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PRIMER NOTES

Polymorphic microsatellite DNA markers in red drum (*Sciaenops ocellatus*)

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The red drum (*Sciaenops ocellatus*, family Sciaenidae) is a marine fish species that is distributed in estuaries and nearshore habitats along the northern Gulf of Mexico and southeastern Atlantic coasts of the USA. Successful recruitment of red drum and co-occurring sciaenid fish species depends on estuarine habitats that serve as nursery areas for larvae. One goal of our research program is to determine whether fine-scale genetic structure of larvae is associated with heterogeneity in nursery habitat. Resolution of fine-scale structure in this case will require several highly polymorphic DNA markers that can be reliably scored from larvae. Microsatellite DNA markers have proven useful for studying fine-scale genetic structure and parentage analysis of fish larvae (Ruzzante *et al*. 1996; Jones & Avise 1997). This study describes 30 microsatellite markers developed for red drum, 26 of which are polymorphic. Each locus was also tested for amplification of microsatellites in three co-occurring sciaenid species.

Red drum genomic DNA, isolated by phenol–chloroform extraction, was digested with *Dpn*II (New England Biolabs) and size-selected in the range of 200–1000 bp. Fragments were purified using Prep-A-Gene (Bio-Rad) DNA purification kits, ligated into pUC18, and heat-shock transformed into *Escherichia coli* strain DH5α following Sambrook *et al*. (1989). The resulting library, consisting of 5280 clones, was screened separately with three classes of synthetic oligonucleotide probes: (i) di-($[CA]_{15}$, $[GA]_{15}$); (ii) tri-($[CCT]_7$, $[ATT]_7$); and (iii) tetranucleotide ($[GATA]_5$, $[GAGC]_5$, $[GTCA]_5$, $[CTCA]_5$, $[GACT]_5$, $[CTAG]_5$, $[GCAT]_5$, $[GCAC]_5$) repeats (GenoSys Biotechnologies). Nucleotide sequencing of 300 positive colonies indicated 144 dinucleotide repeats, 20 trinucleotide repeats, and 19 tetranucleotide repeats. A total of 117 colonies did not contain microsatellites.

Polymerase chain reaction (PCR) primers were designed from unique nucleotide sequence regions flanking microsatellites using the computer progam OLIGO™ (Macintosh version 4.0, National Biosciences). PCR conditions were optimized for 30 primer pairs. Each locus was screened for variation using a panel of 7–11 red drum collected from Apalachicola Bay, Florida, USA. PCR amplification was carried out in 10 μL volumes containing 1 μL (200 ng) of sample DNA, 1 μL of 10× reaction buffer (500 mM KCl, 100 mM Tris [pH 9.0], 10% Triton-X 100), 200 μM of each dNTP, 1 mM $MgCl_2$ (2 mM in Soc177 and Soc204), 5 pmols of each PCR primer, and 0.1 μL of *Taq* DNA polymerase (isolated in our laboratory following Engelke *et al*. 1990). One primer of each pair was end-labelled with [γ^{32}P]-dATP. PCR amplification was performed in an Omn-E thermal cycler (Hybaid) and consisted of 25 cycles of denaturation at 94 °C for 30 s, annealing at 56–62 °C for 30 s, and extension at 72 °C for 30 s, preceded by an initial denaturation step at 94 °C for 2 min. PCR products were analysed in 6% polyacrylamide gels (Sequagel, National Diagnostics) and visualized by autoradiography. Alleles were scored by length in base pairs relative to the known size of the cloned fragment.

Red drum microsatellite primers were tested for their ability to amplify microsatellites in three additional sciaenid species: (i) spotted sea trout, *Cynoscion nebulosus*; (ii) black drum *Pogonias cromis*; and (iii) Atlantic croaker, *Micropogonias undulatus*. DNA isolation and PCR were conducted as above, except that no radioactively labelled primer was used and annealing temperatures were set to 56 °C for all species. Amplification was checked by agarose gel electrophoresis of PCR products. Size standards and positive and negative controls were used in each experiment. For a subset of nine loci, PCR was conducted with radiolabelled primer (n = 7–11 individuals for each species and locus), and analysed in 6% polyacrylamide gels. Number of alleles observed at each locus for each taxon was counted as a measure of variability. Patterns of variability at each locus were tested for association among taxa by correlation analysis ($\alpha = 0.05$).

