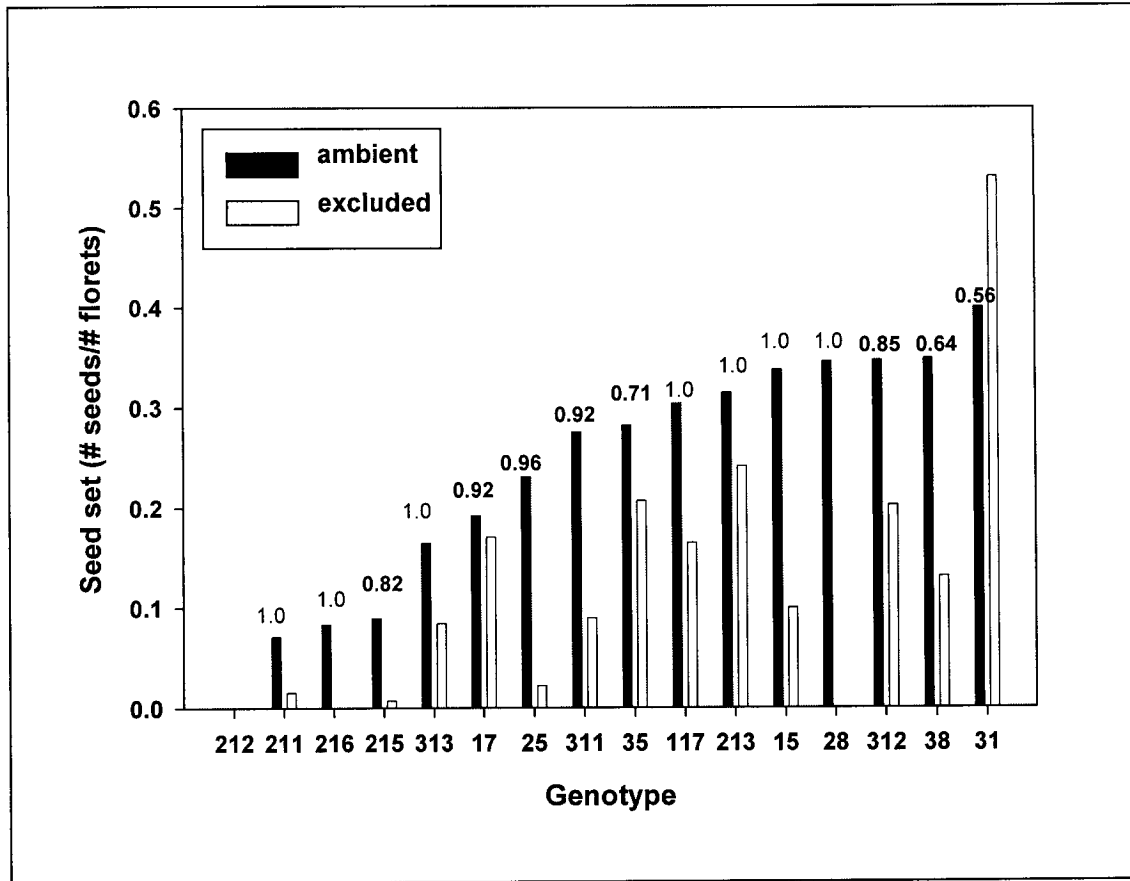


Fig. 2.6: Individual hybrid *Spartina* seed set in a meadow at Cogswell Marsh. Black bars: ambient inflorescences; white bars: excluded inflorescences. Values above black bars indicate the proportion of out-crossed seeds in offspring from ambient inflorescences determined via RAPD marker analysis. Progeny were scored for alleles absent in the mother to determine out-crossing.

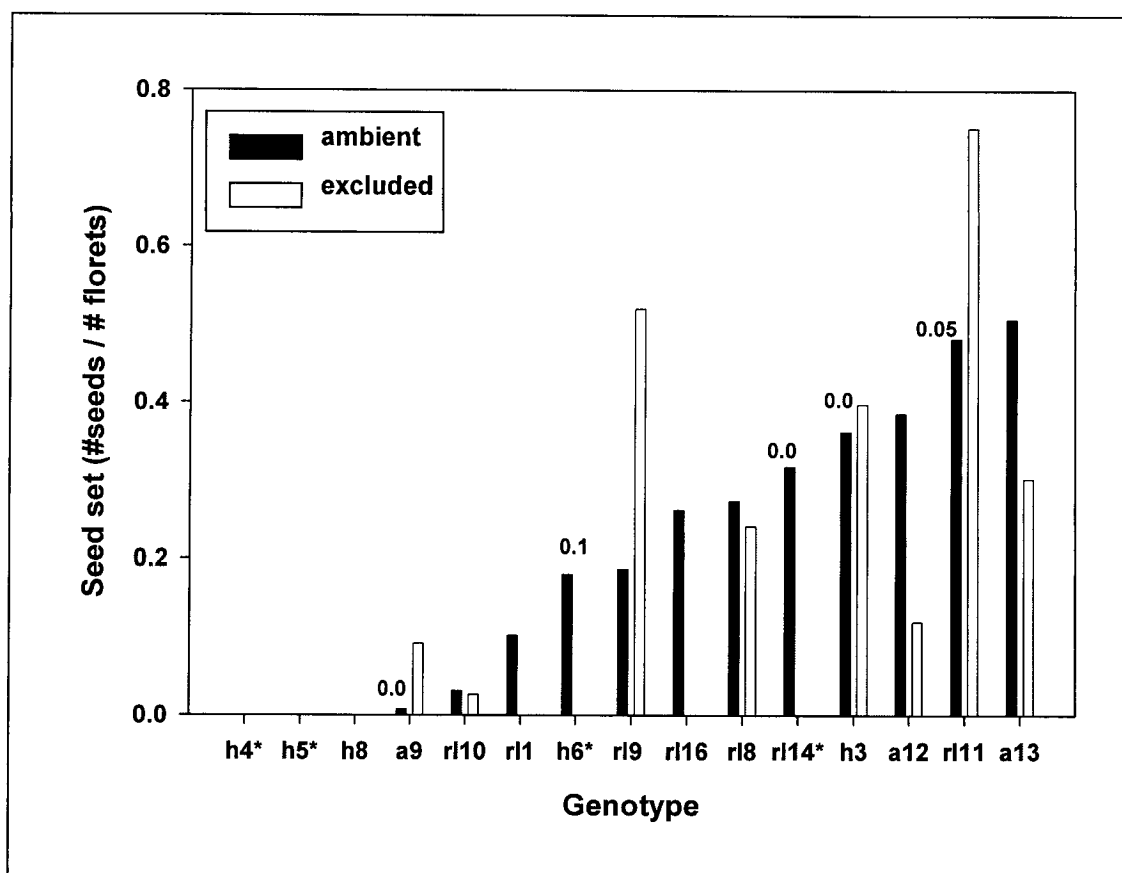


Fig. 2.7: Individual hybrid *Spartina* seed set in open SFB tidal flats. Black bars: ambient inflorescences; white bars: excluded inflorescences. Values above black bars indicate proportion of out-crossed seeds in offspring from ambient inflorescences, determined via microsatellite marker analysis. Progeny were scored for novel alleles, absent in the mother, to determine out-crossing. * Excluded treatment failed: absence of bar does not indicate zero seed set in excluded inflorescences.

Table 2.1: Statistical results.

Analysis	Species	Site/year	Source	df	Type III MS	F	P
Ambient, Pollen added & excluded	<i>Spartina foliosa</i>	Blackie's Pasture 2002	Treatment	2	47492.4	6.05	0.0075
			Error	24	94186.9		
			Total	26			
Ambient, Pollen added & excluded	<i>Spartina alterniflora</i>	Elsie Roemer 2004	Treatment	2	0.66	13.98	<0.0001
			Error	40	0.95		
			Total	43			
Ambient, Pollen excluded	Meadow hybrid <i>Spartina</i>	Cogswell 1999	Treatment	1	0.51	35.90	<0.0001
			ID	14	1.87	9.41	<0.0001
			Rep(ID)	56	0.80	1.00	0.49
			Error	63	0.89		
			Total	134			
Ambient, Pollen excluded: habitat (meadow vs. isolated)	Hybrid <i>Spartina</i>	Cogswell 1999 & ALA, RL, HAY 2004	Treatment	1	0.042	2.01	0.16
			Treatment x habitat	1	0.30	13.25	0.0004
			ID (habitat)	30	5.09	8.05	<0.0001
			Rep (ID x habitat)	84	2.06	1.17	0.23
			Error	103	2.17		
			Total	219			
Ambient downwind & upwind, Pollen added, & excluded	Isolated hybrid <i>Spartina</i>	ALA, RL, HAY mudflats 2004	Treatment	3	43.75	12.82	<0.0001
			ID	16	812.14	14.88	<0.0001
			Rep(ID)	30	366.99	3.59	<0.0001
			Error	101	344.57		
			Total	151			
Germination: Ambient, pollen excluded	Meadow hybrid <i>Spartina</i>	Cogswell 1999	Treatment	1	0.19	0.32	0.57
			ID	14	28.19	5.51	<0.0001
			Rep(ID)	50	16.37	0.90	0.65
			Error	45	16.46		
			Total	109			
Greenhouse survival: Ambient, pollen excluded	Meadow hybrid <i>Spartina</i>	Cogswell 1999	Treatment	1	0.00076	0.00	0.96
			ID	13	6.30	1.43	0.19
			Rep(ID)	45	9.69	0.57	0.96
			Error	37	12.56		
			Total	95			

Table 2.2: Estimates of inbreeding parameters. t_m = the multi-locus population out-crossing rate; t_s = the (minimum variance) single-locus population out-crossing rate.

	Cogswell Marsh	Open Mudflat
Year sampled	1999	2004
Molecular marker type	RAPDs	Microsatellites
No. of loci	9	7
No. of families	11	5
No. of progeny	307	85
Locus type	dominant	co-dominant
Multi-locus t estimate (SD)	0.522 (0.099)	0.035 (0.038)
Single-locus t estimate (SD)	0.438 (0.099)	0.037 (0.027)

Table 2.3: Chi-square test for Mendelian segregation for inbreeding for seven presumptive disomic loci. Individuals from five families with out-crossed phenotypes are indicated in bold. n = sample size, d. f. = degrees of freedom, P = probability at alpha 0.05.

Locus	Family	Maternal phenotype	n progeny	Progeny Phenotypes			X^2	d.f.	P ($\alpha=0.05$)
SPAR.08	RL9	180/184	20	180/180	180/184	184/184	0.670	2	0.715
				4	12	4			
	RL11	180/184	19	180/180	180/184	184/184 180/193	0.132	2	0.936
				2	8	8 1			
	RL10	180/193	20	180/180	180/193	193/193	0.861	2	0.650
				4	11	5			
SPAR.09	RL14	187/187	13	187/187					
				13					
	HAY3	187/187	8	187/187					
				8					
	RL9	273/291	16	273/273	273/291	291/291	0.135	2	0.935
				2	12	2			
SPAR.23	RL11	273/291	8	273/273	273/291	291/291 273/296	0.174	2	0.917
				4	2	1 1			
	RL10	285/296	14	285/285	285/296	296/296	0.276	2	0.871
				1	8	5			
	RL14	273/296	12	273/273	273/296	296/296	0.717	2	0.699
				4	6	2			
SPAR.26	RL9	274/274	20	274/274	248/274	null/276	0.895	1	0.639
				18	1	1			
	RL11	274/274	19	274/274	264/274		0.973	1	0.324
				18	1				
	RL10	264/274	20	264/264	264/274	274/274	0.387	2	0.824
				4	13	3			
SPAR.26	RL14	248/248	13	248/248					
				13					
	HAY3	248/262	10	248/248	248/262	262/262	0.165	2	0.921
				1	8	1			
	RL9	285/291	20	285/285	285/291	291/291	0.259	2	0.878
				2	11	7			
SPAR.26	RL11	285/291	19	285/285	285/291	291/291 263/285	0.398	2	0.820
				2	11	5 1			
	RL10	263/285	20	263/263	263/285	285/285	0.819	2	0.664
				4	10	6			
	RL14	285/291	13	285/285	285/291	291/291	0.141	2	0.932
				1	10	2			
SPAR.26	HAY3	277/291	6	277/277	277/291	291/291	0.004	2	0.998
				5	1	0			

Table 2.3 continued:

Locus	Family	Maternal phenotype	n progeny	Progeny Phenotypes			X^2	d.f.	P ($\alpha=0.05$)
SPAR.27	RL9	320/324	20	320/320	320/324	324/324	0.861	2	0.650
	RL11	320/324	19	320/320	320/324	324/324 313/324	0.378	2	0.828
	RL10	313/327	19	313/313	313/327	327/327			
	RL14	324/324	13	324/324			0.340	2	0.844
SPAR.28	RL9	419/419	12	419/419					
	RL11	419/419	15	419/419	416/419		0.796	1	0.372
	RL10	416/419	20	416/416	416/419	419/419			
	RL14	419/419	10	419/419			0.259	2	0.878
SPAR.29	RL9	358/360	19	358/358	358/360	360/360	0.491	2	0.782
	RL11	358/360	19	358/358	358/360	360/360			
	RL10	353/358	20	353/353	353/358	358/358	0.172	2	0.918
	RL14	358/358	13	358/358			0.549	2	0.760

Chapter 3

Recruitment dynamics of invasive hybrid cordgrass (*Spartina alterniflora* x *S. foliosa*) seedlings accelerate hybrid invasive spread and threaten the San Francisco Bay tidal flat ecosystem

Abstract

Hybrid cordgrasses (*Spartina alterniflora* x *S. foliosa*) originated ca 30 years ago in a cross of native *Spartina foliosa* with the introduced *S. alterniflora*. Hybrids have spread through San Francisco Bay (SFB) at a rate currently exceeding exponential growth, and are largely expanding within native *Spartina* marshes. As demonstrated in Chapter 2, isolated individuals, capable of self-pollination, are also colonizing, and have established in the vast, un-vegetated SFB tidal flats. Hybrid cordgrass, if unchecked, will overgrow much of these tidal flats, resulting in the loss of valuable shorebird foraging habitat. My work verified that seedling recruitment is a key to the spread rate of invasive plants. I examined SFB hybrid population genetic structure and the local, fine-scale seedling recruitment dynamics to identify the source populations and parents of tidal flat recruits, at three hybrid cordgrass populations. I combined molecular microsatellite marker with geographical (GPS/GIS) data, using multivariate ordination, Bayesian clustering, parentage/sib-ship analyses, and spatial genetic association. I found the most distant population to be genetically distinct from both southern, sympatric populations, which were closely related. For each population, meadow and tidal flat adult plants were genetically related. In the northern population, meadow adults produced the majority of

seedlings, while at both southern populations, tidal flat hybrid plants produced most seedlings. A single isolated tidal flat individual produced up to 55% of all surveyed seedlings at each southern site, the majority of which resulted from self-pollination. All of the seedlings genotyped were hybrids; there were none from the parental species even though native *S. foliosa* occurs along the shoreline at Hayward, and in the nearby heavily invaded tidal flat marsh south of San Lorenzo creek. Because seedlings were chosen randomly, this alone indicates that the hybrid is increasing much more rapidly than the native *S. foliosa*. Seed dispersal followed a stepping stone model where most recruitment occurs locally, while some dispersal may occur alongshore, according to tidal currents between adjacent populations. Positive spatial genetic structure up to ~ 200 meters and negative structure above ~440 or 540 meters showed seedling recruitment to be spatially non-random, and suggested that many seedlings establish close to their mothers in sheltered 'safe sites.' Seedling recruitment increased on average 6-fold from 2003 to 2004, and seedling size and shoot number surveys show 9 %, 12 %, and 45 % seedling survival to one-year or greater. I propose that rapid evolutionary changes in this hybrid *Spartina* swarm have resulted in a few extremely fit individuals producing the majority of locally recruiting seedlings at the forefront of the tidal flats invasion. By producing large numbers of self-pollinated, highly fit offspring in isolation, these transgressive plants ensure further colonization of the tidal flat habitat they have adapted to, and also contribute disproportionate numbers of seeds for long-distance dispersal. This successful recipe for invasion, coupled with pollen swamping close to native marshes, may explain greater than exponential hybrid spread, and if unchecked, will assure the continuing invasion of SFB by hybrid *Spartina*.

