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TECHNICAL NOTE

Isolation and characterization of dinucleotide microsatellites in greater amberjack, *Seriola dumerili*

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Abstract Thirteen nuclear-encoded dinucleotide microsatellites were characterized from a genomic DNA library of greater amberjack, *Seriola dumerili*. The microsatellites include 12 perfect-repeat motifs and one imperfect-repeat motif. The number of alleles at the 13 microsatellites among a sample of 29 fish ranged from 3 to 25; gene diversity (expected heterozygosity) ranged from 0.296 to 0.948, while observed heterozygosity ranged from 0.276 to 0.897. Following Bonferroni correction, genotypes at all 13 microsatellites fit expectations of Hardy-Weinberg equilibrium. One pairwise comparison of microsatellites deviated significantly from expectations of genotypic equilibrium, suggesting that these two microsatellites may be linked. Greater amberjack support commercial and recreational fisheries along both the Atlantic and Gulf coasts of the U.S. and represent a species with potential for worldwide aquaculture. The microsatellites developed will be useful for conservation and population genetic studies of 'wild' and domesticated populations of greater amberjack.

Keywords Greater amberjack · Genomic library · Microsatellites · *Seriola dumerili*

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Greater amberjack, *Seriola dumerili*, is a large, reef-associated member of the family Carangidae that has a circumglobal distribution typically in subtropical-temperate ocean waters (Manooch and Potts 1997). Increased commercial and recreational fishing pressure has caused concern over the status of this species along the Atlantic and Gulf of Mexico coasts of the U.S. (Thompson *et al*. 1999), and the species is presently listed as overfished in the Gulf (NMFS 2006). There also is a developing worldwide interest in aquaculture of greater amberjack due to its relatively rapid growth rate (Thompson *et al*. 1999) and high market demand driven by its desirable flesh quality (Nakada 2000). In this note, we report development of polymerase chain-reaction (PCR) primers for 13 nuclear-encoded dinucleotide microsatellites from a greater amberjack genomic DNA library. Nuclear-encoded microsatellites are codominantly inherited markers that have become popular in a variety of fisheries applications due in part to their easy amplification with PCR, high levels of allele polymorphism, and abundant genome-wide distribution (Chistiakov *et al*. 2006).

A more detailed account of the genomic library development can be found in Renshaw *et al*. (2006). Briefly, genomic DNA was digested separately with *Dpn*II and *Apo* I (New England BioLabs), ligated into a *Bam*HI- or *Eco* RI- (New England BioLabs) digested and dephosphorylated (Calf Intestinal Alkaline Phosphatase, New England BioLabs) pBluescript vector (Stratagene), using T4 DNA ligase (New England BioLabs), and transformed into XL10-Gold Ultra-competent cells (Stratagene). Recombinant colonies were picked using a GENETIX Q-BOT, inoculated into 384 well plates that contained 50μl of LB freezing media [36 mm K_2HPO_4, 13.2 mm KH_2PO_4, 1.7 mm

sodium citrate, 0.4 mm $MgSO_4$, 6.8 mm $(NH_4)_2SO_4$, 4.4% (v/v) glycerol, 100 μg/mL ampicillin, LB], and incubated overnight at 37°C prior to freezing at – 80°C. Clones were spotted and fixed in a 4 × 4 array onto 22.5 × 22.5 cm Hybond nylon membranes (Amersham), with each clone being spotted twice to eliminate false positives.

The genomic library was probed with two [gamma] ^{32}P-labelled oligonucleotides: $[CA]_{13}$ and $[GA]_{13}$. A total of 106 positive clones were screened. Plasmid DNA was isolated (alkaline lysis) with a BioRobot 8000 (Qiagen). Miniprep DNA was quantified, normalized, and both strands sequenced, using M13 forward and reverse sequencing primers and ABI BigDye Terminator v3.1. Products were electrophoresed on an ABI 3100 DNA Analyzer (Applied Biosystems). SEQUENCHER (Gene Codes Corporation) was used for DNA sequence base calling and vector trimming. A total of 50 complete sequences containing microsatellite arrays were obtained from the positive clones. Twenty primer pairs, flanking dinucleotide microsatellite arrays, were designed using AMPLIFY 1.2 (Engels 1993) and NETPRIMER (http://www.premierbiosoft.com/netprimer).

