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Short Communication

Isolation and Characterization of Twelve Microsatellite Loci for Rockfish (*Sebastes*)

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Abstract: We describe the first microsatellites for rockfishes in the diverse genus *Sebastes.* Clones containing microsatellites were isolated from the genomic library of a quillback rockfish, *Sebastes maliger.* Twelve microsatellites are characterized; six of these are polymorphic in quillback rockfish, and eight are polymorphic in at least one rockfish species on which they were tested. The number of alleles per variable locus ranged from 4 to 15 and averaged 6.8. The expected heterozygosities ranged from 0.38 to 0.79 and averaged 0.60 in these loci. These loci should prove valuable in studies examining species identification, population genetics, hybridization, paternity, kinship, and microsatellite evolution.

Key words: *Sebastes,* rockfish, microsatellites.

INTRODUCTION

Rockfishes (genus *Sebastes*) comprise an important biological, cultural, and economic resource along the Pacific Coast of North America. The genus, which numbers around 100 species in the North Pacific, represents an evolutionary radiation of unusual scale among temperate marine fishes, and contributes richly to the biodiversity of coastal waters in the region (Barsukov, 1981; Kendall, 1991). Many species also support significant commercial and sport fishing industries (Laidig et al., 1996). Unfortunately, most populations have declined in recent years due to overexploitation (Gunderson, 1997). Knowledge of the genetic structure of populations is important to successful management of these species, and may provide useful insights into the population genetic processes that gave rise to so many species. Attempts to characterize population structure of *Sebastes* using allozymes and DNA sequence data have been hampered by the low variability of these genetic markers (Wishard et al., 1980; Seeb, 1986; McGauley and Mulligan, 1995; P. Wimberger unpublished data). Neutral genetic markers with greater polymorphism would provide better resolution of fine-scale population structure in the genus. Most microsatellite loci meet these criteria (Wright and Bentzen, 1994); however, no *Sebastes* microsatellite loci have yet been published. We cloned a suite of 12 microsatellite markers from quillback rockfish, *Sebastes maliger,* and tested them on quillbacks as well as representatives of 10 other species of *Sebastes* and one related genus (*Helicolenus*). Six of the loci are polymorphic in quillbacks, and all amplify in at least a majority of species tested. These microsatellite primers should prove to be useful tools in a wide range of population and evolutionary studies of this large group of species. In addition to permitting the examination of population genetic structure of different rockfish species, these micro-

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satellite loci should prove useful in studying paternity in *Sebastes* (females are internally fertilized and give live birth). They should also aid in the species identification of rockfish larvae because larvae and juveniles of many rockfish species are virtually impossible or difficult to distinguish morphologically (Matthews, 1988; Kendall, 1991; Moser and Boehlert, 1991). In some north Pacific regions, rockfish larvae make up the majority of ichthyoplankton during certain times of year.

Materials and Methods

DNA from liver, heart or fin clip tissue was extracted from a single quillback rockfish (*Sebastes maliger*), digested with *Mbo*I, and size fractionated on a 1% agarose gel. We excised DNA fragments between 300 and 800 bp and extracted them using the phenol-chloroform method. The fragments were ligated into pZErO-2.1 vector (Invitrogen Corp., San Diego, Calif.) cut with *Bam*HI. TOP10F′ (Invitrogen Corp.) competent cells were transformed with ligated vector, plated, and allowed to incubate overnight at 37°C. Approximately 2200 colonies were lifted using nylon membranes and probed with fluorescein-labeled oligonucleotides: $(GT)_{15}$, $(GA)_{15}$, $(AAT)_{10}$, $(AAG)_{10}$, $(AAC)_{10}$ (Amersham Life Science Inc., Arlington Heights, Ill.). Following stringency washes and signal amplification using the Vistra signal amplification kit (Amersham Life Science Inc.), we used a Molecular Dynamics FluorImager 575 to visualize positive colonies on probed membranes. Seventy positive colonies were selected from the initial screen; 44 of these were positive following a second screen. DNA from 34 of the positives with inserts of 300 to 1500 bp was extracted using the Qiaprep plasmid DNA prep kit (Qiagen Inc., Santa Clarita, Calif.) and sequenced using Applied Biosystems Inc. (ABI) Prism *Taq* DyeDeoxy terminator chemistry and an ABI 373A automated sequencer. Microsatellite sequences were identified in 29 of 34 positives; however, the majority of these were short, imperfect microsatellites with the longest contiguous stretch of repeats ranging from 5 to 7. We designed primers for 12 sequences with seven or more repeats (see Table 1) using the program CPrimer (G. Bristol and C.D. Andersen, 1995, Regents of the University of California) and purchased those from Gibco/BRL (Gaithersburg, Md). We examined all microsatellite-containing sequences for homology with other nucleotide sequences in GenBank using a simple BLAST search with default parameters (Altschul et al., 1990). Few of the resultant hits had very high probabilities, and none of them had high similarity to any known vertebrate coding regions. The highest probability regions were usually protist or *Caenorhabditis elegans* sequences, and the regions with over 50% similarity were relatively short (50 to 100 bp).

