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PRIMER NOTES

Isolation and characterization of polymorphic microsatellite markers in the North Atlantic redfish (Teleostei: Scorpaenidae, genus *Sebastes*)

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Redfish (genus *Sebastes*) are marine, viviparous and commercially important fish of the North Atlantic. For the last 30 years, a considerable amount of effort has been invested to study the evolutionnary relationships and the population genetic structure of this species complex. The complex is composed of four currently recognized taxa: *Sebastes fasciatus*, *S. mentella*, *S. marinus* and *S. viviparus*. However, genetic studies relying on proteins and/or mitochondrial DNA markers have generally revealed low levels of genetic variation within and between redfish taxa (McGlade *et al.* 1983; Nedreaas & Naevdal 1991a), and hampered the analysis of young stages (Nedreaas & Naevdal 1991b). Consequently, there was a need for genetic markers with a higher resolution that could detect differences at any level of genetic differentiation (species, stocks), and any developmental stages. We report here the isolation and characterization of eight microsatellites from the Atlantic redfish complex that may be useful for a wide range of applications throughout the Atlantic Ocean.

Total DNA was extracted from each of the four redfish taxa using standard proteinase K phenol–chloroform techniques (Sambrook *et al.* 1989). Fifty μg of pooled DNA from all four taxa was digested with *Sau*3AI restriction enzyme and fragments were separated on a 0.8% agarose gel. Fragments ranging in size from 300 to 800 bp were isolated, purified (QIAquick Gel extraction kit, Qiagen) and ligated into a pUC18 circular vector ('Ready to go' kit, Pharmacia). Ligation products were transformed into XL-1 Blue competent cells following standard procedures (Sambrook *et al.* 1989). Transformed cells were grown on a Luria–Bertani (LB) agar medium and then transferred onto nylon membranes (Hybond-N, Amersham). DNA fixation was carried out by exposing the membranes for 3 min under UV light. Transformants were screened for inserts containing microsatellites, with an equal mix of $(CT)_{10}$ $(GT)_{10}$ $(CAC)_5(CA)$, $CT(CCT)_5$, CT $(ATCT)_6$ and $(TGTA)_6$ labelled with [γ^{32}P]-dATP (Estoup & Cornuet 1994) using T4 polynucleotide kinase. Hybridization procedures followed standard protocols (Sambrook *et al.* 1989). Among the 1400 clones of the partial library screened with specific oligonucleotides, 47 (3%) were identified as positive and isolated. DNA from 29 positives was extracted using the QIAprep plasmid DNA prep kit (Qiagen) and sequenced using an ABI 373 automated sequencer using universal primers. Primers for PCR amplifications were chosen from specific flanking regions of microsatellites and designed using both OSP (Hillier & Green 1991) and OLIGO™ version 4.0 (National Biosciences) software programs. Eleven microsatellites allowed specific primer pair design, but three failed to produce PCR products. Characteristics of the remaining eight primer pairs are described below (Table 1).

The eight microsatellites were amplified using either radioactive (α^{35}S, γ^{35}S) or fluorescent labelling method. α^{35}S radioactive PCR amplifications were carried out in a 15-μL reaction volume using 2 μL of DNA template (25–100 ng), 0.133–0.532 μM of each primer (Table 1), 75 μM of each of dCTP, dGTP, dTTP, 5 μM dATP, 1.5 μCi of [α^{35}S]-dATP, 1.2 mM $MgCl_2$, 1× *Taq* buffer (10 mM Tris-HCl (pH 9), 50 mM (KCl) and 0.25 units of *Taq* polymerase (Perkin-Elmer). For γ^{35}S PCR amplifications, the same conditions were used except that one of the primers was end-labelled with γ^{35}S using T4 polynucleotide kinase. Duplex amplifications were conducted either for SEB31 and SEB33, or for SEB37 and SEB46. The following PCR profile was used for all loci: an initial denaturing step of 3 min at 95 °C, 30 cycles of 1 min at 94 °C, 40 s at 62 °C, 40 s at 72 °C, followed by a final elongation step of 5 min at 72 °C. PCR amplifications were performed with a Perkin-Elmer 480 DNA thermocycler. Electrophoresis, fixation, drying and autoradiography followed standard procedures (Sambrook *et al.* 1989). An M13-mp18 (USB) sequence ladder, as well as a subset of four standard samples run on all gels, were used to estimate allele size at each locus.