Unlabelled PCR primers were purchased from Invitrogen (Carlsbad, CA) and tested for amplification by screening two individuals obtained from John's Island, South Carolina. PCR amplifications were performed with a PTC-200 thermocycler (MJ Research) in 10μl reaction volumes containing 100 ng DNA, 1x PCR buffer (50 mm KCl, 10 mm Tris, 1% Triton-X 100), 0.1 U *Taq* DNA polymerase (Gibco-BRL), 0.5μm of each primer,200μm of each dNTP, and 1 mm $MgCl_2$. PCR conditions consisted of an initial denaturation at 95°C for 3 min, followed by 38 cycles of denaturation at 95°C for 30 sec, annealing at 45–65°C for 45 sec, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide, and visualized under UV light. Following the establishment of optimal primer annealing temperatures, microsatellite arrays were tested for polymorphisms with an additional seven individuals from John's Island. A total of 13 microsatellite repeats were chosen for

Table 1 Summary data for dinucleotide microsatellites developed from the greater amberjack, *Seriola dumerili*

Msat	GenBank[a]	Primer sequence (5'–3')[b]	Repeat sequence[c]	T_A^d	N^e/N_A^f	Size Range[g]	H_E/H_O^h	P_{HW}^i
*Sdu*29	DQ883568	**CCTTGCCATACCGATGCCAG*** GACTGCTCTGCCTGCTTGTTG	$(GA)_{14}$	60	29/11	311–377	0.847/0.793	0.487
*Sdu*31	DQ883569	**CACATTTGGACGGATTCTTC*** GCTGTTATCCTCCAGTGCT	$(CA)_{14}$	56	29/7	84–98	0.738/0.759	0.650
*Sdu*32	DQ883570	**CCTGTGAGAGCATTTGGTAT**** GTGCTTGTCTCTTCTGTCAT	$(CA)_{17}$	53	29/21	99–177	0.948/0.862	0.231
*Sdu*33	DQ883571	**CCTCTAACAGCCACAATCA**** GCTCTTCACCTTCCTCATA	$(GA)_{13}$	56	29/3	202–208	0.296/0.276	0.598
*Sdu*34	DQ883572	**CCTTGTGTTGTATCTGCTGTAA***** GGAATAAACCTCGTCTGTCA	$(GA)_{20}$	56	29/8	84–118	0.778/0.517	0.028
*Sdu*36	DQ883573	**CTGTTATGAAGCAGTGAAGAGG*** GGACCATCCTGCTCTGACA	$(GA)_{23}$	56	29/9	200–226	0.815/0.690	0.132
*Sdu*37	DQ883574	**CCTCTAATGGACTTCAGCG***** GGTTATTTTGAGAGCCGTC	$(CA)_{16}$	53	29/25	160–278	0.889/0.828	0.448
*Sdu*39	DQ883575	**AGTGGCTTCTGCTGCTGT**** CGTGTGCGTGCTTGTAAA	$(CA)_{16}$	56	29/6	154–180	0.338/0.345	0.688
*Sdu*40	DQ883576	**CGATGCTTTCAACTCCGACACAC***** CCATCCTTCATCAGCAACAACATCC	$(CA)_{17}$7 bp$(CA)_5$	64	28/9	197–235	0.825/0.893	0.884
*Sdu*41	DQ883577	**AGCGTGGACAGTTTATGG**** GTCTGTTTACTGGTCGCA	$(CA)_{18}$	53	29/10	96–130	0.636/0.724	0.548
*Sdu*43	DQ883578	**CAGAAGAAGAGCGTGGTGGAGAG** CAGAAGAAGAGCGTGGTGGAGAG***	$(CA)_{28}$	60	29/11	276–298	0.835/0.828	0.654
*Sdu*44	DQ883579	**GGTAATGGGAGGTGTGAGTGT**** CCTTCTCCTGTTAATCCATCTCC	$(GA)_{12}$	56	29/3	114–124	0.402/0.379	0.574
*Sdu*46	DQ883580	**GCAGTGTGAGCCATACATTAC***** CTACAGGACAAAAGCCATT	$(GA)_{30}$	53	29/13	217–259	0.817/0.897	0.628

