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PRIMER NOTE

Isolation and characterization of eight microsatellite loci for white hake (*Urophycis tenuis*)

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Abstract

Eight tetranucleotide microsatellite loci were characterized for white hake, *Urophycis tenuis*, a commercially exploited demersal gadoid fish distributed in the Northwest Atlantic. The eight loci are polymorphic with number of alleles and observed heterozygosities ranging from seven to 40 and from 0.544 to 0.978, respectively, in samples of between 39 and 47 individuals collected from throughout the species range. These loci were developed for population genetic studies.

Keywords: microsatellites, *Urophycis tenuis*, white hake

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White hake (*Urophycis tenuis*, Mitchill 1815, Gadidae) is a demersal fish species distributed in the Northwest Atlantic from southern Labrador to Cape Hatteras (Musick 1974; Scott & Scott 1988). Within this latitudinal range, the species is most abundant in the area that extends from the southern Grand Banks and the Gulf of St Lawrence to the Scotian Shelf, and the Gulf of Maine (Kulka *et al*. 2005). White hake are most common in soft bottom areas at depths from 50 to *c*. 800 m on the continental shelf and upper continental slope waters. Although, historically, one of the most important fisheries in the region, in particular in the southern Gulf of St Lawrence (Kulka *et al*. 2005), the fishery for white hake has been under a moratorium since the mid-1990s. Critically low abundance estimates in the early 2000s has prompted initiatives for the species to be considered by the Committee for the Status of Endangered Wildlife in Canada (COSEWIC). Little information exists, however, on the biology and life history of the species throughout its range and in particular on the spatial distribution of population or stock components (Melendy *et al*. 2005). Here, we present eight new microsatellite DNA loci specifically developed to examine the population genetic structure of white hake, *U. tenuis* in the Gulf of St Lawrence, the southern Grand Banks, the Scotian Shelf and the Bay of Fundy, in the Northwest Atlantic.

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Whole genomic DNA was extracted from ethanol-preserved hake fin-clip tissue using a standard phenol–chloroform method. DNA from one individual was used to create microsatellite-enriched libraries for CATC and GACA repeats, following previously published protocols (Hamilton *et al*. 1999; Diniz *et al*. 2004). The microsatellite libraries were cloned using pDrive Cloning Vector (QIAGEN), transformed into QIAGEN EZ Competent Cells and plated on imMedia Amp Blue agar (Invitrogen). Positive colonies were screened for suitably sized inserts (400–1000 bp) by direct polymerase chain reaction (PCR) amplification of colony picks using M13 primers under standard PCR conditions. Aliquots of 3 μL of the 20 μL total volume of each PCR amplification were electrophoresed on agarose for size screening. The remaining 17 μL of PCRs showing appropriate-sized fragments was purified using Montage PCRμ96 microwell filter plates (Millipore), in preparation for sequencing using a Beckman Coulter Quick Start sequencing kit and M13 primers. Sequencing was performed on CEQ 8000 (Beckman Coulter). PRIMER 3 software (Rozen & Saletsky 2000) was used for primer design.

We tested the eight loci on a maximum of 47 *U. tenuis* individuals (up to 12 chosen from each of four regions: Newfoundland, Scotian Shelf, Bay of Fundy and Gulf of St Lawrence). DNA was extracted from 1 × 1 mm pieces of finclip using the HotSHOT alkaline lysis protocol (Truett *et al*. 2000). Individuals were genotyped using PCR

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Table 1 Characterization of eight polymorphic microsatellite loci for white hake (*Urophycics tenuis*, Mitchill 1815): T_a, annealing temperature (°C); number of alleles and (sample size, *N*), and allele size range; H_O and H_E, observed and expected heterozygosities, respectively; HWE within-samples (top) and overall (bottom) *P* values; and GenBank Accession nos