Introduction

Maritime cordgrasses in the genus *Spartina* (*S. alterniflora* and *S. anglica*) are potent invaders of naturally open intertidal mudflats. *Spartina anglica* eventually colonized 10,000 ha of British salt marshes (Gray, in press) in the decades following its origination in the late 1800's by allopolyploid speciation between native *S. maritima* and introduced *S. alterniflora*. Introduced into tidal flats in New Zealand (Partridge 1987), Tasmania (Pringle 1975), and elsewhere, and more recently Puget Sound, WA (Hacker *et al* 2001), *S. anglica* has proved to be a successful invader in these places. *Spartina alterniflora*, deliberately planted in China in 1981 for tideland reclamation, spread to 260 ha by 1985, to 1300 ha by 1990, and currently exceeds 120,000 ha (An *et al*, in press). In Willapa Bay, WA, *S. alterniflora* has covered 7,300 ha since its accidental introduction 120 years ago (Civille *et al*, in press). Successful colonization appears to be due to large open tidal mudflats providing regeneration sites for seedlings, and the ability of large cordgrasses to survive in this harsh environment. Rate of spread is exponential in Willapa Bay, where careful studies of spread rate have been made (Civille *et al*, in press). An expected endpoint to the invasion, without control, would be extensive meadows of cordgrass, as are seen within the native range of *S. alterniflora*.

In San Francisco Bay (SFB), the *Spartina* invasion differs from these other situations in that the introduced species, *S. alterniflora*, hybridized with a native species, *S. foliosa* (Daehler and Strong 1997, Ayres *et al* 1999). Subsequent introgressive hybridization has left only a small number of extant SFB *S. alterniflora*, and produced a diverse array of

hybrid genotypes (Ayres *et al* 1999). Hybrid plants differ widely in morphology (Zaremba 2001), salt tolerance (Pakenham-Walsh 2003), tolerance to immersion (Ayres *et al* 2004, <http://www.spartina.org/>) and reproductive capabilities (Anttila *et al* 1998, Daehler 1998, Zaremba 2001, Chapter 2). Some hybrids exceed both parental species in one or more traits deemed adaptive for colonization and successful invasion, such as the ability to set self-fertilized seed (Chapter 2). The invasion in SFB is most rapid near existing invaded marshes, where former salt ponds have been restored to tidal marsh by the breaching of levees, and in the conversion of existing native marshes to hybrid-dominated marshes (Zaremba, in press). Historical evidence further suggests that in the late 1970s the native Alameda *S. foliosa* meadow population was supplemented by plantings of both *S. foliosa* and *S. alterniflora*, as part of a shoreline erosion demonstration project (U.S. Army Corps of Engineers 1978). These native and introduced cordgrass planting materials were obtained from a previous USACE restoration project site at Cargill Pond 3, situated next to Alameda creek. *Spartina alterniflora* seeds collected from Virginia (D. Ayres pers. comm.) had been introduced there in the early 1970s, and were grown amidst native *S. foliosa* (Faber 2000). Colonization has occurred, but at a slower rate, along the exposed shoreline tidal mudflats. Over time, *Spartina* hybrid tidal flat invasion will turn from isolated single plants to cordgrass meadows, and, if unchecked, will result in the loss of valuable shorebird foraging habitat, seriously impacting migrating birds on the Pacific Flyway route.

In salt marsh plants, disseminules, such as seeds, germinated seedlings, and separated vegetative shoots, can be transported over great distances, drifting with tidal currents (Cain *et al* 2000, Koutstaal *et al* 1987, Guppy 1906, 1917, Nelson 1978, Schimper 1891). Water transport and drift distance of disseminules chiefly depend on their buoyancy (Darwin 1857, 1859, Martins 1857). *Spartina anglica* seeds, for example, remain buoyant between 4 to over 60 days (Huiskes *et al* 1995), and within a week were transported by tidal currents over a net distance of 60 km (Koutstaal *et al* 1987). Huiskes *et al* (1995) found that floating seeds are transported only when the water reaches a certain height above the salt-marsh surface. Accordingly, the incoming water may lift disseminules from the plant directly, and may also pick up those seeds that have fallen to the ground, even though these may take some time to float to the surface (Huiskes *et al* 1995). Few seeds were imported into the marsh from elsewhere by the incoming tide, but the flood stream carried many low-marsh seeds towards higher zones (Huiskes *et al* 1995). Many meadow seeds got trapped in the marsh vegetation along the soil surface and traveled only short distances, mainly landward, with the stronger incoming tide (Huiskes *et al* 1995). In contrast, the net transport of propagules in the tidal flat was seaward, with the outgoing tide, a phenomenon present in many tidal wetlands (Huiskes *et al* 1995). However, while the marsh vegetation was successful in trapping most seeds, 10% of all *S. anglica* seeds observed in the study were found in mudflat traps on the outgoing tides (Huiskes *et al* 1995). Given the potential production of many millions of hybrid seed in hybrid-dominated marshes (e.g. a single hybrid plant produced over 3 million seeds in 1998, Zaremba 2001), a 10% export of seed could represent a sizable number of potential colonizers. In light of the successful invasion of tidal flats by maritime cordgrasses

elsewhere, I conclude that sites in SFB, receiving tidally dispersed seed from invaded marshes, are vulnerable to invasion.

Hybrid *Spartina* seeds are, like *S. anglica* seeds, buoyant (Huiskes *et al* 1995, K. Sayce unpublished data), and remain viable for 6 to 8 months, germinating the spring following seed set. Mature seeds are shed into the water column, or fall onto the mud surface.

Average water depths in San Francisco Bay at mean higher high water (MHHW) range from 4.6 m in the South Bay to an average depth of 13.4 m in the Central Bay, and median depths range from 3.2 m to 10.9 m, respectively

(<http://sfbay.wr.usgs.gov/access/Bathy/geostats.html>). The tidal currents along the eastern and western SFB shoreline extend from south to north

(http://sfports.wr.usgs.gov/SFPORTS/vec_map/centbay.html), consistent with the general observation that hybrid seed disperses northward from the hybrid origin site(s) in SFB.

Tidal water containing a genetically diverse mixture of seeds could be carried long distances from their source populations and deposited randomly along the shoreline in accord with the island dispersal model of Wright (1969). The island model assumes many discrete populations where migration occurs randomly, irrespective of spatial proximity.

On the other hand, the stepping stone model of Kimura and Weiss (1964) suggests that migration (or seed and pollen dispersal in plants, Hu and Ennos 1999) mainly occurs between neighboring populations, with the remainder of gene flow occurring through random long-distance dispersal.

For successful seedling establishment, seed must be delivered to a suitable environment.

In terrestrial systems, seedlings have been observed to establish at a greater rate near their seed parents in ‘safe sites,’ where the presence of the adult plant favorably affects the surrounding space for seedling establishment, causing spatially distinct recruitment patterns (Harmon and Franklin 1989), and complex population genetic structures (Alvarez-Buylla *et al* 1996). This has been observed on cobble beaches behind *S. alterniflora* in New England (Bruno and Kennedy 2000). *Spartina* hybrids, like the parental *S. alterniflora*, are potent ecosystem engineers (Crooks 2002), accreting sediment and in some cases creating seedling safe sites: raised areas of sediment accreting in back of the clone form a suitable ‘nursery’ by decreasing water level and wave action. The pattern of seedling distribution along the eastern shoreline of SFB is not random; rather, seedlings almost always occur only in the vicinity of large hybrid cordgrass plants, scattered on the tidal flat (Sloop, Ayres, Pakenham-Walsh, personal observations). Understanding seedling distribution dynamics was the over-arching goal of this study.

In general, the number of seeds produced and exported on tidal waters from shoreline hybrid meadows far exceeds that of isolated tidal flat individuals. Tidal flat seedling populations should be genetically well mixed, and unrelated to nearby cordgrass plants, if tidal flat seedling recruitment mainly stems from meadow hybrids. In contrast, if isolated self-fertilizing tidal flat colonizers are the source of local recruitment, then tidal flat seedlings will be strongly related to each other, and to the existing plants, reflecting their selfed origins. In order to examine overall SFB hybrid population dynamics, and local tidal flat recruitment dynamics, my goals were 1) to determine the allelic diversity and

the genetic structure of geographically distant and proximate hybrid populations in SFB, 2) to reveal inter-relatedness of established adult plants, within and among sites, and infer colonization history via sib-ship analysis, 3) to determine yearly, site-specific seedling abundance, and through parentage analysis, identify potential source populations of isolated tidal flat populations, and investigate whether hybrid recruitment occurs at a local versus regional scale, and 4) to determine the fine scale spatial-genetic structure of colonizing seedlings, to identify whether specific, highly fit individuals contribute disproportionately to tidal flat seedling populations.

Materials and Methods

Field Sites and Sampling

Isolated hybrid plants from the open tidal flats to the north, at Elsie Roemer marsh in Alameda (ALAtf, $n = 9$), and two southern tidal flats at Robert's Landing (RLtf; San Leandro shoreline; $n = 19$), and the Hayward shoreline (HAYtf, $n = 24$) were genetically and geographically surveyed (Fig. 3.1). These sites are separated consecutively by ~16 km and ~2 km along the eastern SFB shoreline from north to south. Tidal creeks link the closer two, southern tidal flat populations to a nearby, levee-bound *Spartina* hybrid meadow at Robert's Landing (Fig. 3.2, arrow). The more distant, northernmost Alameda population is directly adjacent to a hybrid meadow (Fig. 3.3B, arrow). Additionally, 10 *S. foliosa* plants from the HAY shoreline (Fig. 3.3A), 5 *S. alterniflora* individuals from Elsie Roemer meadow at Alameda (Species determined via RAPD markers), and meadow *Spartina* hybrid adults from 1) Elsie Roemer marsh in Alameda (ALm; $n = 25$,

Fig. 3.3B), and 2) an inland RL marsh (RLm, $n = 30$) were sampled (Fig. 3.2A, arrow). In the spring of 2003 and 2004 all three tidal flats sites (ALAtf, RL, HAY) were inspected for seedling recruitment during extreme low tides. Each site was exhaustively searched by teams of 2 – 5 people walking transects ca 15 m apart (We were able to clearly discern *Spartina* seedlings from a distance of 10 meters). All located seedlings were GPS logged in each year; telltale footprints on either side of a seedling allowed us to distinguish those already logged. To measure seedling survival we counted the number of seedlings with more than one shoot, and exceeding 10 cm in height. In 2003, leaf samples for DNA extraction, were haphazardly collected from about 30 percent of all GPS surveyed seedlings per site, resulting in 42 RLtf, 32 HAYtf, and 33 ALAtf samples (Figs. 1, 3). In 2004 seedling density had increased more than 500-fold from the previous year at RL and HAY. DNA samples there were thus collected by walking two parallel transects along the length of the surveyed shorelines, collecting a sample each 20 paces. This resulted in 54 RLtf, 69 HAYtf, and 67 ALAtf samples in 2004 (Figs. 1-3).

Genetic Structure

Gene flow and spatial genetic structure of adult and seedling cohorts at each site were measured via a combination of genetic and geographic analyses, using multilocus haplotypes from 17 microsatellite markers: 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, SPAR.33 (Blum *et al* 2004, Sloop *et al* in press), and GPS/GIS (Geographic Positioning System/Geographic Information System) methods, respectively. DNA was extracted from leaf samples using Qiagen DNeasy Plant

Mini Kits (Qiagen, Valencia, CA). PCR (polymerase chain reaction) volumes and genotyping procedures are outlined in Sloop *et al* (in press). Allelic frequencies, and observed (H_o) and expected (H_e) heterozygosity were calculated using GENETIX (Belkhir *et al* 2004). Hardy-Weinberg- (HWE) exact tests (using Markov chain default settings) and genotypic linkage equilibrium (LE) analyses were carried out for all eight adult populations using GENEPOP (Raymond & Rousset 1995, <http://wbiomed.curtin.edu.au/genepop/genepop/>). GPS data were obtained with Trimble GeoExplorer and Pathfinder data loggers (Trimble Inc., Sunnyvale, CA). Spatial points were differentially corrected using GPS Pathfinder Office 2.80. X- and y-position coordinates per seedling, and adult plant center, were calculated using ArcView 8.3 (2002 ESRI Inc.).

Multivariate ordination and Bayesian cluster analysis

In order to depict genetic distinctions between hybrid populations and both *Spartina* parent species, factorial correspondence analysis (FCA, Benzécri 1973), a multivariate ordination technique on haplotypes was performed using GENETIX (Belkhir *et al* 2004). FCA allows the construction of an orthogonal system of axes (called factors and denoted Axe1, Axe2, etc.) where observations (individuals) and variables (loci/alleles) can be jointly displayed graphically. The factors are presented in a decreasing order of importance, according to the information they represent. The ability of graphically displaying observations and variables simultaneously on the same factorial space makes it easy to discover the salient information given by the data, but it does not allow assignment of individuals to clusters quantitatively (probabilistically).