For the fluorescent method, PCRs were performed in a 10-μL reaction volume with a Perkin-Elmer 9600 thermocycler (version 2.01) (1.2 mM $MgCl_2$, between 0.05 and 0.3 μM of each primer (Table 1), 1× *Taq* buffer, 0.25 units of *Taq* polymerase, 75 μM of each dNTP and 25–100 ng of DNA template). The second primer of each pair (Table 1) was end-labelled with one of the three fluorescent labels: yellow (HEX) was used for SEB9, SEB31 and SEB33, green (TET) for SEB37 and SEB46 and blue (6-FAM) for SEB30 and SEB45. SEB25 was not analysed via fluorescent labelling. Multiplex amplifications were optimized for SEB9, SEB31 and SEB33 (A), and for SEB37, SEB46, and SEB30 (B). The PCR profile was as follows: a denaturing step of 2 min 15 s at 95 °C, 30 cycles of 30 s at 94 °C, 20 s at 62 °C, 20 s at 72 °C, and a final step of 3 min at 72 °C. Electrophoresis was conducted using an ABI 377 sequencer in Genescan™ mode. For each sample, 0.9 μL of A, 0.6 μL of B and 0.6 μL of SEB45 were mixed

Table 1 Repeat sequence for eight *Sebastes* loci, primer sequences, primer concentration (μM) for radioactive and fluorescent methods. *indicates γ^{35}S end-labelling. GenBank Accession nos are AF103018–AF103024

Locus	Repeat array of the cloned allele	Primers (5′–3′)	Primer concentration (μM)	
			Radioactive	Fluorescent
SEB	$(GA)_{22}$	1-AAGGCTGACTCTGAGTGGGA	0.399	0.250
		2-CTCTGAGTCTATGTATCTGGCT	0.133*	0.250
SEB25	$(CA)_{15}$	1-CAGCTTGACGTGAGGGGA	0.266	–
		2-GTGCCTGTTTAGGGTGTTCTT	0.266	–
SEB30	$(CT)_7TT(CT)_{15}TT(CT)_{29}$	1-CTGTTGGACAGATAAAGACGC	0.133	0.075
		2-GGTGATATTGCTGCTGGTAGAT	0.133	0.075
SEB31	$(GA)_2GGGG(GA)_{22}GGG(GA)_4$	1-GTGAGACCAGTAATAAGGGCA	0.399	0.300
		2-TACTTCTCGACTGTGGTG	0.399	0.300
SEB33	$(GA)_{39}$	1-CAGATGTTGGTAGACGCAAGCA	0.133	0.300
		2-AGTCCAGTGTCCATCCTCCTT	0.133	0.300
SEB37	$(GT)_{10}(GA)_{16}$	1-GTACAGTCCATTCAGCTTTGA	0.399	0.250
		2-AGGGTGTGTGGAAGAAATAGT	0.399	0.250
SEB46	$(GT)_{20}$	1-GCTGATGTTGCTCCTAAAGAA	0.532	0.050
		2-CTCTTCATGTCAATCCTGCCT	0.532	0.050
SEB45	$(GT)_{24}$	1-GAGGAGGAAAAGACTGGACAGA	0.133	0.150
		2-GAAAGATGGTGAGCAGCGATGA	0.133*	0.150

Table 2 Expected (H_E) and observed (H_O) heterozygosity, number of alleles (A) for each locus/species and overall, and size range of alleles (in base pairs)

Taxa		SEB9	SEB25	SEB30	SEB31	SEB33	SEB37	SEB45	SEB46
S. fasciatus	A	8	14	17	5	19	14	7	10
	H_E	0.799	0.902	0.882	0.586	0.939	0.867	0.500	0.800
	H_O	0.759	0.833	0.793	0.667	0.931	0.886	0.544	0.789
S. mentella	A	9	15	22	14	24	15	15	10
	H_E	0.772	0.893	0.930	0.835	0.958	0.886	0.878	0.832
	H_O	0.964	0.828	0.793	0.893	0.893	0.720	0.714	0.741
S. marinus	A	9	19	17	12	21	12	12	13
	H_E	0.786	0.886	0.693	0.854	0.948	0.823	0.821	0.912
	H_O	0.759	0.964	0.600	0.767	0.929	0.759	0.733	0.867
S. viviparus	A	5	4	26	6	23	17	11	20
	H_E	0.628	0.606	0.960	0.612	0.956	0.933	0.761	0.918
	H_O	0.483	0.714	0.897	0.926	1.000	0.611	0.667	0.667
Total	A	12	22	46	21	41	27	20	24
	H_E	0.755	0.826	0.865	0.725	0.951	0.885	0.752	0.867
	H_O	0.740	0.838	0.769	0.820	0.938	0.822	0.655	0.768
Size range		99–125	195–237	173–353	150–188	222–314	219–283	114–164	116–186