We initially tested the 12 microsatellites in seven quillback rockfish and found six loci to be polymorphic. We then measured levels of polymorphism for these six loci in 132 to 160 individuals. We subsequently tried the primers on 10 other *Sebastes* species (1–5 individuals per species; see Table 2), as well as blackbelly rosefish (*Helicolenus dactylopterus*). Initial polymerase chain reaction (PCR) was carried out in 20-μl volumes comprising 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.8 units *Taq* polymerase, 0.5 μM each primer, and approximately 100 ng DNA template. DNA amplifications involved the following profile: one cycle of 94°C (2 minutes); seven cycles of 94°C (1 minute), 55°C (30 seconds), 72°C (15 seconds); and 18 cycles of 94°C (30 seconds), 58°C (30 seconds), 72°C, or a standard three-temperature PCR profile using annealing temperatures ranging from 50°C to 58°C. Initially microsatellites were run on an Owl Scientific vertical electrophoresis system. Typically 5 μl of each PCR product and 1 μl of loading buffer (15% wt/vol ficoll 400, 0.06% wt/vol bromophenol blue, 0.06% wt/vol xylene cyanol, 30 mM ethylenediaminetetraacetic acid [EDTA]) was loaded on a 20-cm, 6% nondenaturing polyacrylamide gel and electrophoresed for approximately 2 hours at 150 V. Each gel was stained with a 1:10,000 solution of SYBRGreen 1 nucleic acid gel stain (Molecular Probes Inc., Eugene, Ore.) and 1× TBE buffer for 30 minutes and scanned on the FluorImager, or with ethidium bromide and visualized on a Bio-Rad gel imaging system.

We estimated microsatellite polymorphism and heterozygosity in variable *S. maliger* loci by genotyping 132 to 160 individuals using the ABI 373A in GeneScan mode (ABI, 1993). Following PCR, 1.0 μl from each sample was combined with 2.92 μl of formamide, 0.58 μl of 25 mM EDTA, and 0.50 μl (1.0 fmol) of Perkin-Elmer GS500 internal size standard. The samples were denatured at 95°C for approximately 3 minutes, chilled on ice, and loaded on a 6% denaturing polyacrylamide gel. Each gel was run for approximately 10 hours at 25 W. Data were analyzed using the internal lane sizing standard and local Southern sizing algorithm in GeneScan 2.0. Scoring of alleles for each locus was performed with Genotyper 2.1 (ABI, 1996).

Table 1. Quillback Rockfish Microsatellites*

Locus	Core sequence	Primers	Size (bp)	T (°C)	*N*	No. of alleles	H_e
*Sma1**	$(GT)_{21}$	AAGTGAGTGGGTTTCATTGAGATAC TACCTGTGTCAGCAAGTAAACTCTG	110–138	58	158	15	—
Sma2	$(TG)_3TA(TG)_2TC(TG)_{16}$	TTACTTGTTTTCTTTGTCTCATGTGG CACAGGACTATCAGCAGGGAAG	163–173	58	157	4	.47
Sma3	$(GT)_{12}$	GCAGACTTACAGCGGT TTCAC ACCATCCAGTCATACGAGCAC	146–154	58	152	5	.68
Sma4	$(AAT)_{11}ATAAT$	CATAACATATGATGGAAAATAAAACCC CAAATTGCCCCCACTGAAG	212–228	58	158	6	.66
Sma5	$(GT)_2GCGTGC(GT)_8$	ATTCCCACCCACTCACACTT GAGATTTCTGGAGTCCACGC	110–116	58	132	4	.38
Sma6	$(GT)_7$	ATGATGAAGTGTCGGTTGCTC AAGGGAGGGCACCCCAAAAC	175	58	7	1	0
Sma7	$(TG)_{10}$	CATAGGTCATTTCTCAAAGGTGTG GAGAAACAAACAGGAACTGAGAGAG	117	58	7	1	0
Sma8	$(AC)_3ATGC(AC)_{10}$	TCACACATCGTCTGGCTATTTC AATTCTAAAGTCTATGGGGACACG	176	58	10	1	0
Sma9	$(GT)_8$	GATCACCGTTATTGCTTGAGTG CTTTTAACGGTTTTTACCGATAGC	127	58	5	1	0
Sma10	$(TG)_4TC(TG)_{12}GG(TG)_9$	CAGCAGCATTTGAAGCACATC CACCAGTGGAACACACGCAC	92–163	58	160	15	.79
Sma11	$(CT)_2CCCTCC(CT)_{10}TT(CT)_2CC(CT)_7$	AATATGAGGACGGGCAACG TAGTAGGAGTGAGCATCGTACCAG	196	56	17	1	0
Sma12	$(ACC)_5(AC)_7$	AACTGAGAAGAAAAGGGAGCG GACAAAGTGGAGCAGAGAGTCC	251	56	7	1	0

