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PRIMER NOTE

Isolation and cross-species amplification of microsatellite loci from the fucoid seaweeds *Fucus vesiculosus*, *F. serratus* and *Ascophyllum nodosum* (Heterokontophyta, Fucaceae)

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Abstract

The Fucaceae is a family of brown seaweeds that dominate and frequently co-occur on North Atlantic rocky shores. We developed nine polymorphic microsatellite markers for the fucoid seaweeds *Fucus vesiculosus*, *F. serratus* and *Ascophyllum nodosum* using a combined, enriched library. Six of these loci were polymorphic in at least two species, showing from two to eight alleles with heterozygosities ranging from 0.41 to 0.85. Loci were also tested on *F. spiralis*, revealing five polymorphic microsatellite loci in this species.

Keywords: cross-species amplification, Fucaceae, microsatellites, seaweeds

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The Fucaceae (Phaeophyceae) are a family of closely related dioecious and hermaphroditic brown seaweeds that have undergone recent radiation (Serrão *et al*. 1999). Several species are ecologically dominant on temperate North Atlantic rocky shores and frequently occur together. The close phylogenetic relationships of these sympatric species and the possibility of interspecific hybridization (e.g. Kniep 1925; Bolwell *et al*. 1977; Scott & Hardy 1994; Kim *et al*. 1997) provide an excellent opportunity to explore the microevolutionary, population-level processes of reproductive isolation, hybridization and speciation. Furthermore, dioecious *Fucus vesiculosus* and hermaphroditic *F. spiralis* are poorly defined species (Serrão *et al*. 1999) that co-occur throughout most of their range, raising the additional intriguing question of the evolution and maintenance of mating systems. Co-dominant genetic markers are valuable tools for assessing mating patterns and are useful for detecting hybridization (Pritchard *et al*. 2000). Here, we describe polymorphic microsatellite markers for *F. vesiculosus*, *F. serratus*, *F. spiralis* and *Ascophyllum nodosum*.

Sperm DNA was isolated from *F. vesiculosus*, *F. serratus* and *A. nodosum* by a cetyltrimethyl ammonium bromide (CTAB)/Sephaglas procedure (Coyer *et al*. 2002). Genomic DNA (300 ng, weighted 2 : 1 : 1 of *F. vesiculosus* : *F. serratus* : *A. nodosum*) was restricted with *Rsa*I (Pharmacia) and simultaneously enriched for CT, CA, AT, GC, CAA, GCC, GATA, CATA and ATAT motifs according to Edwards *et al*. (1996). An aliquot of the ligation mixture was transformed into DH5α competent cells. Plasmid DNA was extracted from 96 colonies and sequenced on an ABI 373 automated sequencer (PE Applied Biosystems) using the BigDye kit (PE Applied Biosystems). Of the 59 clones containing significant microsatellite sequences, primers were designed for 28 clones using Primer3 software (Rozen & Skaletsky 2000).

Fucus vesiculosus and *F. spiralis* DNA for genotyping was extracted from *c.* 1 mg of dried tissue using DNeasy™ Plant Mini kit (QIAGEN) and diluted 1 : 500. Polymerase chain reactions (PCRs) were performed in 20 μL containing 3.7 μL H_2O, 0.2 μg/μL bovine serum albumin, 10 mM Tris-HCl, 50 mM KCl, 2.0 mM $MgCl_2$, 0.25 μM of each forward and reverse primer, 200 μM of each dNTP, 0.5 U Ampli*Taq* DNA polymerase (PE Applied Biosystems) and 5 μL of DNA (*c.* 0.2 ng). PCRs were run on a PerkinElmer GeneAmp PCR System 9700 thermocycler. After an initial denaturation step (95 °C, 5min), 'touchdown' PCR was carried out for five cycles of 30 s at 95 °C, 30 s at the annealing temperature (T_a, Table 1) reduced by 1 °C each subsequent cycle and 30 s at 72 °C, followed by 35 cycles of 95 °C for

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Table 1 Primer sequences and characteristics of nine microsatellite loci in fucoid seaweeds. Cloned sequences were registered with the GenBank database under the Accession nos AY158011–AY158019