Using this multivariate ordination, I used two sample sets to measure population structure. The first data set consisted of all hybrid adult sub-populations, consisting of 25 ALm (meadow), 9 ALAtf (tidal flat), 24 HAYtf (tidal flat), 30 RLm (meadow), and 19 RLtf (tidal flat) samples (Table 3.1), plus 29 *S. foliosa* individuals from 6 sites in SFB and the northern coastline to Tomales Bay, and 2 locations in southern California/northern Mexico, and 27 *S. alterniflora* plants from 6 East Coast/Gulf Coast locations (New York, Georgia, Florida, Alabama, and Mississippi), and 3 sites in Willapa Bay, WA (Palix River, Tower Slough, and Peninsula), and 5 SFB *S. alterniflora* adults from Elsie Roemer marsh, Alameda. These latter 5 individuals were initially genetically identified to species via RAPD (randomly amplified polymorphic DNA) analysis; microsatellite data confirmed that these plants were *S. alterniflora*. In a second analysis, I added tidal flat seedling data to this set, from three sample sites and two years: ALAs '03 = 33, ALAs '04 = 67, RLs '03 = 42, RLs '04 = 54, HAYs '03 = 32, HAYs '04 = 69 (Table 3.1).

In a second approach, a model based Bayesian clustering method was applied to all haplotypes of the second sample set (including all seedlings) using STRUCTURE (Pritchard and Wen 2004, Falush *et al* 2003, Pritchard *et al* 2000). In this analysis individuals are probabilistically assigned to either a single cluster (the population of origin), or more than one cluster (the parental populations), if there is genetic admixture due to hybridization (Falush *et al* 2003). The program assumes the neutral unlinked markers to be in Hardy-Weinberg equilibrium and linkage equilibrium and that recent hybridization or migration would likely produce departures from Hardy-Weinberg

equilibrium and linkage equilibrium. STRUCTURE identifies the K unknown populations (genetic clusters) of origin of individuals 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 10,000 iterations. To detect the true number of clusters (K) I followed the graphical methods and algorithms outlined in Evanno *et al* (2005). Under the assumption that the sampled plants belong to an unknown number of K genetically distinct clusters, I 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 true value of K .

Sib-ship and Parentage Analysis

In order to assess relationships within the genotyped adult study stand, FaMoz software (Gerber *et al* 2003) allows half-and full-sib-ship analysis, which was employed separately on all adults within each site and among ALAm and ALAtf, and RLtf, RLm, and HAYtf sites, respectively. Log of odds (LOD) values of 1 and 2 for half- and full sib-ship, respectively, determined from sib-ship simulation results, served as threshold values. Parentage analysis was used to establish the parents of seedling recruits, by calculating within population parentage likelihoods for all tidal flats adult plants in both sampling years using the multi-locus genotype data outlined in the spatial genetic analysis above. FaMoz reconstructs parent/offspring relationships using such genotypic arrays (Gerber *et al* 2003), based on the assumptions of independent Mendelian inheritance, allele frequency estimates as true allele frequencies, panmixia (split into a number of separately

evolving populations (demes) but with the level of emigration sufficiently high that they continue to evolve as if a single population), and equal allelic frequencies ‘inside’ and ‘outside study stand’. The software calculates LOD ratio scores for any potential parentage relationship with a value greater than zero (Gerber *et al* 2000). The higher the LOD score for a given parent/offspring pair, the higher the likelihood that the parentage relationship is true. Simulations were used to calculate the threshold values of the LOD score for sib-ship and parentage assignment. The intersection of the LOD distributions of ‘inside’ versus ‘outside the study stand’ was then used to determine a LOD threshold with known first and second type errors (user can choose simulation and LOD calculation error beforehand). Additionally, parentage test simulations were used to examine the test quality of the genotyped data set. These simulations (per site/year) indicated the percentages for correct single parent and parent pair choice in this data set to vary between 35% and 91%, and 61% to 100%, respectively (parameters used: simulated offspring number = 100, size of total reproducing population = 200, simulation error = 0.0005, calculation error = 0.0005, departure from H-W = 0). Simulated LOD distributions ranged between 0.67 to > 25, and since over 75% of all observed LOD scores were above 3, the LOD threshold value for correct single parent and parent pair choice was set at this value.

Spatial Genetic Analysis

In order to determine whether genetically related individuals are situated close to each other, I employed analysis of within population spatial genetic structure. This analysis shows whether a relationship between geographical distance and genetic relatedness

between individuals exists. 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 (SGS) v.1.0d (Degen *et al* 2001, Degen 2000), comparing genetic distances with geographic distances, employing 1) mean genetic distance calculations by Gregorius (1978), and 2) the number of alleles/haplotypes in common. Permutation tests were used to evaluate the statistical significance of all measures, in order to assess deviations from a spatially random distribution. 1,000 permutations were performed to obtain 95% confidence intervals under the hypothesis of no spatial correlation. The data set consisted of all adults and seedlings (Table 3.1).

Results

Genetic Variation

In all tested populations the average observed heterozygosity (over all 17 loci) was slightly lower, but not significantly different than expected (Table 3.1). The average number of alleles per locus was highest at 8.3 for *S. alterniflora* in its native range, and lowest at 1.59 for *S. foliosa*. *S. alterniflora* in SFB had 3.76 alleles per locus. Hybrid allelic diversity ranged between 3.65 and 5.65 alleles per locus (Table 3.1). For all 17 loci, one to ten alleles per locus were found only in hybrid individuals. These ‘unique’ hybrid alleles may point to the allelic composition(s) of one or more, most likely, *S. alterniflora* ancestors not present in this sample, as most are only present separately in individuals either at Alameda or Robert’s Landing/Hayward. However, most of the ‘unique’ hybrid alleles are at relatively low frequencies (0.02 to 0.13), which may either

suggest that present hybrid populations have greatly diverged from their original ancestors (e.g. mainly via back-crossing to *S. foliosa*, or via inbreeding), that these alleles either stem from recent mutations, or from individual genotyping error, or all three. Hardy-Weinberg (H-W) exact tests, examining the random union of gametes, showed that more loci were in H-W equilibrium in all populations at Alameda (ALm = 77%, ALAtf = 94%, ALA *S. alterniflora* = 88%), and in the meadow at Robert's Landing (RLm = 65%), than in the tidal flats at RLtf (12%), and HAYtf (18%). Loci in *S. foliosa* populations were either monomorphic (65%), or deviated from H-W (35%), confirming extremely low gene flow in this native species. In a mix of *S. alterniflora* populations from the native range, only 12% of tested loci showed H-W equilibrium, as expected for a combination of individuals from several geographically distinct source populations.

Linkage disequilibrium was far greater in the two isolated tidal flat populations: RLtf = 74%, HAYtf = 96%, as compared to Alameda and meadow populations: ALm = 27%, ALAtf = 0%, ALA *S. alterniflora* = 0%, RLm = 44%. These differences may reflect non-equilibrium population dynamics (inbreeding), recent population admixture, mixed sexual and asexual reproductive modes, and/or the presence of null alleles. Genotypes were in linkage disequilibrium in *S. foliosa* in only 2%, and in *S. alterniflora* in 16% of all possible loci combinations.

Multivariate and Bayesian cluster analysis

Multivariate ordination showed four distinct groupings among the 162 genotyped adult individuals (Fig. 3.4A). Once all genotyped seedlings were added in the analysis, all

seedlings grouped with adults from their site of origin (Fig. 3.5). In the adult-only factorial correspondence analysis (8 populations, Fig. 3.4) the first three principal correspondence factors explained 78.76 percent of the variation, while in the adult-plus-seedling run ($8 + 6 = 14$ populations, Fig. 3.5), this value decreased to 67.10 percent.

Bayesian cluster analysis for the combined parent species, hybrid adult, and seedling samples showed at least two distinct hybrid membership groups (Fig. 3.6, Alameda vs. Hayward/Robert's Landing). At $K = 4$ all ALA samples are most similar to each other, and local ALAm *S. alterniflora* (Fig. 3.6, group 3), while different from the rest of the parent species and hybrid groups (Fig. 3.6). At each of the three sites, seedling groups (Fig. 3.6, groups 6, 7, 9, 10, 13, 14) are most like adults at the same location, depicting similar color patterns (Fig. 3.6, groups 4, 5, 8, 11, 12). All Hayward (Fig. 3.6, groups 8 to 10) and Robert's Landing (Fig. 3.6, groups 11 to 14) sample groups are overall more similar to each other (Fig. 3.6), suggesting gene flow across these groups. In more detail, however, the color patterns of all line segments show a slightly darker pattern at HAY, whereas at RL, especially the seedling populations (Fig. 3.6, groups 13 and 14) are predominately lighter. This may indicate differences in the more recent seedling recruitment patterns, as at each site a different, selfing individual dominated seedling production. The color representing *S. alterniflora* (range wide) ancestry (Fig. 3.6, group 2) is different from that displayed in the ALAm *S. alterniflora* (Fig. 3.6, group 3), and all other ALA groups (Fig. 3.6, groups 4 - 7). The distinction of the Alameda populations as a single, separate group may thus suggest a discrete *S. alterniflora* ancestor at Alameda.

Sib-ship analysis

Within population sib-ship analysis revealed that four of the nine ALAtf plants were siblings, and that all but two ALAtf plants were related to ALAm plants within four family groups (Fig. 3.7, A-D). This supports common gene flow between the Alameda meadow and tidal flat plants, and may suggest that most tidal flat plants originated from this meadow.

At Robert's Landing, sib-ship analysis of the tidal flat plants revealed two distinct family groups with 3 and 9 inter-related plants, respectively (Fig. 3.8). All but two RLtf plants (RLtf8 and RLtf11) showed either a direct sibling or more removed relationship to one or more plant(s) from the nearby inland RLm *Spartina* meadow (Fig. 3.8). This relationship could indicate that tidal flat plants may have originated from these meadow plants, in particular RLtf 14, related to four RL meadow plants, and the biggest contributor to seedling abundance at this site (Table 3.2a). Two plants (RLtf 12 and RLtf 10), were unrelated to any other plant in the study stand (Fig. 3.8). All members of the family group C (RLtf 8, RLtf 9, RLtf 11, Fig. 3.8, Table 3.2a), and five individuals of, *S. foliosa*-like (only one/two loci difference) family group D (RLtf 7, RLtf 16, RLtf 106, RLtf 107, RLtf 108, Fig. 3.8, Table 3.2a) also contributed to reproduction.

At Hayward, only ~2 km to the South of Robert's Landing, the data show three distinct family groups: *S. foliosa*-like hybrids in one family (Fig. 3.9: C; only one/two loci difference, 7 plants), and two other hybrid groups with 4 (Fig. 3.9: A) and 9 (Fig. 3.9: B) closely related plants, respectively. The plant with the largest reproductive contribution at

Hayward (Haytf M1, Fig. 3.9, Table 3.2b) is part of group A, with 4 hybrids. All other group A members (Hay22M5, HayM2B, Hay24, Fig. 3.9, Table 3.2b) are also likely parents. Most plants in hybrid family group B also produced offspring in either or both years (Haytf 21, Haytf 28, Haytf CL29, Haytf 46, Haytf 47, Haytf 48, Haytf 49, Haytf M2, Fig. 3.9, Table 3.2b). Five *S. foliosa*-like hybrids (Haytf 31, Haytf 33, Haytf39, Haytf 42, Fig. 3.9, Table 3.2b) and Haytf M4 were among the mainly out-crossing parents.

Exploring sib-ship relationships of all tidal flat adults from Robert's Landing and Hayward combined, indicated that only two RLtf adults (RLtf 1, RLtf 14, Fig. 3.9, Table 3.2:) were related to a total of 7 HAYtf hybrids. The largest contributor to reproduction at Robert's Landing (RLtf 14) is linked to 6 of the 9 inter-related hybrids in HAYtf group B, of which most also produced progeny. RLtf1, which is not a member of any RLtf family, is sibling to Haytf M4, which is also without family ties (Fig. 3.9). This might indicate that, instead of the inland meadow, RLtf 14 may have directly arisen from the southern tidal flat plants, or vice versa. It may have possibly originated further to the south, from Cogswell marsh, or directly from Alameda creek via long-distance dispersal.

All members of one Alameda family group (Fig. 3.7, B) were related to both *S. foliosa*-like groups at RLtf (Fig. 3.9, E) and HAYtf (Fig. 3.9, C), and another HAYtf family group (Fig. 3.9, A), including the most successful HAYtf parent (Haytf M1), which is unrelated to any plants at the RL tidal flats. This family affinity may not be due to

common ancestry, or identity by descent, however, but may instead be a result of convergent evolution, or the proportion of shared *S. foliosa* alleles.

Seedling abundance

The abundance of first-year hybrid seedlings on tidal flats from 2003 to 2004 was extremely variable, indicating episodic recruitment. In 2003, the number of first-year seedlings was relatively low (three site average 329.33 ± 197.70), while in 2004 it increased ~6-fold (1597.67 ± 977.61) (Table 3.3). In 2005, however, the abundance of new, first-year seedlings fell to close to zero at Robert's Landing and Hayward (Alameda was not surveyed in 2005). All sites differed in the amount of older seedlings that survived from the previous year(s). I considered seedlings exceeding 10 cm in height, and having more than one shoot, to be older than one year. At Alameda, 45 % of surveyed seedlings fell into this category, while survival was 12 % at Robert's Landing, and only 9 % at Hayward. Survival is a direct function of seedling deposition patterns (e.g. in 'safe sites' behind maternal plants versus sand banks along the tide line), and the site-specific environmental pressures, causing seedlings to either perish or flourish. At the Alameda tidal flat, seven adults are relatively tightly clustered together spatially, providing a large safe-site area, whereas at both other sites established tidal flat plants are more widely spread apart. Since the survival percentage at the more clustered Alameda site exceeded both other sites about four-fold, spatial clustering, or lack thereof, may have a direct influence on the number of surviving seedlings.