Locus	Primer sequence (5′–3′)	Repeat motif	T_a	Size of cloned allele (bp)	No. of alleles/ sample size (*N*)	Allele size range	H_O	H_E	HWE	GenBank Accession no.
Ute1	F: GGGGCGATTTAGACCAAAGT R: GTGGGTCTGTCCAGGATGTT	$(GACA)_8$	54	157	7 (39)	148–172	0.8810	0.7768	0.98 0.99	DQ 294931
Ute12	F: CATCCTCTCCGAACACTGGT R: GCAGAAGCACCGGTTACATT	$(CTGT)_{13}$	62	190	13 (44)	166–214	0.9070	0.8947	0.42 0.63	DQ 294932
Ute13	F: CCCTGCACTCCCTCCTCT R: AGAGGGCCGGGGCTATAT	$(CTGT)_{10}$	65	128	10 (40)	126–170	0.8750	0.8509	0.80 0.55	DQ 294933
Ute19	F: GTCTGCCTGCCTGTCTAT R: GCAAAGGAAGAGAAGAAAAA	$(CTGT)_{14}$	54	182	7 (46)	156–180	0.7826	0.7413	0.67 0.65	DQ 294934
Ute22	F: CTGTTTACCTCCGCTTTTAG R: ATCACTGCAAGAGACATGAT	$(CTGT)_9$	60.5	264	24 (45)	222–362	0.8000	0.9039	0.001 0.001	DQ 294935
Ute27	F: TTTGTCGGTGCACTGTTACC R: CCGGTTCTTCGTCTTTCCAC	$(GACA)_{12}$	58	195	15 (42)	158–214	0.7674	0.8577	0.011 0.017	DQ 294936
Ute34	F: ACTGACAGACGGGTCGATG R: ACCTGAATGCTGCTGATGTG	$(GACA)_{11}$	68	119	9 (45)	105–130	0.5435	0.6636	0.171 0.054	DQ 294937
Ute35	F: CCCGAGGGTAACTCGATTG R: CGCTATTGGAGAGGTGAAGC	$(GACA)_{12}GGCA(GACA)_7$	58	229	40 (47)	150–460	0.9783	0.9689	0.345 0.283	DQ 294938

amplifications of 10-μL volumes containing 0.5–1 μL of extracted DNA, 2.5 mm $MgCl_2$, 50 μm of each dNTP, 0.5 U *Taq* polymerase (Sigma), 1 μm of each primer (forward primers were 5′ end-labelled with IR700 or IR800 dye) and 1× PCR buffer. PCRs included: 94 °C for 3 min, followed by 30 cycles at 94 °C for 45 s, primer-specific T_a (Table 1) for 30 s and 72 °C for 30 s. Reactions were run in MJ Research thermocyclers and imaged on a LI-COR IR2 DNA Analyser model 4200.

Gels were scored by eye and with SAGA automated microsatellite software (LI-COR). Tests for departure from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium were conducted with FSTAT version 2.9.3.2 (Goudet 1995). Observed and expected heterozygosities are reported (Table 1). There was no evidence for departures from HWE (either within samples or overall) for six out of the eight loci; the exceptions being loci Ute 22 ($P < 0.001$) and Ute 34 ($P < 0.011$) (Table 1). No evidence of null alleles or large allele dropout was detected when the data were analysed using MICRO-CHECKER (Goudet *et al*. 1995). Similarly, there was no evidence of genotypic disequilibrium between any pairs of loci even before correcting for multiple tests ($P > 0.1046$, $\alpha_{(95\%)} = 0.0017$ with 28 pairwise comparisons). Preliminary (due to small sample size) tests for population differentiation (not assuming random mating within samples, i.e. log-likelihood *G*-test, Goudet *et al*. 1995) not surprisingly indicated that, though markers may differ in their ability to discriminate among (these) populations ($P \leq 0.01$ to $P \leq 0.82$), this ability increases when all markers are considered jointly ($P \approx 0.002$). Stronger tests of markers' ability for detecting population differentiation must, however, await the scoring of further samples of mature individuals in spawning condition. Primer sequences were deposited in GenBank [(Accession nos DQ 294931–DQ 294938 (Table 1)]. Although no cross-species amplification tests were conducted with these markers, markers developed for Atlantic cod, *Gadus morhua* (Gmo8, Gmo19, Gmo34, Gmo37, Miller *et al*. 2000; Gmo2 and Gmo132, Brooker *et al*. 1994), and for walleye Pollock, *Theragra chalcogramma* (Tch5, Tch11, Tch14, Tch22; O'Reilly *et al*. 2000), were initially tested on our samples of white hake but failed to amplify successfully.

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