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

Christina M. Sloop*, Heather G. McGray, Michael J. Blum & Donald R. Strong
*Section of Evolution and Ecology, University of California, Davis, One Shields Avenue, Davis, CA, 95616, USA (*Corresponding author: Phone: +1-530-752-6852; Fax: +1-530-752-1449; E-mail: cmsloop@ucdavis.edu)*

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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. 2005), where *S. foliosa* does not occur. We initially developed 11 microsatellite loci to elucidate how *Spartina* invasions proceed (Blum et al. 2004). Here we 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 ($> 100\mu g$) isolated from a plant sampled from North Carolina. Following GIS polymerase chain reaction (PCR) library screening guidelines, we 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 and Skaletsky 1998) and http://www.gramene.org (Ware et al. 2002). The reaction volume for each PCR amplification trial per microsatellite locus was 14μl, consisting of 5μl of DNA (20 ng), 0.15μl (1U/μl) *taq* DNA polymerase (Promega, buffer A), 0.5μl of dNTPs (10μm), 0.375μl of each primer (40μm), 2μl of 10× Promega PCR buffer, and 3.25 to 4.35μl of *dd*H_2O; relative to the individually optimized amount of $MgCl_2$ used per locus listed in Table 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) for 45 s, 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 3730 × l 96-capillary DNA analyzer and ABI GeneMapper 3.0 software (Applied Biosystems, Cupertino, CA) (Table 2). PCR amplifications were successful for 145 loci. We 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*×*S. foliosa* hybrids; and 3–12 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

Table 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[a]	T_a (°C)	$MgCl_2$ (μM)	*S. densiflora* (No. alleles/ no. individuals)
AY758209	*SPAR.13*	F:CCGTGTACTCTAGCCTCTTG R: AGTCGAGTTGCTGTTCAGAT	$(CGC)_2GCG(CGC)_1$	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: CTTCCTGCTTGAATTGGTAG R: ACATCGGTGGCAGTAGTAAC	$(CT)_{11}AT(CT)_3$	55	30.00	−(0/3)
AY758213	*SPAR.17*	F: TACTTTGGTGTTTGCTTTATC R: GAGTTAGAGGAGTTATTGCTG	$(AAG)_8$	60	25.00	+(2/3)*
AY758214	*SPAR.18*	F: AACTTCTTGTTCTGGGATTG R: TAGGGAGATAGGACTGGACTG	$(CTT)_8$	64	25.00	+(2/3)
AY758215	*SPAR.19*	F: CTAATTCCTCACCCTACGC 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: GACACCAACGGAACTG	$(GA)_{23}$	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: CTATGAGTAGTTGGCCGTTC R: TGTTGTACTTCTTCTGGATGC	$(AT)_{3...}(GA)_{4...}(GA)_3$	53	21.67	+(1/12)*
AY758227	*SPAR.31*	F: GATCGGACAACTCTATGGAC 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: ACCGTAACAACTGAACTCTG R: TAGACGACGACCACTGCTTG	$(TC)_8$	58	21.67	+(2/3)*
AY758230	*SPAR.34*	F: TCATCATCGACCGAAAAC R: TCACCAGTGTCAAGCAGAG	$(CT)_{12}C(CT)_2$	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: TTTAGGTACTGCTGGGATTC	$(CTT)_{10}$	58	31.67	−(0/3)

[a]Core repeat from original clone sequence.
T_a, optimized annealing temperature; $MgCl_2$, optimized concentration per locus (μM);
+ indicates successful amplification; − indicates no amplification; *indicates presence of species-specific allele.