Parentage Analysis

Parentage likelihood analyses, using 17-loci genotypic data per site and year, revealed a large proportion (from 0.38 at HAYtf '03 to 0.59 at RLtf '04) of selfing in HAYtf and RLtf tidal flats populations (Table 3.3), and relatively high proportions of seedlings with a single likely parent among the tidal flat plants, either as pollen or seed parent (RLtf: 95% in 2003, 83% in 2004; HAYtf: 87 % in 2003, 81% in 2004). The addition of 30 genotyped adults from the inland hybrid *Spartina* marsh (RLm, location see Fig. 3.2) to the 2004 analysis did not significantly change inbreeding proportions. However, the proportions of seedlings with at least a single likely parent in the study stand increased to 99 %. This shows that the great majority of seedlings were produced locally in the tidal flat, or in the nearby meadow.

In contrast, the data showed a smaller proportion of seedlings having likely parents among the tidal flat plants at Alameda Island (38% in 2003, 27% in 2004, Table 3.3). The proportion of seedlings having a likely parent pair in the population increased to 64% when all the ALAtf seedlings from both years were analyzed with a combination of 9 tidal flat and 30 meadow plants (including 25 hybrids and 5 *S. alterniflora*). Therefore, 36% of seedlings either originated from the many other, non-genotyped hybrid and *S. alterniflora* plants in the adjacent meadow, or from elsewhere.

The proportion of the tidal flat plants at each site producing seedlings in either year ranged between 0.50 and 0.77; of those that did, the proportion that produced seedlings both years ranged from 0.44 to 0.71. At RL and HAY, the successful seedling producers

were, in general, those that predominately selfed. Plant size and the distance to the nearest neighbor were no direct predictors of individual seedling production (Table 3.2 a - c). Individuals that did not produce any offspring tended to be more closely related to *S. foliosa*, differing only by one or two alleles (Figs. 7 – 9).

The proportion of reproducing adults that were selfing at HAYtf and RLtf varied between 0.44 and 0.78, while similarly, 0.58 to 0.80, were producing out-crossed seedlings (Table 3.3). Fewer plants at these sites, 0.08 to 0.45, displayed mixed mating, contributing both selfed and out-crossed progeny (Table 3.3). In both sites and years, a single plant produced the majority of mainly inbred seedlings (Table 3.2 a - c, red/bold). In 2003 and 2004, tidal flat plant RLtf 14 likely produced 85% and 78%, respectively, of all inbred seedlings, and 38% and 46%, respectively, of all surveyed RLtf progeny. Out of 42 total seedlings produced in both years by this individual, just a single seedling was the result of out-crossing; all the rest arose via self-pollination. Given the position of this plant far out in the mudflat, 193 meters from its nearest neighbor, and the prevailing onshore breeze, it is likely that RLtf 14 was the pollen parent to the out-crossed seedling (average LOD score $\pm$ stdev: 16.33 ± 4.65 (2003), 8.16 ± 2.94 (2004); Table 3.2a). At Hayward, tidal flat plant HAYtf M1 generated 33% of the total and 55% of all inbred progeny, and 13% and 29% of all surveyed offspring in 2003 and 2004, respectively (average LOD score $\pm$ stdev: 8.30 ± 3.73 (2003), 7.40 ± 1.51 (2004)). HAYtf M1 was also the most likely single parent to an additional 3 and 5 out-crossed seedlings per respective year (Table 3.2b). Single parent contribution per individual (out-crossing) ranged from 1 to 7 offspring at all three sites (Table 3.2a, b). At Alameda, only 28% (2004) or 40% (2003) of tidal flats plants produced a small proportion (0.07 to 0.09) of inbred offspring, while

four (2003) or six (2004) tidal flat plants were producing out-crossed seedlings (0.18 to 0.30), and a single plant per year underwent mixed mating (Tables 3b, 4).

Spatial Genetic Analysis

Two spatial analyses, using different metrics, showed significant positive spatial structure between adult and seedling individuals up to approximately 200 meters at all three sites, and negative spatial structure above a distance of approximately 440 meters at HAY and above ~ 530 meters at RL (Fig. 3.10). The two distograms, one using genetic distance (Gregorius 1978), and the other using only alleles in common, showed similar results (Fig. 3.10). At Alameda there were too few individuals in a distance class greater than 200 meters to include in the analysis (minimum value = 30 individuals per distance class, Degen 2000). Using genetic distance (Gregorius 1978), the distances below the reference value indicate positive spatial structure, where proximal individuals are genetically more similar than expected for a spatially random distribution of genotypes. Distances above the reference indicate negative structure, where individuals show greater genetic divergence than expected for a spatially random distribution of genotypes (Degen 2000). Spatial genetic structure according to the number of alleles in common show positive spatial structure above the reference line, and negative spatial structure below the reference line (Degen 2000).

Discussion

In order to shed light on SFB hybrid population structure, and the dynamics of hybrid seedling recruitment in open tidal flats, my first goal was to determine the allelic diversity and genetic structure of SFB hybrid *Spartina* populations. *Spartina foliosa* was genetically depauperate throughout its range with an average of 1.6 alleles per locus. *Spartina alterniflora* had roughly 5 times more diversity range wide than *S. foliosa*. SFB *S. alterniflora* at Alameda had lower allelic diversity (3.76 alleles per locus) than the range wide value, which was consistent with the introduction history of *S. alterniflora* into SFB from a single Virginia *S. alterniflora* source population (Faber 2000). I found *Spartina* allelic hybrid diversity (from 3.65 to 5.65) was an additive value between the two parent species consistent with the joining of 2 divergent genomes (Table 3.1).

Multivariate ordination confirmed clear genetic divergence between hybrids and the parental species. *Spartina alterniflora* and *S. foliosa* are genetically distinct from each other at opposite ends of the ordination space along axis 1, and vary to different degrees from all other hybrid population groups in between (Fig. 3.4a, b: CL 1 & CL 2). The distinct *S. alterniflora* group (CL 2) is genetically highly variable, reflecting its large within-group diversity, including individuals from five separate populations along the Eastern seaboard (NY, NC, FL, MS, AL), and three in the Pacific Northwest. SFB *S. alterniflora*, clustered closely with ALAm and ALAtf hybrids (Fig. 3.4b: CL 4), showing a clear divergence from the other eight *S. alterniflora* populations. This may be due to the fact that the purported SFB *S. alterniflora* source population in Virginia was not

represented in the surveyed *S. alterniflora* populations from the native range. At the opposite end of axis 1, the tight grouping of *S. foliosa* (Fig. 3.4b: CL 1) shows a less clear distinction from other groups due to the close similarity of backcrossed, *S. foliosa*-like hybrids at HAYtf and RLtf (Fig. 3.4b: CL 3).

Multivariate ordination analysis assigned hybrid *Spartina* individuals into at least two largely geographically distinct clusters, one more like *S. foliosa*, different by only one or two loci from the parental species (RL and HAY; Fig. 3.4: CL 3: HAYtf & RLtf & RLm), and the other genetically more similar to *S. alterniflora* (ALA; Fig. 3.4: CL 4: ALAm & ALAtf). Bayesian cluster analysis confirmed at least two distinct hybrid membership groups, where Alameda plants are aligned with a divergent *S. alterniflora* founder (Fig. 3.6, group 3), largely distinct from the range wide *S. alterniflora* group and the rest of the HAY and RL hybrid groups (Fig. 3.6). RL (tf and m) and HAYtf populations are similar to each other in their more common ancestry (Fig. 3.6). In more detail, however, the color patterns of all line segments show a slightly darker pattern at HAY, whereas at RL, especially the seedling populations (Fig. 3.6, groups 13 and 14) are predominately lighter. This may indicate differences in the more recent seedling recruitment patterns, as at each site a different, selfing individual dominated seedling production.

In light of this hybrid genetic structure, I wanted to examine inter-relatedness of established adult plants, within and among sites, to infer colonization history, my second goal. All tidal flat populations are tidally linked to a nearby meadow population, either

situated directly adjacent to a shoreline meadow at Alameda, or via tidal channels to an inland, levee-bound *Spartina* hybrid meadow at Robert's Landing. Sib-ship analysis revealed sibling adults between and within the meadow and tidal flat plants in the south (HAYtf, RLtf, and RLm; Figs. 8 & 9). Plants at the Alameda population (ALAtf and ALAm) were also closely inter-related across habitats (Fig. 3.7). These results confirm gene flow between mudflat and meadow at both of these geographically distinct locations, as well as movement along the shoreline at the southern sites.

Long-distance dispersal may also be a factor, however, since one ALAm and ALAtf family group (Fig. 3.7, B) showed close relation to the *S. foliosa*-like families at HAYtf (Fig. 3.9, C) and RLtf (Fig. 3.9, E), and another HAYtf group (Fig. 3.9, A), including the highest reproducer at this site. This family affinity is caused either by common ancestry, or simply by the proportion of shared *S. foliosa* alleles; a result of convergent evolution rather than identity by descent. The two genetically dissimilar hybrid populations at ALA and HAY/RL may be the result of different founding trajectories caused by distinct establishment histories through natural or human-aided means, involving discrete ancestors for both groups. After the initial founder(s) established at each site, gene flow, and spatial spread may have been restricted locally within the last 30 years to sustain the observed genetic structure. Hybrid long-distance dispersal trajectories are variable, and depend on tidal current flow for shoot or seed dispersal. In contrast, human-aided hybrid dispersal could have occurred accidentally during past SFB restoration efforts.

Historical evidence suggests that in the late 1970s the native Alameda *S. foliosa* meadow population was supplemented by plantings of both *S. foliosa* and *S. alterniflora* as part of a shoreline erosion demonstration project (U.S. Army Corps of Engineers 1978). These native and introduced cordgrass planting materials were obtained from a previous USACE restoration project site at Cargill Pond 3, situated next to Alameda creek, to the south of HAY (Fig. 3.1). *Spartina alterniflora* seeds collected from Virginia (D. Ayres pers. comm.) had been introduced there in the early 1970s, and were grown amidst native *S. foliosa* (Faber 2000). After an initial hybridization event, newly formed hybrid *Spartina* may have proliferated at this site alongside both parent species, and been unknowingly transplanted with the parental species into Alameda. This would suggest that at Alameda, a distinct group of *S. alterniflora* and early hybrids, mixed with native and transplanted *S. foliosa*, formed an isolated and locally reproducing hybrid population.

At the same time, seeds of *S. alterniflora* and hybrid individuals at Cargill Pond 3 escaped naturally, and were subsequently transported from Alameda creek via a northerly tidal current, slowly establishing in marshes with native cordgrass and tidal flats until they reached Hayward and Robert's Landing. In light of this, natural colonization would have taken much more time than the proposed human aided hybrid establishment at Alameda, explaining the current lack of genetic structure between populations at Robert's Landing and Hayward, and supporting the genetic distinctions between Alameda and these two southerly populations. At Alameda, *S. alterniflora* had been planted, probably together with early F1-type hybrids, and backcrosses must have occurred with local *S. alterniflora* plants, resulting in a more *S. alterniflora*-like population over time. *Spartina*

foliosa-like hybrids were either less frequent, or possibly lost out in competition early on. Alameda plants remained isolated in the north, as hybrids in the south slowly moved northward with the tide, reaching tidal flats and native marshes along shore. There, crossing with native *S. foliosa* and other hybrids, more diverse hybrid seed was produced to further colonize the tidal flats. This natural *Spartina* hybrid dispersal trajectory would therefore fit the stepping-stone model (Kimura and Weiss 1964), as the most geographically and genetically distant ALA population (Fig. 3.1) has to date remained relatively isolated, while gene flow occurs between the two proximate and genetically more similar Hayward and Robert's Landing populations.

My third goal was to investigate recent seedling abundance and contemporary scales of gene flow (local versus regional), and to identify potential source populations of the newly recruiting isolated tidal flat seedlings through parentage analysis. All of the seedlings genotyped were hybrids; there were none from the parental species even though native *S. foliosa* occurs along the shoreline at Hayward, and in the nearby heavily invaded tidal flat marsh south of San Lorenzo creek. Because seedlings were chosen randomly, this alone indicates that hybrid numbers are increasing much more rapidly than the native *S. foliosa*. Seedling recruitment increased on average 6-fold from 2003 to 2004, and seedling size and shoot number surveys show 9 %, 12 %, and 45 % seedling survival to one-year or greater, from south to north. Following a year with high seedling abundance, these survival percentages would represent a successful recruitment of about 274 individuals at each site, numbering in the thousands when considering all of SFB.

The high seedling abundance in 2004 was followed by an almost complete absence of new seedlings in 2005 at the southern sites, indicating that recruitment is episodic.

If most tidal flat seedlings originated from more distant hybrid meadows, the adult and seedling population structure should be genetically well mixed. In contrast, if established tidal flat plants are the main seedling source, the seedlings genotypes should be more similar to that of the isolated tidal flat adults. My sib-ship analysis data showed some adults at HAYtf, RLtf and RLm were related (Figs. 8, 9), and most tidal flat adults at ALAtf likely stemmed from the adjacent ALAm meadow. It is therefore probable that most tidal flat plants originated from adjacent or nearby hybrid meadows, which are connected to the tidal flats via ebbing and flowing tidal channels (see Fig. 3.2). I found using multivariate ordination and Bayesian clustering that most seedlings, in both years, were likely the offspring of local plants. Further, in the southern sites, the parents were not in the nearby meadow, but rather in the isolated plants on the tidal flats. At the Alameda Island population, the meadow plants were the most likely parents for 2/3 of the seedlings. This makes sense as the meadow at this site is contiguous with the tidal flat where as the meadow at the southern site is located behind a levee.

My geographic seedling recruitment surveys and parentage analyses in 2003 and 2004 also support local gene flow, as all seedlings clustered with certain established adults at their locality (Figs. 2B, 3A). Parentage analysis further shows prevalent inbreeding at two of the three surveyed tidal flat sites (Table 3.2). Due to the proximity of Elsie Roemer marsh, local meadow *Spartina* plants most likely created the majority of ALAtf seedlings.

This was confirmed, as the number of ALAtf seedlings with likely parents in the genotyped stand increased by 50 percent, once ALAm meadow parents were added to the parentage analysis. The remaining ALAtf seedlings probably arose as offspring of the many non-genotyped *Spartina* at Elsie Roemer marsh. A small number of them may, however, have originated elsewhere, and more analysis on a greater number of hybrid populations in SFB is needed to assess this further.

