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PERMANENT GENETIC RESOURCES

Expressed sequence tag-derived polymorphic SSR markers for *Fucus serratus* and amplification in other species of *Fucus*

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Abstract

The seaweed genus *Fucus* is a dominant component of intertidal shores throughout the North Atlantic and North Pacific and has been the focus of considerable developmental, ecological, and evolutionary research for the past century. Here, we present details of 21 expressed sequence tag-derived simple sequence repeat markers (microsatellites). All 21 were polymorphic for *F. serratus*, which also display considerable cross-reactivity with the sister species *F. distichus* (18) and the more distantly related *F. vesiculosus* (13), and *F. spiralis* (5).

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The seaweed genus *Fucus* (Fucaceae, Phaeophyta) dominates the intertidal biomass of warm and cold temperate shores throughout the Northern Hemisphere, where several species co-occur from the highest intertidal to shallow subtidal (Lüning 1990). Recent studies have used anonymously derived microsatellites to examine phylogeography, mating systems, and hybridization in several species (e.g. Coyer *et al*. 2002a; Coyer *et al*. 2003; Billard *et al*. 2005; Engel *et al*. 2005).

Genomic research often produces high numbers of molecular markers that potentially are under selection. One strategy for detecting selection is to perform 'genome scans' (Luikart *et al*. 2003), using markers that are tightly linked to selectively relevant target loci via 'genetic hitchhiking' (Smith & Haigh 1974). The occurrence of polymorphic microsatellites (simple sequence repeats, SSR) within untranslated regions of expressed sequence tags (ESTs) provides markers that covary with genomic regions potentially under selection (Li *et al*. 2004). Here, we report development of EST-SSR markers from cDNA libraries of abiotically stressed *F. serratus* (temperature + high light) and *F. vesiculosus* (desiccation), as an initial step to identify genes putatively under selection.

A total of 4902 unigene sequences were searched with MsatFinder 2.0.7, (Thurston & Field 2005), which scanned for repeated sequences, as well as designed primer pairs flanking the repeated sequences, using the following parameters: (i) perfect motifs of 2, 3 or 4 repeat units, (ii) a product size of *c*. 200 bp, (iii) and primers of 20–25 bp with an annealing temperature of 60 C. Of the 269 candidate SSRs identified, 128 were selected for further screening in *F. serratus*, yielding 21 polymorphic loci.

Fingernail-sized pieces of meristematic tissue were excised from individuals in the field, blotted dry, and placed in silica crystals for dehydration and long-term storage. DNA was extracted from *c*. 10 mg of dried tissue using a modification of the technique in Hoarau *et al*. (2007): (i) a 96-well glass fibre microtiter plate (MultiScreen HTS, FB; Millipore) replaced the microtiter filtration plate with silica fines, (ii) the glass fibre microtiter plate (plus supernatant and binding buffer) was centrifuged at 182 *g* for 15 min, followed by 729 *g* for 10 min, (iii) the microtiter plate was dried at room temperature for > 30 min following elution of wash buffer, and (iv) DNA was eluted with 100 L elution buffer, incubated for 5 min at 55 C, then centrifuged at 182 *g* for 5 min and 729 *g* for 5 min.

Polymerase chain reactions (PCR; 10 L total volume) contained 1 L of the DNA template, 1× Hotmaster *Taq* polymerase buffer (5Prime), 0.2 mM of each dNTP, 2.0 M of each primer (forward primer of each primer pair 5′ end-labelled with FAM or HEX; Biolegio, BV), and 0.5 U HotMaster *Taq* polymerase (5Prime). PCR was performed with either a MyCycler (Bio-Rad) or Veriti (Applied Biosystems)

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thermocycler (94 C, 2 min; followed by 94 C, 20 s, annealing temperature, 10 s, and 65 C, 35 s for 35 cycles; and a final extension at 65 C for 10 min).

Amplification products were mixed with formamide and GeneScan ROX 350 size standard (Applied Biosystems); denatured at 90 C for 2 min; then analysed with an ABI 3730 autosequencer (Applied Biosystems). Genotypes were determined with GeneMapper 4.0 software (Applied Biosystems). Observed and expected heterozygosities (nonbiased), as well as linkage disequilibrium (2000 permutations with sequential Bonferroni corrections; Rice 1989) were estimated using Genetix 4.02 (Belkhir *et al*. 2001) for 43 individuals of *F. serratus* collected from Perharidy, Brittany, France.

All of the 21 loci were polymorphic with three to 19 alleles (Table 1). Values of unbiased expected heterozygosity

Table 1 Characteristics of 21 polymorphic EST-SSRs derived from a *Fucus vesiculosus–F. serratus* library. The number of alleles and heterozygosities of each locus and significant deviations from Hardy–Weinberg equilibrium (F_{IS}) at the < 0.001 level (*) were calculated for 43 individuals of *F. serratus* (*Fs*) from one population in Brittany, France. Cross-species amplification was tested using 18 individuals of *F. distichus* (*Fd*), eight of *F. vesiculosus* (*Fv*), and 48 of *F. spiralis* (*Fsp*). NT, not tested; 0, no amplification; +, scorable amplification

