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PRIMER NOTE

Development of 11 microsatellite loci for population studies in the swordfish, *Xiphias gladius* (Teleostei: Scombridae)

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Abstract

Eleven microsatellite loci were developed in the swordfish, *Xiphias gladius*, to examine population structure. Loci were amplified in samples from two geographically distant populations (Ecuador and Mediterranean Sea). Observed heterozygosity ranged from 0.150 to 0.960. Most loci conformed to Hardy–Weinberg expectations with the exception of locus Xg-59, which had a heterozygote deficit caused probably by null alleles. Locus Xg-402 did not show significant divergence between the populations. The remaining nine loci showed statistically significant spatial heterogeneity (genic differentiation) between the two populations, making them useful for future studies of population structure in swordfish.

Keywords: microsatellites, swordfish, Xiphias gladius

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The swordfish, *Xiphias gladius*, is a highly migratory pelagic species exploited heavily as a commercial fishery worldwide. Analysis of mitochondrial DNA (mtDNA) control region sequences have shown populations to be subdivided between ocean basins (Alvarado-Bremer *et al*. 1995; Rosel & Block 1996) and more recently, within basins (Chow *et al*. 1997; Reeb *et al*. 2000). Microsatellite loci can supplement and enhance conclusions gleaned through mitochondrial studies. Rapid evolution of these loci allows detection of additional population structure. Biparental inheritance combines both male and female ancestry and results can be compared to mtDNA's history of matrilines for evidence of sex-biased dispersal. Here we describe the development and characterization of 11 microsatellite loci for the study of population structure in swordfish.

The DNA of a single Pacific swordfish (from Hawaii) was isolated from frozen tissue using methods described in Rosel & Block (1996) then digested with three restriction enzymes (*Hin*dII, *Rsa*I and *Hae*III, Boehringer Mannheim) to produce blunt-end fragments which were then isolated on a 1% low melting point agarose gel. Fragments between 150 and 500 base pairs (bp) were excised and purified using the Wizard PCR Prep kit (Promega Corp.). Fragments were ligated into dephosphorylated pUC18 cut with *Sma*I to produce a partial genomic library. The ligated vector was transformed into XL1 Blue Epicurean Coli Supercompetent Cells (Stratagene) and plated on Luria broth media selecting for ampicillian-resistant recombinants. Colonies were transferred to replicate Hybond-N filters (Amersham).

Eight single-stranded oligonucleotides composed of di- or tri-nucleotide repeats (CA_{20}, AG_{20}, AAT_{24}, AAG_{24}, AAC_{24}, CCT_{24}, CCA_{24}, TGA_{24}) were purchased (Operon Technologies, Inc.) and end-labelled with alpha ^{32}P. These probes were used to detect repetitive sequences in the library using autoradiography. Positive clones were harvested and cultured. Plasmid DNA was extracted using the Wizard Plus Miniprep Kit (Promega Corp.). Each clone was sequenced to verify the presence of microsatellites and to characterize flanking regions for primer design. The Prism DNA Sequencing Kit and ABI 373 A automated sequencer both purchased from Applied Biosystems, Inc. were used to sequence 403 clones and 150 microsatellite loci were identified. Eleven loci were chosen based on the presence of long, uninterrupted repeat units (> five repeats) and primers were designed and purchased (Operon Technologies, Inc.). Once polymerase chain reaction (PCR) conditions were optimized, fluorescently labelled primers (6-FAM, TET, or HEX) were purchased.

DNA samples from two distant populations, Ecuador (n = 21) and the Mediterranean Sea (n = 26), were examined. PCR reactions contained 10 mM Tris-HCL pH 8.3, 50 mM KCL, 1.5 mM $MgCl_2$, 0.2 mM dNTPs and 10–20 ng of genomic DNA in a final volume of 20 μL. Final

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Table 1 Primer sequences and PCR conditions for 11 microsatellite loci in *Xiphias gladius*

Locus	Motif sequenced	Primers (5′– 3′)	T_a (°C)	Size range (bp)	GenBank Accession no.
Xg-55	$(GT)_{35}$	GGAAAAAGGGGAGTCCAGC ACACGTAGTTGTTACAATTCAC*	55	86–176	AF545835
Xg-56‡	$(CA)_{16}$	ATGGGAAACATCTGGTCAC ACTTTCTTATTCTGTTTCTGTCC*	69	116–154	AF545836
Xg-66‡	$(CA)_{11}$	TTTTCACCTTGTCAGTGTGCG ACAGACGTATACAACCACCTG*	69	126–144	AF545837
Xg-59	$(AT)_5 + (GT)_{16}$	TTACCAAAGCTGTCCGGTAT GAGTGACTGACTGGGGAC*	55	110–178	AF545838
Xg-75	$(CA)_{53}$	CAAACTAACATTTACACAGTCAG TTTGGGTTAGCAGCTGCCAG*	58	132–290	AF545839
Xg-144†	$(GGA)_7$	TTCCACTCATACCTCTGTCATC ACCACATCCATTATAGCATGTTG*	69	157–175	AF545840
Xg-166†	$(CAA)_7$	GTGAGTCATGTGTCAGTGTGG CCTCTGCCTGAAATACTTCAG*	69	130–148	AF545841
Xg-379	$(ATG)_{11}$	GGATGTAGCCTACAACTCA TTACAAATCAGTCCTACAGAG*	59	105–153	AF545842
Xg-394§	$(TCC)_9$	AGCGACAAACAGACCTGCCA GAGGAAACCGGGGCTTCTAC*	66	141–147	AF545843
Xg-396§	$(CCT)_{10}$	TCAGTCAGAAAGCTCCGAC TCCGCTGCCACCACCACT*	66	117–135	AF545844
Xg-402§	$(TCC)_5 + (CTT)_2$	GCGATTCAGGAGATTCCTAAC ATTAACTCGTCATTCAACGGC*	66	186–194	AF545845