I determined the fine-scale spatial-genetic structure of colonizing seedlings, and spatial genetic analysis revealed positive pair-wise structure up to ~200 meters, and negative structure above ~440 or ~530 meters (Fig. 3.10). These results indicate that within a 200-meter distance, plants are genetically more similar to each other than expected by chance, as perhaps a mother plant surrounded by its seedlings in safe sites. Plants more than 440 or 530 meters apart, on the other hand, are more genetically dissimilar than expected by chance. Such negative spatial genetic structure is conceivable above a certain geographic distance, assuming that a large proportion of progeny are inbred, clustering around only a few prominent non-related self-fertilizing mothers. Therefore, more distant plants are likely more divergent from all the closely related seedlings surrounding their mothers, and the overall distribution of seedlings is thus non-random. In support of this, I found the geographically most isolated Alameda hybrid populations (ALAm and ALAtf), and the meadow population at Robert's Landing (RLm) to show less deviation from Hardy-Weinberg equilibrium, and less linkage disequilibrium than the isolated tidal flat hybrid populations at Robert's Landing (RLtf) and Hayward (HAYtf). This shows that the ALAm, ALAtf and RLm populations are mainly out-crossing according to Hardy

Weinberg expectations, while the latter RLtf and HAYtf tidal flat populations display non-equilibrium population dynamics, possibly due to prominent inbreeding.

With my fourth goal I aimed to confirm whether there were specific, highly fit tidal flat individuals that contributed disproportionately to seedling populations. At both HAYtf and RLtf, a single isolated hybrid tidal flat plant predominately contributed to the seedling pool through dissemination of mainly inbred progeny that established within close radius (~ 200 meters) from a geographically isolated, self-compatible mother (Table 3.2, Figs. 2B, 3A). In contrast, at ALAtf only a small number of seedlings were most likely produced by tidal flat parents, and of those, only a few by a single individual (Table 3.2). These results support the existence of specific, highly fit, self-compatible, transgressive hybrid individuals that disproportionately contribute to seedling recruitment in tidal flats. In this context, positive spatial genetic structure below ~ 200 meters further corroborates the existence of safe sites surrounding self-fertilizing, isolated tidal flat plants.

Successful seedling recruitment on exposed tidal flats is directly linked to favorable environmental/climate factors, influencing both, seed set in the previous year, and the survival of seedlings in the recruitment year and beyond. Seedling recruitment is episodic in SFB, as well as in Willapa Bay, WA (J. Lambrinos, pers comm.). The probability of seedling survival increases with increasing numbers of available propagules in the preceeding year (J. Lambrinos, pers. comm.). This is also directly related to fluctuations in climate, evidenced by a doubling of seed set during an El Niño year (Zaremba 2001). A moderate El Niño event was recorded in winter of 2002/03 and spring of 2003,

characterized by increases between 1.0 and 1.4 degrees in precipitation and temperature for 5 consecutive months (Golden Gate Weather Services: <http://ggweather.com/enso/oni.htm>). The 6-fold rise in seedling abundance in the spring of 2004 reflects the increased 2003 seed set due to this El Niño event. In 2004, temperatures and precipitation had again decreased to normal levels, and accordingly, abundance of new seedlings in 2005 dropped considerably to almost zero at the two southern tidal flat sites.

Direct spatial proximity of large isolated plants to a cordgrass meadow also plays a role as a major driver of seed production in SFB (Chapter 2), and at Willapa Bay (Davis *et al* 2004). Pollen from invasive tidal flat colonizers can sire seed in adjacent cordgrass meadows, and can so contribute to the vast numbers of seeds produced in such marshes, as seen at Alameda. Hybrid *Spartina* invaded tidal creeks and channels can further assist in increasing seed numbers on the tidal flat in this way, and source and sink dynamics can have an effect on recruitment trajectories.

Isolated, large clones are inundated by tidal action each day and sediment deposits occur in their lee, toward the shore. In this area, tidal velocities are only moderate, and water depth is reduced, making it a perfect ‘safe site’ for newly recruiting seedlings. I observed this mainly at Hayward, behind HayM1, the largest seedling contributor at this site. The existence of this ‘safe-site’ was reflected in the high frequency of spatial proximity of the HayM1 offspring to their mother (Fig. 3.11A). Most HayM1 seedlings (~ 60 %) are distributed within 100 meters from it, most situated in the shoreward ‘safe-site’ (Fig.

3.3a, yellow circle). While seedlings also occurred in the vicinity of RL14, its extremely isolated position far out from shore (Fig. 3.2, yellow circle), may have caused a smaller effective 'safe-site' area, coupled with a greater tidal mixing effect, as there, seedlings were carried further away from the mother plant (Fig. 3.2). Spatial analysis confirms this, as RL14 seedlings are far more spread out from the mother, with only about 15 percent of the seedlings found within 100 meters (Fig. 3.11 B). In addition to safe sites, I observed offshore sand banks with high seedling abundance in SFB, and matted down, or bare, central areas of clones, resulting from storm damage, have been found to have high seedling recruitment in Willapa Bay (J. Lambrinos, pers. comm.).

Conclusion

The invasion histories of other maritime cordgrasses, having explosively expanded into open intertidal mudflats worldwide, within a relatively short time period, forecast the impact hybrid *Spartina* will likely have in the vast, unvegetated SFB tidal flats, if unchecked. The rate of spread is expected to surpass all other historical *Spartina* in its magnitude, because natural selection favors invasive genotypes. It took less than 30 years for the genetically highly variable *Spartina* hybrid swarm to form extremely invasive genotypes, contributing locally and/or regionally to the current greater than exponential hybrid spread in native marshes, and open tidal flats. In these tidal flats, and near native meadows, it is the presence of large hybrid colonizers, capable of selfing as well as out-crossing through pollination of nearby plants (Chapter 2), that fuels further colonization by seedlings, driving the invasion. In this way, these plants accelerate the rate of spread

in SFB, having successfully overcome pollen limitation, an Allee effect that has slowed the invasion of *S. alterniflora* in open tidal flats in Willapa Bay (Taylor *et al* 2004, Davis *et al* 2004b). Seed production in isolation allows seedling recruitment in this vast, open habitat. As each isolated plants expands vegetatively, increasing in diameter, the number of inflorescences increases linearly with the number of new culms added. In turn, their potential for selfed seed production increases also, since plants can utilize geitonogamous pollination across their growing numbers of inflorescences (Chapter 2).

Hybrid *Spartina* spread greatly threatens SFB tidal flats, mainly due to the accelerating colonization resulting from explosive seedling recruitment episodes. Rapid evolutionary changes in this hybrid *Spartina* swarm were vigorous, self-compatible plants and their offspring are favored by natural selection, have resulted in a few extremely fit individuals being responsible for a large part of locally recruiting seedlings in this as yet unvegetated habitat. Extremely fit, transgressive hybrid plants ensure the successful colonization of the challenging tidal flat habitat they have adapted to, by producing large numbers of equally fit, inbred progeny. The occurrence of episodic seedling recruitment SFB tidal flats, coupled with observed seedling survival, as I documented from 2003 to 2004, may greatly increase the likelihood of permanent local seedling establishment, and also result in long-distance dispersal of highly fit progeny. Hybrid *Spartina* will continue to expand within tidal flats locally, in the vicinity of established plants, but will also have a regional, or bay-wide impact over time, as relatively rare, long-distance dispersal events can have a large influence on the rate of range expansion through time (Hastings *et al* 2005, Cain *et al* 2000). This successful tidal flat invasion, involving explosive dispersal and successful establishment of highly fit propagules, increasing both local and regional

hybrid recruitment, coupled with pollen swamping (Antilla *et al* 1998) in native marshes, results in ever more hybrid *Spartina*. These dynamics may explain the current greater than exponential hybrid spread (Ayres *et al* 2004), and will assure the continuing and accelerating invasion of SFB by hybrid *Spartina*, if this invasion remains unchecked.

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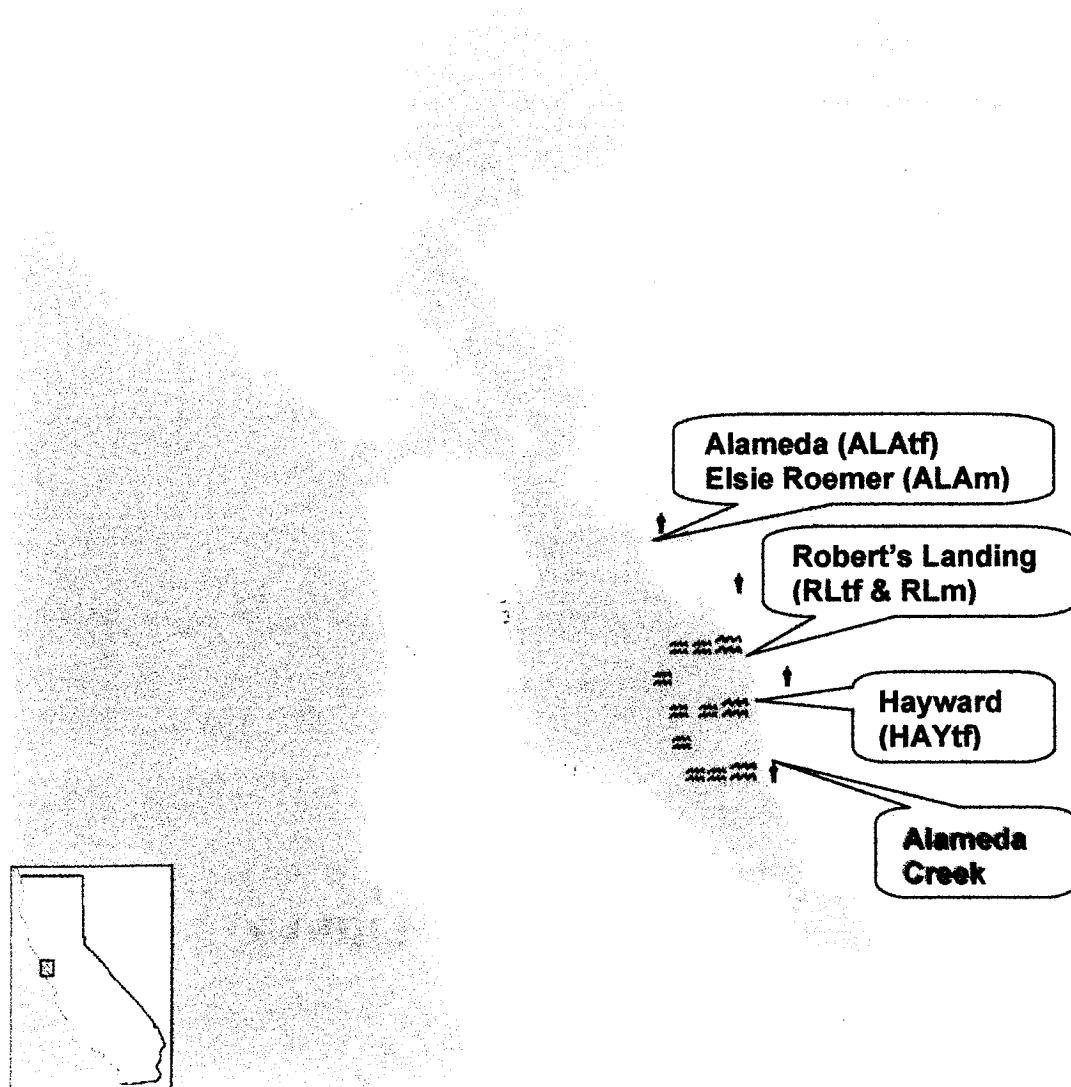


Fig. 3.1: Map of San Francisco Bay (SFB). Arrows indicate study sites: *S. alterniflora* and hybrids at Elsie Roemer marsh, Alameda; and isolated tidal flats hybrids along the eastern SFB shoreline at Alameda, Robert's Landing, and Hayward; and proposed initial *S. alterniflora* SFB introduction site at Alameda creek. † mark proposed human aided dispersal of *S. alterniflora* and possibly hybrids to Alameda. ≈≈ indicate potential natural tidal dispersal from Alameda creek to Hayward and Robert's Landing.

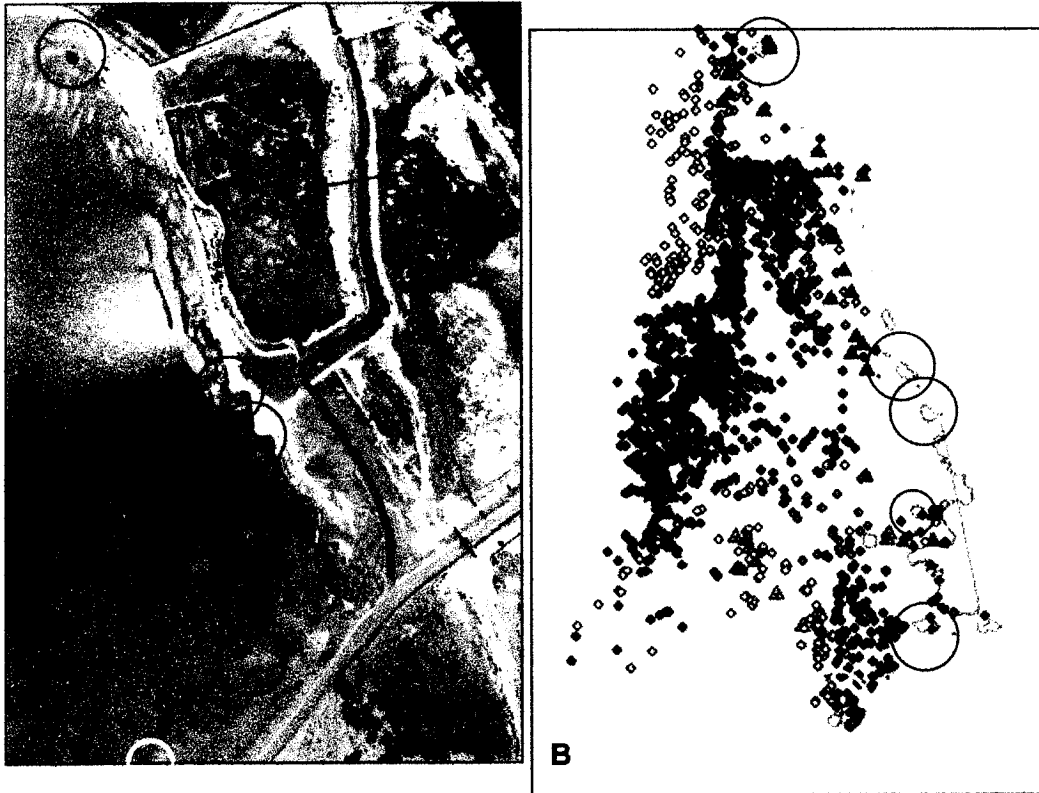


Fig. 3.2: A) Infrared aerial photograph of the Robert's Landing (RLtf) sample site. The arrow indicates the sampled (RLm) hybrid *Spartina* inland meadow, connected to tidal flats via tidal channels. B) GPS points for 2004 seedlings surveyed (dots), and DNA samples collected (triangles, and red outline). For both panels: Circles indicate six of the large isolated reproducing tidal flats plants. A yellow circle indicates the plant with the largest reproductive contribution (RL14).