Locus	Core sequence	Primers (5′–3′)	T_a (C)	Size (bp)	H_E	H_O	F_{IS}	No. of alleles	*Fd*	*Fv*	*Fsp*
F-9	$(GT)_{16}$	F: GGCGGAAGTCGATTTGAATA R: ACTTGGCTGACGTCCAGAAT	55	184–212	0.8752	0.7857	0.10338	12	+	+	NT
F-12	$(GT)_{15}$	F: TATGTGTCCGACGACCTGAG R: TGAAGTCAAATGCTTGTTCG	55	186–218	0.8606	0.6667	0.22746*	12	+	+	NT
F-14	$(AGT)_6$	F: AAGCGCTCCATTCGTAGTACA R: GGCTGGCTGTTTGTCGTATT	55	198–210	0.6139	0.6750	–0.10089	8	+	0	NT
F-17	$(AT)_7$	F: GCAGACAGAGAGGGCAGAAG R: CCCCTCTCTCCCAGGTATTT	55	206–211	0.4203	0.2143	0.49313*	4	+	+	NT
F-19	$(GT)_{15}$	F: AGGTTTCAACCTGCTTCTGG R: TGCTACATCCAAGAATTGCAG	55	163–219	0.9251	0.8333	0.10031	19	+	+	+
F-21	$(TG)_{15}$	F: CATGTAGCGTGAAGCGTTTG R: CACGCAAACAAAACGTCAAC	55	198–207	0.6440	0.5952	0.07658	7	+	+	NT
F-22	$(AT)_6$	F: CCGTCTACGTTTCGTTTCGT R: ATCCGAGAGACGGATAGCAA	55	167–181	0.3543	0.3571	–0.00820	4	+	+	NT
F-34	$(TG)_6$	F: TGCCGAAGTACCGCATCTAC R: CTCCACTGGCATGCTGTTTA	55	187–232	0.7278	0.1667	0.77312*	9	+	+	NT
F-36	$(TG)_8$	F: TTTGCGGGATTGAAAGAGAG R: CCAGAATGGATGGGAAGAAA	55	188–221	0.7197	0.6429	0.10798	6	+	+	+
F-37	$(AT)_7$	F: ATTGTCCTGTCGGGATGAAA R: GCCCTTGTCAAACGAAAGAG	55	183–295	0.7978	0.7143	0.10578	7	0	0	NT
F-42	$(AGC)_5$	F: AGTGTGACTGCCCATTAGGG R: AGACGTAACCCAGTGCTGCT	55	186–197	0.2608	0.2381	0.08788	5	+	+	+
F-45	$(AT)_{10}$	F: CCGATACGTACGTGGGAGTG R: GACGGGAAATTGCTAAGTGG	55	195–209	0.8569	0.5714	0.33581	13	+	0	NT
F-47	$(TG)_7$	F: CCCTTGGCAAAGAGCAAATA R: GCAGAAGGAAGGTGGATGAG	55	193–209	0.4753	0.3810	0.20049	6	+	0	NT
F-49	$(AT)_6$	F: TGCTGTAGAAGGCCGAAGTT R: AACGAGTTCGTCGAGTGTCC	55	193–196	0.2149	0.1429	0.33782	3	+	+	+
F-50	$(CTA)_6$	F: GGTGTTGCTTTTCCGAGTGT R: GGGCGTGTGTCTCTTTGTTC	55	182–202	0.6913	0.2143	0.69263*	10	+	0	NT
F-58	$(TA)_8$	F: CGTGTTTTGTCCGTCCTTTT R: CGGAACAGATGGGAGACAAT	55	190–201	0.5941	0.5238	0.11957	8	+	+	NT
F-59	$(AT)_7$	F: TCGCCATATCTGTGTCAAGG R: AACAAATTGGTGCCGAGTGT	55	181–213	0.5860	0.1579	0.73317*	9	+	+	NT
F-60	$(CA)_8$	F: GGGGTTGTTTTCGATAAAAGG R: GCAATCGACCTCGAGAAATC	55	186–198	0.4223	0.3333	0.21262	7	+	+	+
F-65	$(TA)_{10}$	F: GGTAGAGGTTGGGCGTGTTA R: GGAGATTCGACCAGAGTCCA	53	184–205	0.6819	0.5476	0.19881	12	0	NT	NT
F-69	$(ATGT)_9$	F: TGCGTTGTATGCAAGAGGAC R: CCTTACCCCGTGATTTCGTA	53	194–230	0.8738	0.4390	0.50069*	10	0	NT	NT
F-72	$(AG)_6$	F: ATCTCCGCCTTAACCCAGTC R: CAGCTGGATACGGATGGAGT	53	193–197	0.3680	0.3095	0.16063	6	+	NT	NT

ranged from 0.2149 to 0.9251 and six loci deviated significantly ($P < 0.001$) from Hardy-Weinberg equilibrium. Given the very low rate of amplification failure (0.7%), null alleles are unlikely to be present at a significant level. No linkage disequilibrium was detected. Cross-species amplification was apparent for 18 loci in the sister species *F. distichus* (see Coyer *et al*. 2006), 13 loci in *F. vesiculosus*, and five loci (only five of the 21 loci were evaluated) in *F. spiralis* (Table 1). The combination of EST with anonymously derived loci (Coyer *et al*. 2002b; Engel *et al*. 2003) increases the number of loci, thereby providing additional statistical power in phylogeographical and hybridization studies of *Fucus* spp. Furthermore, large numbers of loci can be used to increase the statistical power of genome scans to identify putative regions under selection.

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