				Fucoid species											
				F. vesiculosus			*F. serratus*			*A. nodosum*			*F. spiralis*		
Locus	Repeat array	T_a (°C)	Primer sequences	N_a (size range)	H_O	H_E	N_a (size range)	H_O	H_E	N_a (size range)	H_O	H_E	N_a (size range)	H_O	H_E
L17	GAGAA$(GA)_4$AATACA $(GA)_{30}$GGGAAA$(GA)_3$	60°	F-GTTGAACAAACTGGCCGAGA	1			2	ND	ND	—			1		
			R-TGGGTTTACGAGCACAAACA	(ND)			(ND)						(ND)		
L20	CTGG$(CTG)_8(TTG)_3$CTT $(CTG)_2$	60°	F-ACTCCATGCTGCGAGACTTC*	8	0.33	0.85	4	0.42	0.41	4	0.58	0.54	4	0.58	0.54
			R-CCTCGGTGATCAGCAATCAT	(120–159)			(135–147)			(135–150)			(120–153)		
L38	$(GCT)_{11}$GCC$(GVT)_7$	63°	F-TGCTAGCTGCTCTTGTGTGC	4	0.58	0.64	5	0.75	0.64	—			2	0.08	0.08
			R-TAACCTGTCGGTCGCAACG*	(169–199)			(193–214)						(169–190)		
L58	$(GA)_{19}$	59°	F-AAACGAAAATGGCACAGTGA*	6	0.58	0.69	9	0.83	0.82	—			3	0.08	0.16
			R-CCTTGCATGTAGGAGGGAAC	(103–115)			(113–138)						(107–113)		
L78	$(TGC)_{11}$TGT$(TGC)_2$TGG CGGTGCTGT$(TGC)_3$	60°	F-CGTGAGGGCAGGAATGTC	4	0.75	0.57	5	0.25	0.71			—	3	0.00	0.49
			R-GATTTCCGGCATCATCAATC*	(137–158)			(140–167)						(121–137)		
L85	$(GT)_3$CG$(GT)_8$GCGT	60°	F-GCTGAGTTGCCTTACCGACA*	3	0.58	0.64	2	0.17	0.44	—			1		
			R-TAGGATGATAGGGCCGGATT	(98–102)			(102–104)						(102)		
L94	$(GCA)_3$GACGAT$(GCA)_5$ ACA$(GCA)_5[GCT(VCA)_6]_2$ $(ACA)_2$	61°	F-TTAGGAATGGGCGGGATG*	5	0.67	0.65	7	0.75	0.78	—			4	0.17	0.45
			R-GATTTCGTGAGGCTGGTTCA	(136–166)			(142–172)						(136–160)		
L132	$(GCA)_8$	59°	F-GACACCAGCAGCCTGAGAG	—			5	ND	ND	—			—		
			R-GGCTCAGAGGCTGAAAACC				(ND)								
L138	$(CAG)_2$CAA$(CAG)_8$	60°	F-GACGAGGCAAGCGATCAG	—			—			4			—		
			R-CGATGGAGGTGGTCTGCT							(ND)					

V: G, A or C; T_a: annealing temperature; *primer labelled with IRDye™700 or IRDye™800; N_a: number of alleles observed; H_O: observed heterozygosity; H_E: expected heterozygosity; '—' indicates no amplification products. ND, not determined.

30 s, T_a –5 °C for 30 s and 72 °C for 30 s. To assess polymorphism, 12 individuals were analysed for each of four species; equal numbers of individuals came from Brittany, Dover Strait and northern Portugal for *F. vesiculosus* and *F. spiralis*, from Oslo, Jutland, Brittany and Galicia for *F. serratus*, and from Maine, Brittany, Donegal and Bergen for *A. nodosum*. PCR products were electrophoresed on 8% polyacrylamide gels visualized by silver staining, or on 3% MetaPhor® agarose gels (FMC BioProducts) stained with ethidium bromide. Primer pairs that amplified simple, polymorphic loci in *F. vesiculosus* were then amplified as described above except that one primer of each pair was end-labelled with infrared fluorescent dye (IRDye™800 or 700, MWG). Labelled PCR products were analysed on an automated DNA sequencer (Li-Cor 4200™) along with an M13 sequence to estimate allele sizes.

Of the 28 primer pairs, six pairs gave no amplification products in any species, six gave multiple-banded profiles in all four species, four gave multiple-banded profiles in at least one species, three were monomorphic in all species showing positive amplification and nine were polymorphic in at least one species (Table 1). Of these nine, six loci were polymorphic in *F. vesiculosus*. The six loci showed three to eight alleles with expected heterozygosities ranging from 0.57 to 0.85. In addition, these loci were polymorphic in at least one other species (Table 1). Our microsatellite library thus revealed eight polymorphic loci in *F. serratus*, two in *A. nodosum* and five in *F. spiralis*. These markers complement those recently reported in *F. serratus* (Coyer *et al*. 2002) and in *A. nodosum* (Olsen *et al*. 2002).

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