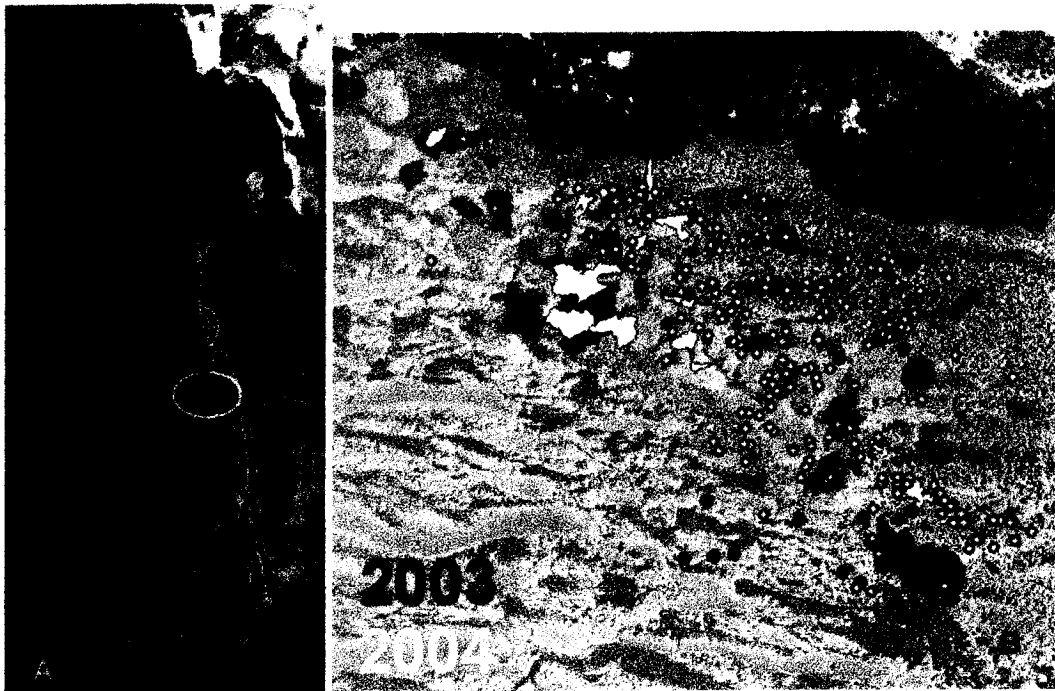


Fig. 3.3: Infrared aerial photograph of two sample sites. A) Hayward Shoreline (HAYtf). Circles: six of the large isolated reproducing tidal flats plants (all adults in purple). Yellow circle: plant with largest reproductive contribution. Green triangles: 2003 seedlings; red stars: 2004 seedlings. B) Alameda (ALAtf). Purple dots/areas: 2003 seedlings; pale pink dots/areas: 2004 seedlings. Dots: individual seedling; areas: >10 seedlings. Arrow: (ALAm) adjacent Elsie Roemer hybrid *Spartina* meadow. Adult plants in orange.

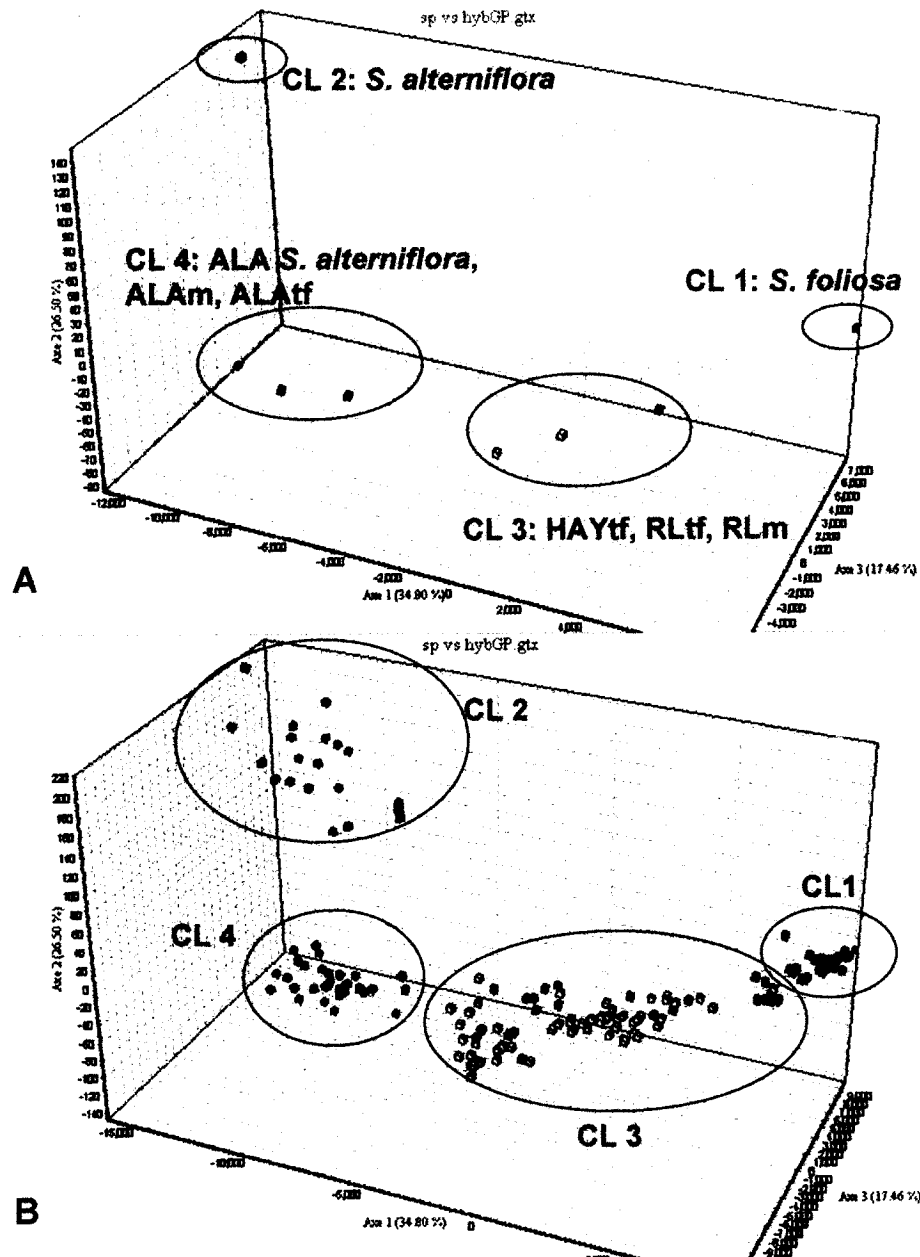


Fig. 3.4: A) Factorial correspondence analysis presenting multivariate relationships among eight pre-determined groups: *S. foliosa* (dark blue, CL 1), *S. alterniflora* (dark red, CL 2), and their hybrids from three sites along the Eastern SFB meadows and isolated tidal flats (CL 3: HAY = light blue, RL tidal flats = yellow, RL meadow = white, ALA meadow = pink; CL 4: ALA meadow = pink, ALA tidal flats = green, ALA meadow *S. alterniflora* = grey). B) All individuals are shown.

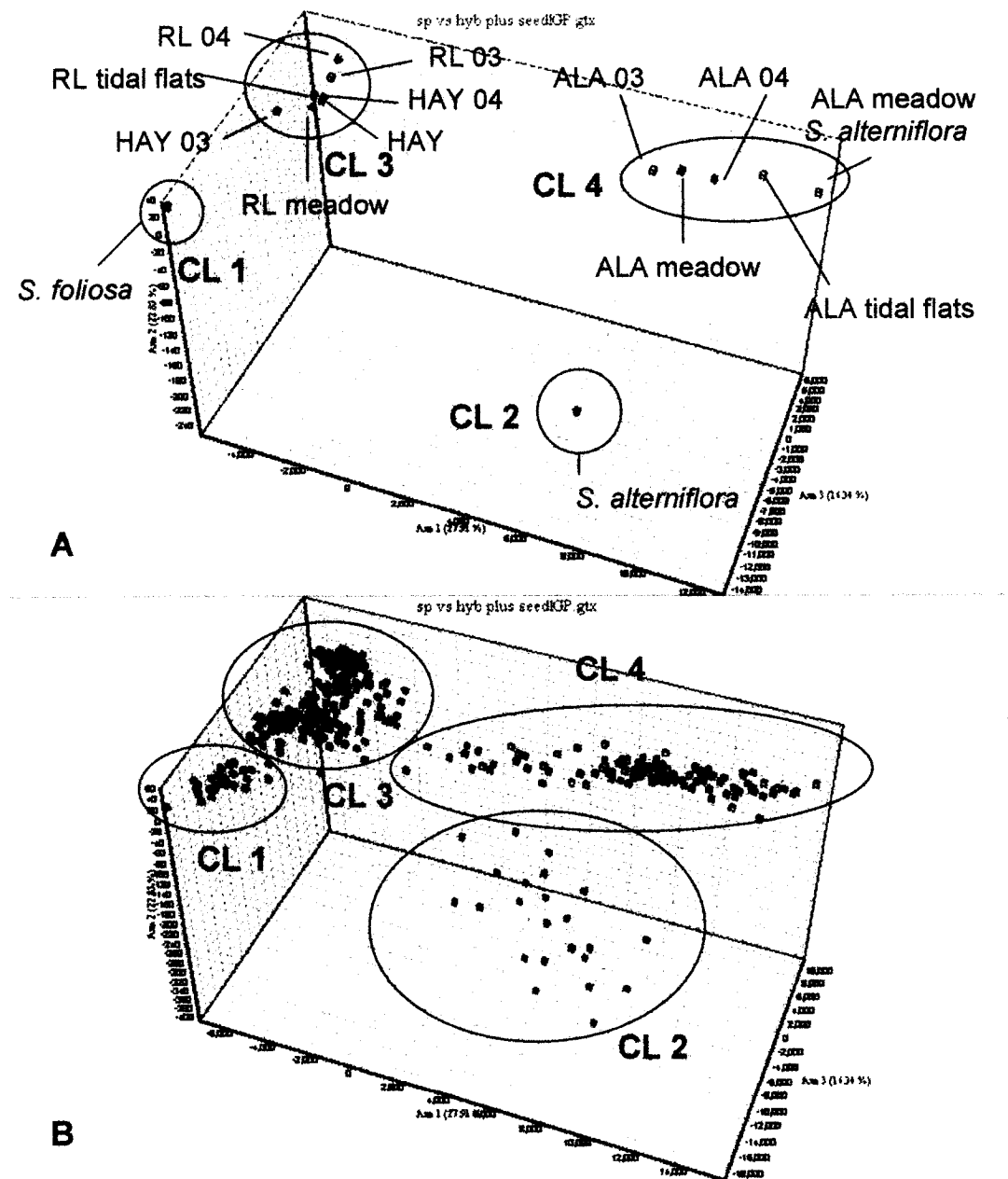


Fig. 3.5: A) Factorial correspondence analysis presenting multivariate relationships among 14 groups: *S. foliosa* (dark blue, CL 1), *S. alterniflora* (dark red, CL 2), and their hybrids and 2003 and 2004 seedlings from three sites along the Eastern SFB meadows and isolated tidal flats (CL 3: HAYtf = light grey, HAYtf 03 = grey, HAY 04 = purple, RLtf tidal flats = green, RLm meadow = pink, RLtf 03 = light green, RLtf 04 = brown; CL 4: ALAm meadow = blue, ALAtf tidal flats = yellow, ALAm meadow *S. alterniflora* = white, ALAtf 03 = turquoise, ALAtf 04 = black). B) All individuals are shown.

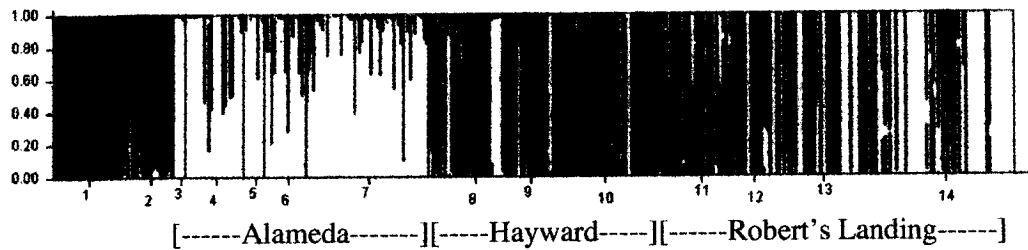


Fig. 3.6: Bar plot estimated by STRUCTURE depicting group membership (hybrid ancestry), representing each individual as a line segment. Each segment is partitioned into $K = 4$ colored components, representing the individual's estimated membership coefficients in the K clusters (y-axis). Numbers (x-axis) represent the initial geographic/demographic groupings: **1** = *S. foliosa*, **2** = *S. alterniflora*, **3** = ALAm *S. alterniflora*, **4** = ALAm meadow, **5** = ALAtf tidal flats, **6** = ALAtf 2003 seedlings (S), **7** = ALAtf 2004 S, **8** = HAYtf, **9** = HAYtf 2003 S, **10** = HAYtf 2004 S, **11** = RLm meadow, **12** = RLtf tidal flats, **13** = RLtf 2003 S, **14** = RLtf 2004 S.