*Indicates fluorescent primer. †, ‡, §: Three different mulitplex sets of primers compatible for PCR.

primer concentrations were 0.3 μM except for Xg-144 and Xg-166, which required 0.06 and 0.07 μM, respectively, to prevent excessive fluorescing and the masking of other loci on the gel. Table 1 includes primer sequences, annealing temperatures and multiplexing compatibility of 11 primer pairs.

Amplifications were performed in a Perkin Elmer Cetus 480 DNA Thermal Cycler. The first three loci in Table 1 were amplified with 1 unit of *Taq* polymerase (Promega Corp.). PCR thermal conditions were as follows: initial denaturation step of 5 min at 95 °C, followed by 33 cycles of 1 min at 94 °C, 1 min at the T_a listed in Table 1 and 2 min at 72 °C. The remaining eight loci were multiplexed. These reactions contained the same components as above except 0.5 units of Ampli*Taq* Gold™ (Perkin Elmer Corp.) hot-start polymerase was used. A stepdown cycling regime (Hecker & Roux 1996) was preformed using the following conditions: initial denaturation for 12 min at 95 °C, followed by three cycles of 1 min at 94 °C, 2 min at T_a and 1 min at 72 °C. The T_a was decreased by 3 °C and three more cycles were repeated. This continued until six rounds of the three-cycle routine had been completed. An additional 17 cycles of the final step were preformed (35 total cycles).

Fragments were separated on an ABI 373 A automated sequencer. Alleles were scored using a comigrating size standard (Genescan-500, Perkin-Elmer Corp.) and identified using the GENESCAN 672 and GENOTYPER software (Applied Biosystems, Inc.). A wide range of scorable polymorphism was found for these loci. Table 2 lists the number of alleles, observed and expected heterozygosity, and genic differentiation between the two populations by locus calculated using GENEPOP version 3.2 (Raymond & Rousset 1995). The expected heterozygosity was often larger than the observed. Locus Xg-59 had a significant heterozygote deficiency in both populations and may suffer from nonamplifying alleles (Pemberton *et al*. 1995). The probability test for Hardy–Weinberg could not be perfomed on Xg-402. This is because only one allele was present in the Ecuador population. Two alleles were detected in the Mediteranean but only a single copy represented the second allele. Sequencing the plasmid clone of Xg-402 showed it contained two trimeric repeat units. However, fragment analysis of the amplified alleles suggested Xg-402 evolves as a tetramer. There is the possibility that the primers may amplify a different target sequence than what was cloned; however, only a single sequence in the genome is targeted. Analysis of genic differentiation between the two populations revealed a significant divergence for every locus except Xg-402. Neither Xg-59 nor Xg-402 may be ideal for population structure studies. We are currently using all 11 loci to examine genetic structure in a global collection of swordfish samples.

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Table 2 Allele statistics for each locus in each population

	Ecuador					Mediterranean					Genic differentiation	
Locus	*n*	*a*	H_O	H_E	P_{HW}	*n*	*a*	H_O	H_E	P_{HW}	*P*	SE
Xg-55	20	20	0.900	0.960	0.459	26	16	0.923	0.904	0.244	0.0016	0.0002
Xg-56	21	11	0.876	0.876	0.169	26	9	0.769	0.848	0.151	0.0010	0.0001
Xg-59	21	10	0.238	0.852	0.000	26	9	0.308	0.865	0.000	0.0000	0.0000
Xg-66	18	8	0.556	0.839	0.005	26	6	0.577	0.677	0.321	0.0058	0.0003
Xg-75	21	21	0.667	0.948	0.001	26	17	0.808	0.888	0.056	0.0000	0.0000
Xg-144	20	3	0.650	0.610	0.804	26	3	0.615	0.658	0.631	0.0097	0.0003
Xg-166	21	5	0.667	0.733	0.656	26	5	0.654	0.788	0.371	0.0356	0.0009
Xg-379	20	10	0.700	0.840	0.143	26	6	0.269	0.342	0.004	0.0000	0.0000
Xg-394	21	3	0.238	0.220	1.000	26	1	—	—	—	0.0150	0.0003
Xg-396	21	6	0.714	0.619	0.677	26	4	0.654	0.654	0.574	0.0000	0.0000
Xg-402	21	1	—	**	—	26	2	0.150	0.140	**	1.0000	0.0000

**Not enough alleles to perform HW test. Number of individuals sampled (*n*), number of alleles (*a*), observed heterozygosity (H_O), expected heterozygosity (H_E), Hardy–Weinberg probability test (P_{HW}), and genic differentiation between populations (probability, *P*, and standard error, SE).

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