with 2 μL of blue formamide, 0.22 μL of GS350 internal size standard (red colour, TAMRA 350 bp) and denatured for 3 min at 95 °C. Polymorphism was screened on a 5% denaturing polyacrylamide gel and run for 2 h 30 min at 3000 V. Data collection and analysis, as well as automated scoring of the alleles for each sample, were performed using the Genescan software. Sizes were determined by reference to the internal standard and by comparison to three samples of known allelic sizes that were run on each gel. Tabulation of data for each locus was conducted with the Genotyper software. Fifteen individuals were compared using both radioactive and fluorescent methods, to ensure the correspondence of the two allelic scoring methods. No differences were found for SEB9, SEB45, SEB46, SEB31 and SEB37.

However, a consistent increase of 4 bp for SEB33, and a decrease of 2 bp for SEB30 were found with the fluorescent method. Allelic sizes were systematically corrected in order to adjust both methods.

In order to estimate microsatellite variation and polymorphism in redfish DNA, 30 individuals of each taxa were screened. DNA from each individual was extracted from muscle tissue using the Chelex extraction method (Walsh *et al.* 1991). All loci showed high variability. The total number of alleles per locus varied between 12 and 46 (Table 2). This also translated into high gene diversity, with a mean overall expected heterozygosity (H_E) varying between 0.500 and 0.960 depending on locus and taxa (Table 2). These values are comparable to those found in other marine fish (de García León *et al.* 1995; Rico *et al.* 1997; Ruzzante *et al.* 1996).

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Characterization of microsatellite loci for red-necked grebes *Podiceps grisegena*

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A wide range of evolutionary forces have been implicated in the evolution of coloniality in birds, including reproductive competition, kin selection, sexual selection, and population structure (Alexander 1974; Wagner 1993). Thorough study of the evolutionary origin or maintenance of coloniality therefore requires that field study be augmented with genetic work (e.g. Hoi & Hoi-Leitner 1996). Microsatellite loci are expected to yield data that allow the roles of these evolutionary factors to be these assessed.

The red-necked grebe, *Podiceps grisegena*, typically nests territorially, while rarely nesting colonially (Cramp & Simmons 1977). Territorial and colonially nesting pairs co-occur at our study population in Minnesota, offering the opportunity of comparative analysis. We developed and characterized seven microsatellite loci in the red-necked grebe to help examine the evolutionary roles of the factors listed above. Additionally, we examined the polymorphism of these loci in five other nearctic grebe species and one hybrid form.

DNA was extracted from blood using a proteinase K, phenol–chloroform extraction procedure modified from Müllenbach *et al.* (1989). Size-selected genomic DNA digested with *Dpn*II was cloned into Lambda Zap Express, Stratagene, La Jolla, CA (Hughes & Moralez DeLoach 1997). We screened (125 000 clones with the oligo $(AAT)_{10}$ (Hughes & Queller 1993), sequenced 35 positives, and developed primers for all seven clones containing ≥ 8 uninterrupted repeats of the sequence AAT.

PCR reactions (5 μL) contained ≈ 5 ng of DNA, 50 mM KCl, 10 mM Tris/Cl pH 8.3, 1.5 mM $MgCl_2$, 0.1% NP40, 250 mM each dNTP, 0.25 U *Taq* DNA polymerase (Perkin-Elmer), 2.5 pmol each primer, and 0.05 μL of ^{35}S-dATP. Primer pairs 3 and 41 yielded scorable product only when *Taq* DNA polymerase was preincubated with Taqstart (Clontech); this produces a 'hot start' effect. Reactions were cycled using the 'tube-control' function of a Hybaid thermal cycler: 90 s at 92 °C, then 5 s at the optimal annealing temperature for each primer pair (Table 1), 5 s at 72 °C, 5 s at 92 °C, 30 times, and finally 90 s at 72 °C. Amplified fragments were resolved using 6% denaturing polyacrylamide gels. Allele length was determined by comparison to the sequencing products of M13.

All loci tested were found to be polymorphic in the red-necked grebe, having between seven and 18 alleles and heterozygosities ranging from 65 to 86% (Table 1).

The six polymorphic loci were tested on five other nearctic grebe species and one hybrid. PCR conditions for all loci