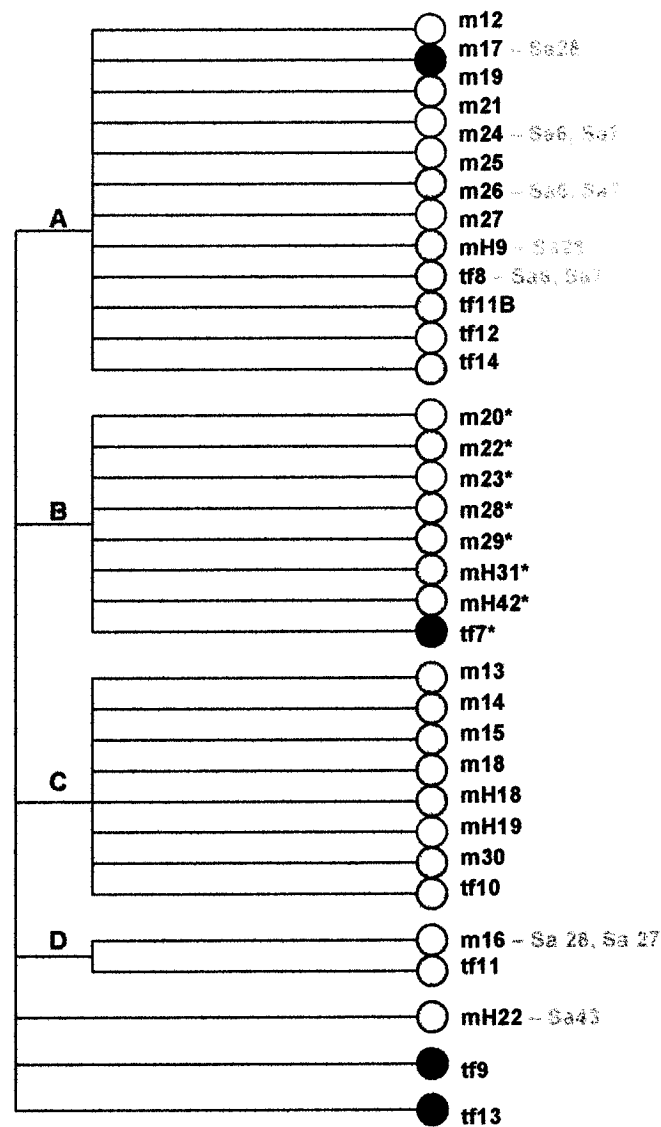


Fig. 3.7: Sib-ship pedigree of 9 ALAtf, 25 ALAm, and 5 *S. alterniflora* (Sa) adults. Numbered circles represent hybrid tidal flat (tf) or meadow (m) individuals. A-D represent family groups. Single individuals were not related to anyone. Colors show the type of parentage: Red, produced both self- and cross-fertilized seedlings; blue, produced only cross-fertilized seedlings; white, produced no seedlings. *Denotes individuals distinguished by only one or two alleles from *S. foliosa*, and showing a relationship to HAY group C and RL group E plants (Fig. 3.9).

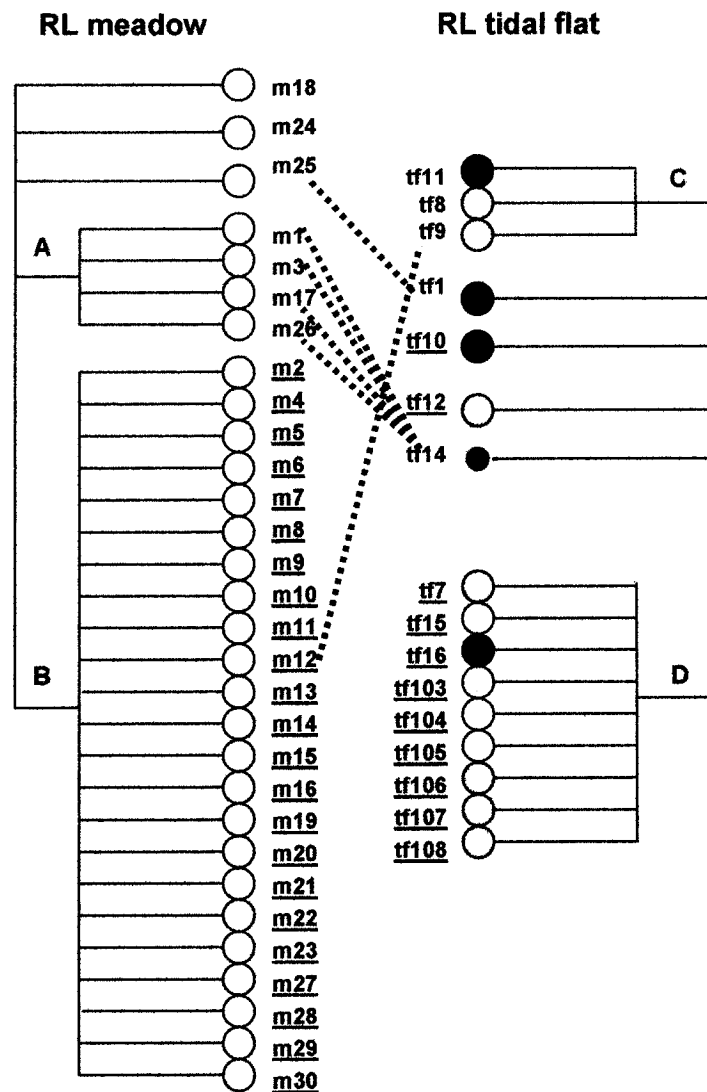


Fig. 3.8: Sib-ship pedigree of 17 RLm and 25 RLtf adults. Numbered circles represent hybrid tidal flat (tf) or meadow (m) individuals. A-D represent family groups. Single individuals were not related to anyone in the study stand. Dotted lines show a direct relation between individuals. Underlined individuals are related to each other across habitats to some degree. Colors represent the parentage type: Red, produced both self- and cross-fertilized seedlings; blue, produced only cross-fertilized seedlings; green, produced only self-fertilized seedlings, white, produced no seedlings. Thick circle outline represents the individual with the most reproductive output per site.

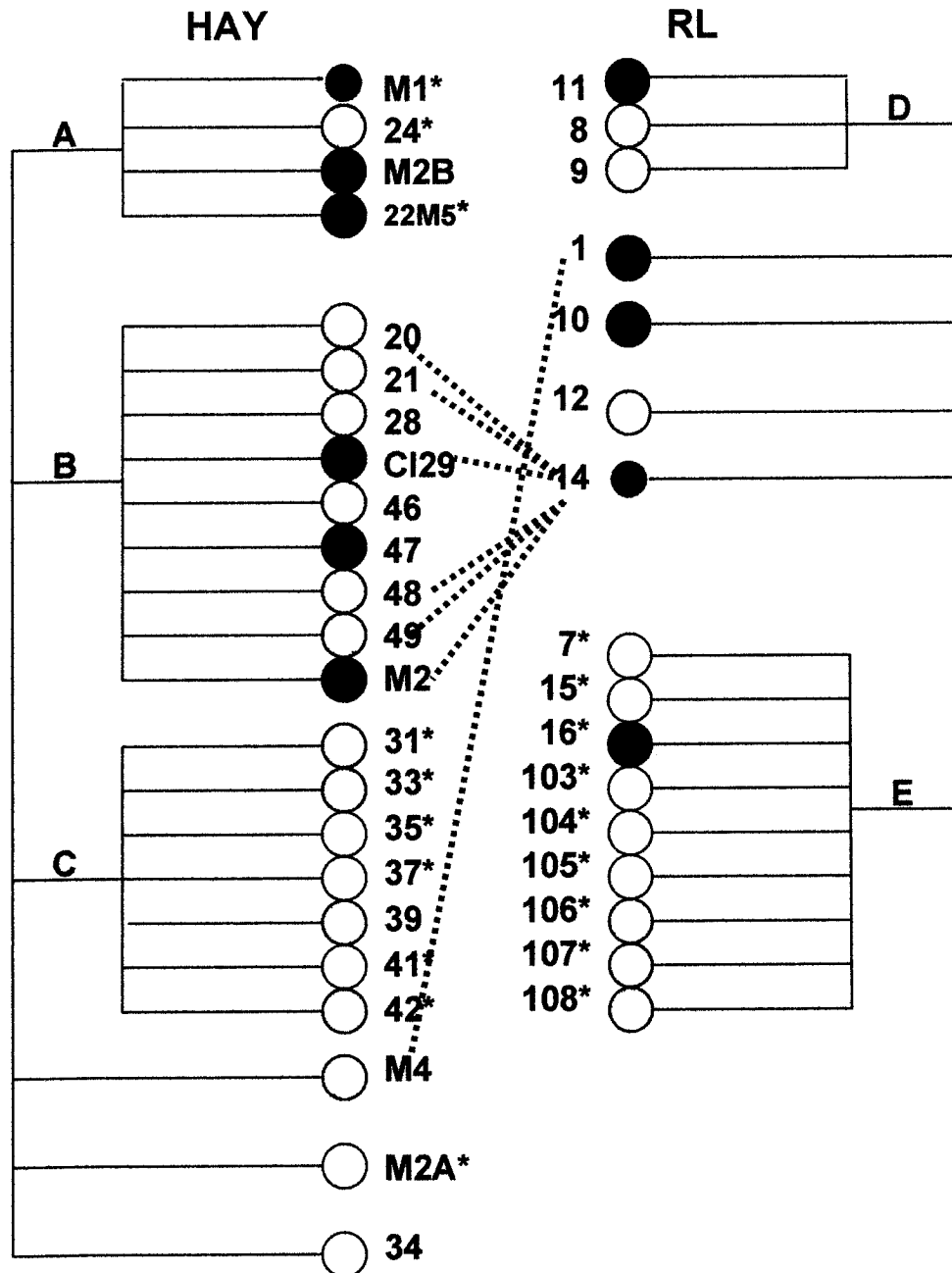


Fig. 3.9: Sib-ship pedigrees of 24 HAYtf and 19 RLtf adults. Numbered circles represent hybrid individuals. A - E represent family groups. Other individuals had no relations. Colors represent the parentage type: Red, produced both self- and cross-fertilized seedlings; blue, produced only cross-fertilized seedlings; green, produced only self-fertilized seedlings, white, produced no seedlings. Thick circle outline represents the individual with the most reproductive output per site. Dotted lines indicate related individuals at both sites. *Denotes individuals distinguished by only one or two alleles from *S. foliosa*, and showing a relationship to ALA B plants (Fig. 3.7).

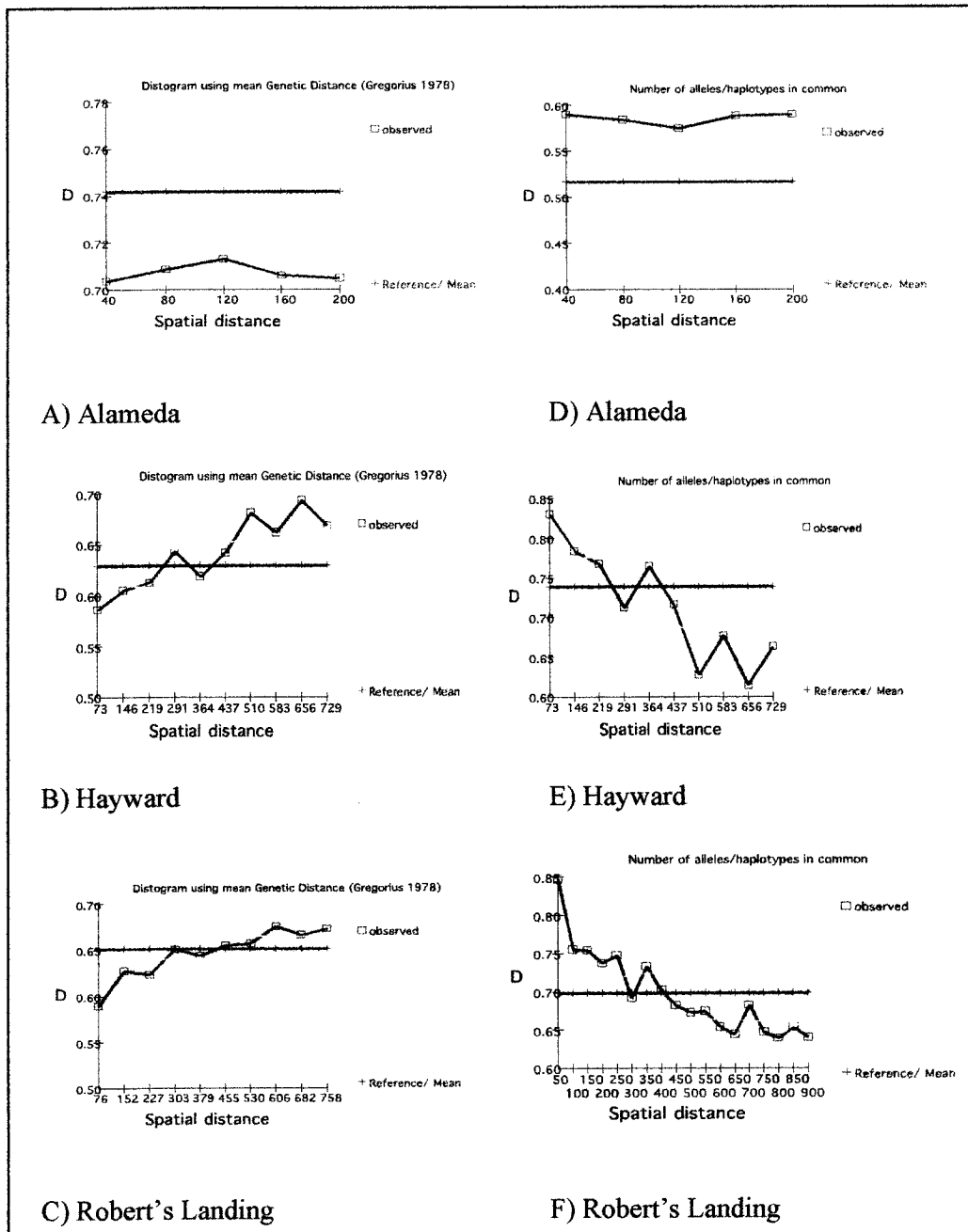


Fig. 3.10: Spatial genetic analysis showing mean Genetic Distance (Gregorius 1978) for hybrid adults and seedlings at the three sample sites (A – C) performed with SGS (Degen 2001). Distances below the reference value indicate positive spatial structure, where proximal individuals are genetically more similar than expected for a spatially random distribution of genotypes (positive structure). Distances above the reference indicate negative structure, where individuals show greater genetic divergence than expected for a spatially random distribution of genotypes (Degen 2002). Spatial genetic structure according to the number of alleles in common (D – F) (positive spatial structure: above reference line; negative spatial structure: below reference line)

