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PRIMER NOTE

Microsatellite markers for the heavily exploited canary (*Sebastes pinniger*) and other rockfish species

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Abstract

The isolation and characterization of nine polymorphic microsatellite loci (eight tetranucleotide and one dinucleotide) from the canary rockfish *Sebastes pinniger* are described. Polymorphism at these loci revealed from six to 28 alleles, with expected heterozygosities ranging between 0.42 and 0.88, enabling high-resolution genetic population structure investigation for this overfished species in the northeastern Pacific. They also amplify in 13 other congeneric species, providing highly variable loci for research on other rockfishes.

Keywords: canary rockfish, genus *Sebastes*, microsatellites, northeastern Pacific, population markers, *Sebastes pinniger*

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The northeastern Pacific rockfish assemblage (genus *Sebastes*) has been the primary resource for a major groundfish fishery since the early 1940s (Methot & Piner 2001). This multispecific stock has been subjected to particularly high harvest rates during the last two decades, mainly by the commercial trawl fleet, resulting in clear signs of overfishing in some of the more vulnerable species (Weinberg 1994; Parker *et al*. 2000; Love *et al*. 2002). Among these, canary rockfish (*Sebastes pinniger*) has suffered a precipitous decline with current estimates at only 8% of the virgin stock abundance (Methot & Piner 2001). Conservation and rebuilding goals are thus urgently required. These should rely not only on accurate abundance estimates but also consider the spatial distribution of genetic variation. Wishard *et al*. (1980) drew inference from an allozyme study to describe only a single continuous stock extending along the coast from northern Washington to southern California. Here, we describe the isolation of microsatellite loci in canary rockfish. The increased average polymorphism observed for this marker type will probably resolve whether genetic differentiation is evident within this northeastern Pacific stock.

Our microsatellite enrichment protocol followed that of Hoffman *et al*. (2003) and is described below in brief. Genomic DNA was extracted from ethanol-preserved fin-clips using a DNEasy Tissue Kit (Qiagen). Degenerate oligonucleotide-primed polymerase chain reaction (PCR), using the K6-MW primer (Macas *et al*. 1996), was used to generate a random set of DNA fragments between 200 and 2500 bp from throughout the *S. pinniger* genome with known flanking sequences. Microsatellite enrichment of the PCR-amplified genomic fragments employed a 3′-biotinylated $(GATA)_8$ repeat motif bound to streptavidin-coated particles (Promega). Microsatellite-enriched DNA was cloned using a TOPO TA cloning kit for sequencing (Invitrogen). We screened 470 clones and found that 42 (9%) appeared to contain microsatellites using the protocol of Cabe & Marshall (2001), which involves a PCR step using three primers (T7, T3 and the $GATA_8$ motif repeat). Positive clones show a distinctive smear, as opposed to a single band (negative), when electrophoresed on a 2% agarose gel. Thirty-four of these were sent to the Nevada Genomics Center (Reno, NV, USA) for automated sequencing and 22 contained repeat motifs.

We utilized VECTOR NTI software (http://www.informaxinc.com) to design primers to amplify 19 unique microsatellite loci. The PCR was carried out in a Peltier Thermal Cycler (MJ Research™) in a 5-μL reaction containing 1 μL of DNA template (25–100 ng), 1× polymerase buffer (500 mM KCl, 100 mM Tris-HCl, 1% Triton® X-100), 1.5 mM $MgCl_2$, 200 μM each dNTP, 0.5 μM each primer (the

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Table 1 Microsatellite loci for canary rockfish (*Sebastes pinniger*)

Locus	Primer sequences (5′–3′)	Repeat motif	Annealing temperature (°C)	N	Size range	A	H_O	H_E	GenBank Accession no.
*Spi*4	*GTCAGAGTTACATAGCGTGCCCT GCACTATGGAACTGTGATTCTGGA	$(GATA)_{11}$	58	83	169–217	11	0.86	0.85	AY192599
*Spi*6	*AGTGGAAGTGAACACGTAGGTTAG CACTATGGAACTGTGATGCTGG	$(GATA)_{21}$	58	108	106–210	28	0.94	0.88	AY192600
*Spi*7	*CTGTCTTTGTCACTGTAATCATAGTCA GATCTGGAGTCAGATGGATAGATG	$(GATA)_{24}$	58	12	136–234	10	0.07	0.88	AY192601
*Spi*9	*CATTCTTACGCACCGATCTG GAGTTTTCTTCATCTCCTTGTATTTT	$(GATA)_5$– $(GATA)_{18}$	58	32	124–186	10	0.11	0.88	AY192602
*Spi*10	*TTTGATGGCCTGAAACTGAG GTTCAAACACACAGTAGCTAAACTATC	$(GATA)_{17}$	58	109	115–155	10	0.77	0.82	AY192603
*Spi*12	*GGGAGTATGAGAGAGGATCATGC CAATACGCCTCCAAGCTAGATC	$(GA)_7$	58	137	77–99	6	0.40	0.39	AY192604
*Spi*14	*CCAGCAGCTTGGATAGATAGTTAG GCTGGAAATACATTACACTGTTTAGTC	$(GATA)_{10}$– $(GACA)_{10}$– $(GATA)_{12}$	55	12	267–317	8	0.91	0.77	AY192605
*Spi*17	*TGTTGGTTAATTACATGCTGGA TATTCCCAGCAGCTTGGATA	$(GATA)_{11}$– $(GACA)_{12}$– $(GATA)_{11}$	55	12	298–342	9	0.91	0.83	AY192606
*Spi*18	*GTACAAGAAGTTAAAAAGCAAGTTGCAG GCGTGTTTGCACTAACCTTTGT	$(GATA)_5$– $(GATA)_{10}$– $(GATA)_8$– $(GATA)_6$	55	25	230–328	8	0.76	0.79	AY192607