*The *Sma1* primers amplify 2 to 4 alleles for each individual (see text for further details).

Results

The loci were only moderately variable. The number of alleles per variable locus ranged from 4 to 15 and averaged 6.8 (Table 1). The expected heterozygosities ranged from 0.38 to 0.79 and averaged 0.60 (Table 1). Locus *Sma1* was variable, but the primers appeared to amplify two similarly sized loci, and individuals displayed 1 to 4 alleles. Despite varying PCR conditions (temperature, Mg^{2+} concentration, and cycling times), we could not reliably reduce the number of alleles per individual to a maximum of two. *Sma1* may be a duplicated locus or a remnant of a polyploidization event.

We have included information for 12 loci in *Sebastes,* even ones that were not variable in any species on which they were tested, because two of the loci that were monomorphic in the source species, *S. maliger,* were polymorphic in other *Sebastes* species (*Sma7* and *Sma11*). This result suggests that microsatellite loci isolated in species that are part of a phylogenetic radiation should be reported regardless of whether they are polymorphic or not because they may be polymorphic in other taxa (for example, see Primmer and Ellegren, 1998). If markers monomorphic in some species are polymorphic in others, they can provide useful data about the evolution of microsatellites across broad time scales when examined in a phylogenetic context (FitzSimmons et al., 1995; Jin et al., 1996; Angers and Bernatchez, 1997; Orti et al., 1998; Streelman et al., 1998). These markers should prove useful for a wide range of studies on *Sebastes* ranging from population genetics, hybridization, and conservation/management issues, to species identification and examining paternity and kinship.

Table 2. Microsatellite Amplification and Variability Across a Range of Rockfish Speices and *Helicolenus dactylopterus**

Species	*Sma1*	*Sma2*	*Sma3*	*Sma4*	*Sma5*	*Sma6*	*Sma7*	*Sma8*	*Sma9*	*Sma10*	*Sma11*	*Sma12*
S. maliger	3	3	3	3	3	1	1	1	1	3	1	1
S. caurinus (4)	3	3	3	3	3	1	1	1	1	3	3	1
S. auriculatus (1–3)	3	3	1	3	2	1	1	1	1	3	2	2
S. pinniger (1)	2	2	2	2	2	2	2	1	2	2	2	2
S. ruberrimus (1)	2	2	3	2	2	2	2	2	2	2	2	2
S. melanops (1)	3	2	3	2	2	2	3	2	2	2	2	2
S. ciliatus (8–12)	3	3	3	0	0	1	3	1	1	3	3	0
S. borealis (5)	3	3	3	3	3	1	3	1	1	3	3	1
S. aleutianus (5)	3	3	3	3	3	1	3	1	1	3	3	1
S. paucispinis (2–5)	3	3	3	3	3	1	3	1	1	3	3	2
S. alutus (5)	3	3	3	3	3	1	3	1	1	3	3	1
Helicolenus (1)	0	2	0	2	2	2	3	2	2	3	0	2

*Sample sizes are in parentheses: 3 indicates amplifies and variable; 2, amplifies, not enough information to determine whether variable; 1, amplifies, not variable; 0, no amplification. Annealing temperatures ranged from 50°–59°C. Annealing temperatures below 56° were not attempted for *S. ciliatus* and may explain the nonamplifications for this species.

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