Table 2. Characteristics of 24 additional disomic microsatellite loci in *Spartina alterniflora*, *S. foliosa* and *S. alterniflora* × *S. foliosa* hybrids

Locus	*S. alterniflora* (Atlantic /Gulf coast)				*S. alterniflora* (Willapa Bay)				*S. foliosa* (California & Mexico)				*S. alterniflora*×*S. foliosa*			
	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	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	295	2/34	0.03†	0.11
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	226–230	3/29	0.79	0.6
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	266–285	9/35	0.37†	0.84
SPAR.16	368–384	8/19	0.53†	0.83	372–384	3/10	0.40	0.62	380	1/30	0.0‡	NA	376–386	6/31**	0.74	0.71
SPAR.17	372–376	3/8	0.63	0.68	374–384	4/8**	1.00	0.81	374	1/18	0.0‡	NA	372–376	3/5	0.60	0.51
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	180–196	5/16	0.31†	0.78
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	317–334	5/22	0.59†	0.76
SPAR.20	167–179	6/13	0.46†	0.73	177	1/6	0.0‡	NA	171	1/40*	0.0‡	NA	169–181	7/35	0.69	0.75
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	224–244	6/35**	0.2†	0.58
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	367–419	5/19**	0.58	0.67
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	248–274	5/33	0.58†	0.79
SPAR.24	173–200	6/20	0.85	0.69	173–191	2/9	1.00	0.53	173	1/39	0.0‡	NA	167–197	7/34**	0.5†	0.64
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	245–283	7/32**	0.56†	0.89
SPAR.26	261–297	14/22	0.73†	0.93	271–285	4/8	0.38	0.74	263	1/38	0.0‡	NA	263–291	8/35	0.86	0.73
SPAR.27	309–337	8/17	0.53†	0.9	306–341	3/9**	0.55†	0.71	314	1/30	0.0‡	NA	304–331	7/30	0.6†	0.79
SPAR.28	419–476	2/11	0.09†	0.44	419	1/8	0.0‡	NA	416	1/30*	0.0‡	NA	416–476	3/20	0.50	0.58
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	353–366	6/35**	0.69	0.76
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	283–300	4/27**	1.00	0.71
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	191–238	4/5	0.20	0.87
SPAR.32	443–507	3/13	0.08†	0.54	445	1/10	0.0‡	NA	443–507	2/29	0.04†	0.32	443–445	2/13	0.08	0.52
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	247–258	4/8	1.00	0.69
SPAR.34	368–380	5/14	0.36†	0.79	368–376	2/4	0.50	0.75	368	1/24	0.0‡	NA	368–378	4/16**	0.56	0.64
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	261–285	4/11	1.00	0.61
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	245–248	2/17	0.24	0.27

†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.

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 2).

We report the sequences for 24 primer pairs in Table 1. All 24 of the *S. alterniflora* loci amplified in *S. foliosa* and *S. alterniflora* × *S. foliosa* hybrids (Table 2), while only 18 loci amplified in *S. densiflora* (Table 1). Only 2 loci (SPAR.17, SPAR. 36) were at Hardy-Weinberg equilibrium in all sample groups (Table 2). All remaining loci exhibited heterozygote deficiency, monomorphism, or heterozygote excess in the various test populations (Table 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 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) and *S. foliosa* (Table 2). We 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 2).

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References

Ayres DR, Smith DL, Zaremba K, Klohr S, Strong DR (2004) Spread of exotic cordgrasses and hybrids (*Spartina* sp.) in the Tidal Marshes of San Francisco Bay, California, USA. *Biol. Invasions*, **6**, 221–231.

Blum MJ, Sloop CM, Ayres DR, Strong DR (2004) Characterization of microsatellite loci in *Spartina* species (Poaceae). *Mol. Ecol. Notes*, **4**, 39–42.

Civille JC, Sayce K, Smith SD, Strong DR (2005) Reconstructing a century of *Spartina alterniflora* invasion with historical records and contemporary remote sensing. *Ecoscience*, **12**, 330–338.

Rozen S, Skaletsky HJ (1998) Primer3 http://www.genome.wi.mit.edu/genome_software/other/primer3.html.

Schneider S, Roessli D, Excoffier L (2000) *Arlequin ver. 2.001: A software for population genetics data analysis*, Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Ware D, Jaiswal P, Ni J, Pan X, Chang K, Clark K, Teytelman L, Schmidt S, Zhao W, Cartinhour S, McCouch S, Stein L (2002) Gramene: A resource for comparative grass genomics. *Nucleic Acids Res.*, **30**, 103–105.