*5′ fluorescent-labelled primer.
N, No. of individuals genotyped to estimate no. of alleles (A), observed (H_O) and expected (H_E) heterozygosity. Multiplex polymerase chain reaction was successful for *Spi*4, *Spi*6, *Spi*10 and *Spi*12, enabling characterization of greater sample sizes. A dash in the repeat motif indicates an intervening sequence (imperfect repeat).

forward primer was fluorescently labelled) and 0.1 U of *Taq* DNA polymerase (Promega). The temperature profile consisted of an initial denaturing step at 94 °C for 3 min, 30 cycles at 94 °C for 30 s, 55–58 °C for 30 s and 72 °C for 30 s and a final extension at 72 °C for 2 min (Table 1). Amplified fragments were electrophoresed in a BaseStation (MJ Research™) using 5% polyacrylamide gels and analysed using CARTOGRAPHER™ software.

Nine primer pairs were polymorphic and amplified consistently, eight of which were tetranucleotide repeats and one was a dinucleotide repeat (Table 1). Five loci were monomorphic and the remaining five either gave no PCR product or amplified inconsistently despite adjusting $MgCl_2$ (1.0–2.5 mM) or dNTP (80–200 μM) concentrations and using an annealing temperature gradient. Analysis of genetic variability was performed utilizing FSTAT (Goudet 1995, 2001). The number of alleles varied between six and 28 and expected heterozygosities ranged from 0.39 to 0.88 (Table 1). Significant departures from Hardy–Weinberg equilibrium ($P = 0.0056$) after Bonferroni correction were observed for *Spi*7 and *Spi*9, probably as a result of unamplifiable (null) variants. Genotypic disequilibrium was not significant in any pairwise comparison after multiple tests adjustment, providing no evidence of linkage among any of these loci.

Canary rockfish primers were screened on 13 additional rockfish species: *S. nebulosus* (china), *S. ruberrimus* (yelloweye), *S. caurinus* (copper), *S. crameri* (darkblotched), *S. diploproa* (splitnose), *S. mystinus* (blue), *S. maliger* (quillback), *S. nigrocintus* (tiger), *S. miniatus* (vermillion), *S. melanops* (black), *S. flavidus* (yellowtail), *S. auriculatus* (brown) and *S. zacentrus* (sharpchin). The PCR cocktails and conditions employed were the same as in canary rockfish. Most showed homologous polymorphic products in at least two species, with the exception of *Spi*17 that did not amplify in any other species (Table 2). These loci provide the first microsatellites isolated from *S. pinniger* and are a source of variable markers for studies on other rockfishes.

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Table 2 Polymerase chain reaction analysis of canary rockfish primers screened in 13 congeneric species

		*Spi*4		*Spi*6		*Spi*7		*Spi*9		*Spi*10		*Spi*12		*Spi*14		*Spi*17		*Spi*18	
	N	A	Size	A	Size	A	Size	A	Size	A	Size	A	Size	A	Size	A	Size	A	Size
China	9	2	169–189	5	111–151	0	—	0	—	4	119–135	4	95–107	0	—	0	—	0	—
Yelloweye	9	6	169–225	8	135–183	0	—	0	—	7	119–151	3	99–103	0	—	0	—	0	—
Copper	9	9	169–237	9	111–175	0	—	0	—	3	123–131	3	81–109	0	—	0	—	8	235–291
Darkblotched	9	8	153–221	4	103–123	0	—	0	—	6	135–167	2	75–93	1	223	0	—	0	—
Splitnose	9	4	165–221	8	139–235	0	—	0	—	6	115–135	3	73–95	0	—	0	—	0	—
Blue	9	3	169–189	6	139–179	6	148–196	7	125–185	3	103–115	1	97	0	—	0	—	0	—
Quillback	9	4	169–185	7	107–151	0	—	0	—	5	123–147	4	105–123	0	—	0	—	2	247–251
Tiger	9	5	157–177	4	119–147	0	—	0	—	7	119–143	3	73–97	4	207–219	0	—	0	—
Vermillion	9	9	173–213	3	147–163	3	152–180	3	129–157	6	115–151	2	95–97	0	—	0	—	3	283–323
Black	9	4	165–197	6	99–143	9	128–192	8	101–169	4	103–119	2	97–99	0	—	0	—	0	—
Yellowtail	2	2	165–201	2	107–111	0	—	3	101–165	4	115–131	2	95–97	0	—	0	—	0	—
Brown	2	2	173–177	2	183–187	0	—	2	149–165	3	119–127	2	79–121	0	—	0	—	0	—
Sharpchin	2	0	—	2	175–199	0	—	0	—	1	127	1	85	0	—	0	—	0	—

The no. of fish genotyped (*N*), no. of alleles (A) and their size range (in bp) are presented.

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