[a] GenBank accession numbers for clone sequences; [b] Primer sequences are forward (top) and reverse (bottom); [c] Repeat sequence indicates repeat motif; [d] T_A is annealing temperature in °C; [e] N is the number of individuals assayed; [f] N_A is the number of alleles detected; [g] Size Range refers to alleles thus far uncovered; [h] H_E and H_O are expected and observed heterozygosity, respectively; [i] P_{HW} represents the probability of deviation from Hardy-Weinberg expectations. The fluorescently labelled primer (FAM*, HEX**, or NED***) is in bold

further screening. Length of cloned alleles ranged in size from 90 to 312 base pairs, and optimal annealing temperatures ranged from 53°C to 64°C (Table 1).

For further screening, one primer from each pair was labelled with one fluorescent label of Set D (Applied Biosystems): FAM, HEX, or NED. DNA was extracted from a total of 29 individuals from John's Island, South Carolina; PCR products were run on an ABI 377 automated sequencer. Alleles were sized using the GENESCAN®-400 HD ROX Size Standard (Applied Biosystems); allele sizing and calling were performed using GENESCAN® 3.1.2 and GENOTYPER® version 2.5 software. Genetic variability of the microsatellite markers was measured by the number of alleles, gene diversity (expected heterozygosity), and observed heterozygosity. Fisher's exact test, as implemented in GDA (Lewis and Zaykin 2001), was used to test significance of departure from Hardy-Weinberg equilibrium (genotype) expectations at each microsatellite and departure from genotypic equilibrium at pairs of microsatellites.

Summary data are presented in Table 1. The number of alleles detected per microsatellite ranged from three (*Sdu* 33 and *Sdu* 44) to 25 (*Sdu* 37). Expected heterozygosity ranged from 0.296 (*Sdu* 33) to 0.948 (*Sdu* 32), while observed heterozygosity ranged from 0.276 (*Sdu* 33) to 0.897 (*Sdu* 46). Following Bonferroni correction (Rice 1989), genotypes at all 13 microsatellites fit expectations of Hardy-Weinberg equilibrium. One pairwise comparison of microsatellites (*Sdu* 32/*Sdu* 34) deviated significantly from genotypic equilibrium ($P = 0.0000$), suggesting that these two microsatellites may be linked. The 13 microsatellites developed in this work will prove useful for conservation and population genetic studies in both 'wild' and cultured populations of greater amberjack.

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References

Chistiakov DA, Hellemans B, Volckaert FAM (2006) Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics. Aquaculture 255: 1–29

Engels WR (1993) Contributing software to the Internet: the AMPLIFY program. Trends Biochem Sci 18:448–450

Lewis PO, Zaykin D (2001) GENETIC DATA ANALYSIS:computer program for the analysis of allelic data. Version 1.0 (d16c). Free program distributed by the authors via the Internet at http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php

Manooch CS, Potts JC (1997) Age, growth and mortality of greater amberjack from the southeastern United States. Fish Res 30:229–240

Nakada M (2000) Yellowtail and related species culture. In: Stickney R (ed) Encyclopedia of aquaculture. Wiley. London

NMFS (2006) Annual report to Congress on the status of US fisheries–2005. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Silver Spring, MD, 1–20

Renshaw MA, Patton JC, Rexroad CE, Gold JR (2006) PCR primers for trinucleotide and tetranucleotide microsatellites in greater amberjack, Seriola dumerili. Molecular Ecology Notes (in press)

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Thompson BA, Beasley M, Wilson CA (1999) Age distribution and growth of greater amberjack, *Seriola dumerili*, from the north-central Gulf of Mexico. Fish Bull 97:362–371