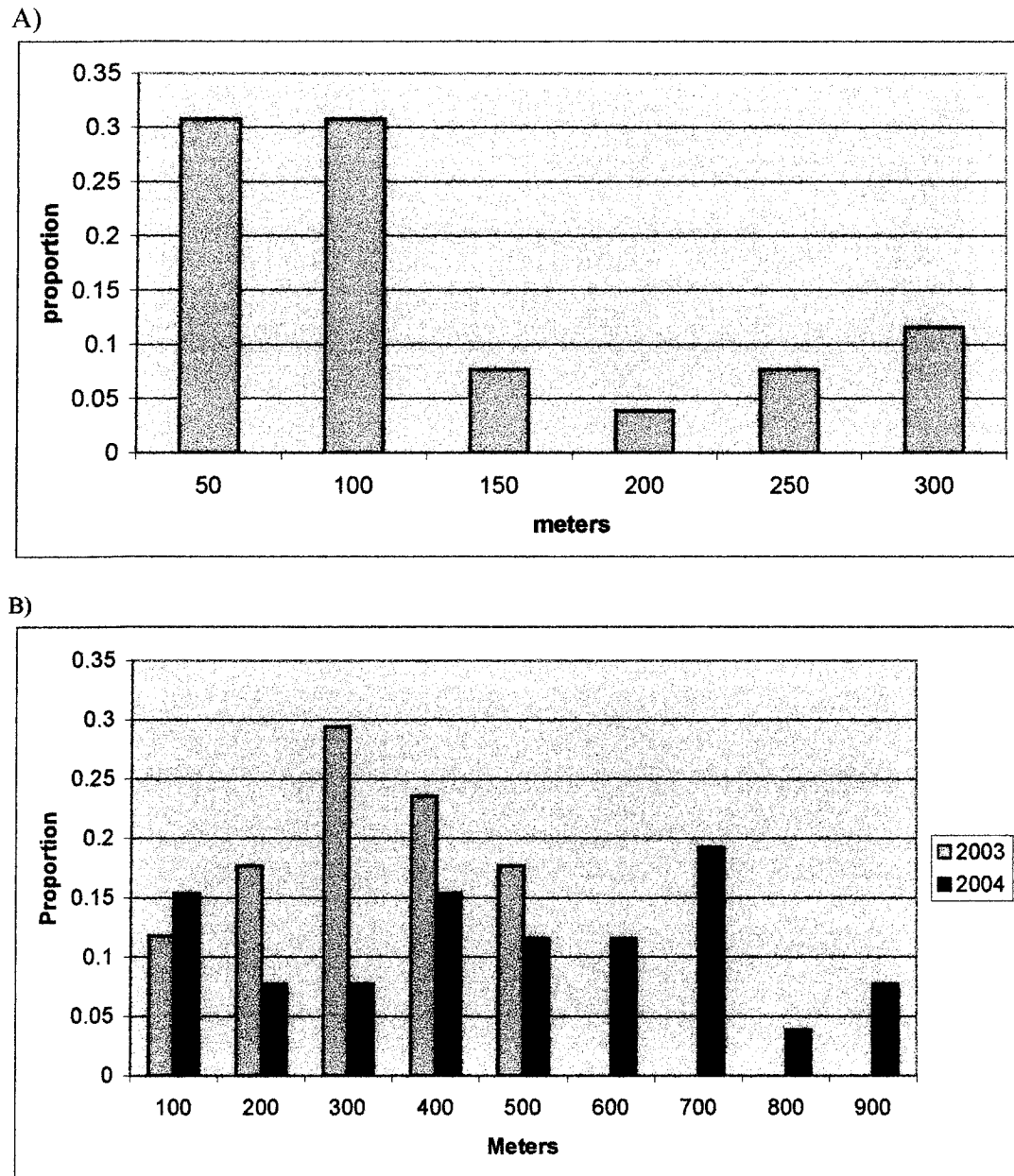


Fig. 3.11: Frequencies of seedlings establishing at different dispersal distances from their mainly selfing mother plants: A) Hay M1 in 2004, B) RL 14 in 2003 and 2004.

Table 3.1: Population mean observed and expected heterozygosities over all 17 loci. * H_e calculated without bias (Nei 1978); n = number of plants sampled, s.d. = standard deviation; P = polymorphism (threshold); N alleles = mean number of alleles per locus. Ho of selfing tf plants?

Population	n	H_e (s.d.)	H_e^* (s.d.)	H_o (s.d.)	P(0.95)	P(0.99)	N alleles
<u><i>S. foliosa</i> (range wide)</u>	50	0.11 (0.18)	0.12 (0.19)	0.09 (0.25)	0.29	0.35	1.59
<u><i>S. alterniflora</i> (range wide)</u>	35	0.75 (0.12)	0.78 (0.13)	0.46 (0.20)	1.00	1.00	8.06
<u>ALAm <i>S. alterniflora</i></u>	5	0.64 (0.12)	0.71 (0.13)	0.61 (0.32)	1.00	1.00	3.76
ALAm meadow	25	0.65 (0.13)	0.66 (0.12)	0.59 (0.22)	1.00	1.00	4.76
ALAtf tidal flat	9	0.64 (0.12)	0.69 (0.13)	0.49 (0.22)	1.00	1.00	4.29
ALAtf seedlings '03	33	0.69 (0.10)	0.71 (0.10)	0.54 (0.22)	1.00	1.00	5.24
ALAtf seedlings '04	67	0.69 (0.09)	0.70 (0.09)	0.52 (0.20)	1.00	1.00	5.65
RLm meadow	30	0.60 (0.17)	0.61 (0.18)	0.52 (0.23)	1.00	1.00	5.06
RLtf tidal flat	17	0.66 (0.12)	0.68 (0.13)	0.40 (0.22)	1.00	1.00	4.41
RLtf seedlings '03	42	0.60 (0.13)	0.61 (0.14)	0.41 (0.18)	1.00	1.00	4.59
RLtf seedlings '04	54	0.50 (0.14)	0.51 (0.15)	0.30 (0.15)	1.00	1.00	5.47
HAYtf tidal flat	24	0.61 (0.11)	0.62 (0.11)	0.46 (0.20)	1.00	1.00	5.18
HAYtf seedlings '03	32	0.62 (0.09)	0.65 (0.10)	0.36 (0.16)	1.00	1.00	3.65
HAYtf seedlings '04	69	0.54 (0.13)	0.54 (0.13)	0.43 (0.19)	1.00	1.00	5.12

Table 3.2a: FaMoz (Gerber *et al* 2003) parentage analysis results showing the most likely parents of inbred or out-crossed seedlings at RL by sample year. Plants are ordered by size from largest to smallest. Red/bold: plants with largest parentage contribution (mainly selfed). * Nearest neighbor was in inland *Spartina* meadow (RLm), separated from tidal flat by levees.

Robert's Landing Tidal flats	Parent Plant ID											
	RL11	RL9	RL7	RL108	RL1	RL14	RL12	RL16	RL10	RL8	RL106	RL107
Distance to nearest neighbor (m)	78	28	54	84	123*	193	84	8	84	28	9	9
Self-fertilized 2003 (n)	1	-	-	-	-	16	1	1	-	-	-	-
Prop. of all selfed seedlings	0.05	-	-	-	-	0.85	0.05	0.05	-	-	-	-
Prop. of all seedlings	0.02	-	-	-	-	0.38	0.02	0.02	-	-	-	-
Average LOD score	30.9	-	-	-	-	16.33	13.83	8.6	-	-	-	-
St. dev. LOD score	-	-	-	-	-	4.65	-	-	-	-	-	-
Single parent 2003 (n)	-	-	-	4	4	-	-	3	5	-	1	2
Total Parentage 2003 (n)	1	-	-	4	4	16	1	4	5	-	1	2
Self-fertilized 2004 (n)	2	2	-	-	1	24	1	1	1	-	-	-
Prop. of all selfed seedlings	0.06	0.06	-	-	0.03	0.78	0.03	0.03	0.03	-	-	-
Prop. of all seedlings	0.04	0.04	-	-	0.02	0.46	0.02	0.02	0.02	-	-	-
Average LOD score	16.94	23.87	-	-	27.22	8.16	25.97	19.16	12.85	-	-	-
St. dev. LOD score	2.37	8.35	-	-	-	2.94	-	-	-	-	-	-
Single parent 2004 (n)	-	-	2	-	1	2	-	4	1	2	-	-
Total Parentage 2004 (n)	2	2	2	-	2	26	1	5	2	2	-	-

Table 3.2b: FaMoz (Gerber *et al* 2003) parentage analysis results showing the most likely parents of a) selfed and out-crossed or b) out-crossed only seedlings at HAY by sample year. Size rank lists plants from largest to smallest. Red/bold: plants with largest parentage contribution (mainly selfed).

Hayward Tidal flats									
a) Selfed and out-crossed	Parent Plant ID								
	HayM1	Hay31	Hay22M5	Hay47	HayCL29	HayM2	HayM2B	Hay49	Hay21
Size rank	1	2	4	5	6	7	8	11	12
Distance to nearest neighbor (m)	50	14	70	21	8	16	32	21	104
Self-fertilized 2003 (n)	4	1	1	-	2	1	2	-	-
Prop. of all selfed seedlings	0.33	0.08	0.08	-	0.17	0.08	0.17	-	-
Prop. of all seedlings	0.13	0.03	0.03	-	0.06	0.03	0.06	-	-
Average LOD score	8.3	12.83	4.77	-	15.55	3.3	5.06	-	-
St. dev. LOD score	3.73	-	-	-	4.52	-	1.06	-	-
Single parent 2003 (n)	3	-	1	-	-	-	1	-	2
Tot. Parentage 2003 (n)	7	1	2	0	2	1	3	0	2
Self-fertilized 2004 (n)	20	-	4	2	4	-	4	3	-
Prop. of all selfed seedlings	0.59	-	0.12	0.06	0.12	-	0.12	0.09	-
Prop. of all seedlings	0.29	-	0.06	0.03	0.06	-	0.06	0.04	-
Average LOD score	7.4	-	4.97	24.27	23.84	-	5.85	25.23	-
St. dev. LOD score	1.51	-	2.66	8.78	1.02	-	0.24	1.6	-
Single parent 2004 (n)	5	-	3	2	2	1	4	-	-
Tot. Parentage 2004 (n)	25	0	7	4	6	1	8	3	0

Hayward Tidal flats								
b) Out-crossed only	Parent Plant ID							
	Hay33	Hay42	Hay48	Hay29	Hay28	HayM4	Hay46	Hay24
Size rank	3	9	10	13	14	15	16	17
Distance to nearest neighbor (m)	44	16	21	8	8	61	17	25
Single parent 2003 (n)	2	2	-	2	2	2	-	1
Tot. Parentage 2003 (n)	2	2	0	2	2	2	0	1
Single parent 2004 (n)	-	-	1	1	1	1	1	-
Tot. Parentage 2004 (n)	0	0	1	1	1	1	1	0

Table 3.2c: FaMoz (Gerber *et al* 2003) parentage analysis results showing the most likely parents of inbred or out-crossed seedlings at ALA by sample year. Plants are ordered by size from largest to smallest. Red/bold: plants with largest selfed parentage contribution.

Alameda Tidal flats	Parent Plant ID						
	ALA 13	ALA 9	ALA 14	ALA 11	ALA 7	ALA 10	ALA 11B
Distance to nearest neighbor (m)	45	36	32	7	21	36	7
2003							
Self-fertilized 2003 (n)	2	-	-	-	1	-	-
Prop. of all selfed seedlings	0.67	-	-	-	0.33	-	-
Prop. of all seedlings	0.06	-	-	-	0.03	-	-
Average LOD score	17.15	-	-	-	5.85	-	-
St. dev. LOD score	2.49	-	-	-	-	-	-
Single parent 2003 (n)	-	1	-	1	2	1	-
Tot. Parentage 2003 (n)	2	1	-	1	3	1	-
2004							
Self-fertilized 2004 (n)	3	2	-	-	-	-	-
Prop. of all selfed seedlings	0.6	0.4	-	-	-	-	-
Prop. of all seedlings	0.04	0.03	-	-	-	-	-
Average LOD score	18.13	12.02	-	-	-	-	-
St. dev. LOD score	7.85	1.61	-	-	-	-	-
Single parent 2004 (n)	-	6	1	3	7	2	1
Tot. Parentage 2004 (n)	3	8	1	3	7	2	1

Table 3.3: Total numbers of seedlings found per site and year; and FaMoz (Gerber *et al* 2003) parentage analysis results by sample site and year: Numbers (n) and proportions (parentheses) of A) seedlings (s) a) most likely self-fertilized, b) seedlings with one likely tidal flat (tf) parent, and c) seedlings with no likely tidal flat parent; and of B) tidal flat adults with a) a large likelihood of having undergone self-fertilization, b) mixed mating, or c) out-crossing, or d) and e) a combination of the two. [RLtf = Robert's Landing tidal flat, HAYtf = Hayward tidal flat, ALAtf = Alameda tidal flat by Elsie Roemer marsh.]

A) Seedlings	RLtf '03	RLtf '04	HAYtf '03	HAYtf '04	ALAtf '03	ALAtf '04
Total No. Seedlings in GPS survey	532	2707	137	1244	319	862
Genotyped Seedlings (n)	42	54	32	69	33	67
a) Self-fertilized seedlings (s)	19 (0.45)	32 (0.59)	12 (0.38)	34 (0.49)	3 (0.09)	5 (0.07)
b) Out-crossed s, with tf parent	21 (0.50)	13 (0.24)	16 (0.50)	22 (0.32)	6 (0.18)	20 (0.30)
c) Out-crossed s, without tf parent	2 (0.05)	9 (0.17)	4 (0.13)	13 (0.19)	24 (0.73)	42 (0.63)
B) Adults						
Genotyped tf adults (n)	17	17	24	24	9	9
Reproducing adults (n)	9 (0.53)	9 (0.53)	13 (0.54)	12 (0.50)	5 (0.56)	7 (0.78)
Adults reproducing both years	6 (0.35)		8 (0.33)		5 (0.56)	
a) Only self-fertilizing parents	3 (0.33)	3 (0.33)	3 (0.23)	5 (0.42)	1 (0.20)	1 (0.14)
b) Selfing & out-crossing parents (mixed mating)	1 (0.11)	4 (0.45)	3 (0.23)	1 (0.08)	1 (0.20)	1 (0.14)
c) Only out-crossing parents	5 (0.56)	2 (0.22)	7 (0.54)	6 (0.50)	3 (0.60)	5 (0.72)
d) Total selfing parents (a & b)	4 (0.44)	7 (0.78)	6 (0.46)	6 (0.50)	2 (0.40)	2 (0.28)
e) Total out-crossing parents (b & c)	6 (0.67)	6 (0.67)	10 (0.77)	7 (0.58)	4 (0.80)	6 (0.86)