Twenty-six of 30 microsatellite loci tested were polymorphic in red drum (Table 1). Despite variability in expected product lengths and heterozygosity, optimal conditions for PCR were similar across loci (Table 1). Priming sites were conserved across the four taxa for 18 of 30 microsatellites tested for cross-species amplification. Microsatellite primer pairs generated for red drum produced well-resolved, polymorphic, and scorable products in other sciaenid species tested (Table 2). No significant correlations of locus polymorphism were detected across taxa for the subset of loci examined. Allelic variability in one species may be a poor predictor of variability in another, suggesting that preliminary screening will be required before applying red drum primers to other sciaenid species. However, microsatellites developed in this work should permit study of fine-scale genetic variation in the four sciaenid fishes examined.

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Table 1 Microsatellite DNA loci in red drum (*Sciaenops ocellatus*). Length indicates size in bp of PCR product amplified from recombinant plasmid (clone). Repeat sequence indicates the repeat motif [in brackets], and number of uninterrupted copies observed in the cloned allele. The slash [/] indicates a compound repeat. Observed (H_O) and Hardy–Weinberg expected (H_E) heterozygosities were calculated from a panel of 7–11 red drum. DNA sequences are listed in GenBank under consecutive Accession nos AF73257 to AF73286

Locus	Primer sequences (5' – 3')	Length (bp)	Repeat sequence	Annealing temp. (°C)	H_O	H_E
Soc 008	AGAGGGCTCCGCATGATGGACATG CTTGCTGAAATACTACAGTACAAA	386	$[TG]_5$	62	0.75	0.76
Soc 011	GCCGAGTCACGAAGGAACAGAGAA TGTCGTCTCATCTATCTCCATCTC	219	$[GA]_{11}$	62	0.46	0.46
Soc 012	GCACCATCTTGCCACTGATGAATT GGGCTCTTACAACTCGTTTCAGAT	187	$[GT]_7$	62	0.00	0.00
Soc 019	GGGTACAACTAAACAGACACAATA TTTGAAAATGTTCCTGTGAATCAC	229	$[GATA]_{16}$	58	0.90	0.93
Soc 034	TCTTTTTCTGTCTTTCAGGTAAGC AACCGTCTTCAACAAGGCTGTGAC	176	$[GT]_8$	58	1.00	0.90
Soc 035	TGTCCCATCAATCAAGCAGACTCT CTCTACCTCACACTCCTCAAAGTT	262	$[CT]_5/[CA]_9$	62	0.73	0.80
Soc 038	TAGGTTGACTGGTTGATGAGGT CAAAGGGTTGTGTTTCTATATG	161	$[AC]_8/[GA]_{14}$	62	1.00	0.87
Soc 044	GAGGGTGACGCTAACAGTTGA CACAGCTCCACTCTGATATG	230	$[CA]_{22}/[GT]_5$	58	1.00	0.94
Soc 049	GTTGCCTTCTGACAATACACTGTT CCGGCTCGCCTTGAAATGAATGAT	237	$[CA]_{24}$	58	0.91	0.93
Soc 050	CCCGTGATTTTAGGCTCAGATA CCTTTAGAGTGCAGTAAGTGATTT	183	$[GT]_7$	58	0.13	0.13
Soc 060	TCTATTGAAGCCTGTAAGTTAGTT CAAGGAAGGAGTGGGGAATGACAA	155	$[AGG]_8$	56	0.50	0.50
Soc 077	TAGCCCTTTGCCTCTCAGAA ACCCATAATGGACCTATTTC	147	$[TG]_{22}$	58	1.00	0.67
Soc 083	TGCTGTAATTGAAAAGCAGTGTAC AGCGGAACTAGAATTGGTTTTATA	130	$[TG]_{19}$	56	1.00	0.91
Soc 085	TTTTGGACCTACACTAGAGTAGC CGTGGGAGACTAGCGATGTAGAT	104	$[AC]_{17}$	58	1.00	0.92
Soc 086	TCTGCTTCRATATTTCCACTTTTT TTACACGGTGCCGCTCACAG	135	$[TGTC]_3$	56	0.00	0.00
Soc 099	CACCCACTGACACACACATACAC GGAACCAATATGTCTGCCATGAT	185	$[CA]_{23}$	58	1.00	0.94
Soc 105	TGGGGAAGAAAAACAGGGAG AAACCCCTGCATCTCTCTAAAC	191	$[AG]_5$	56	0.00	0.00
Soc 125	CCGCCGGCCACTCTGAGGACTCAT ACACTTGCGCTCATACAGTTAGCT	124	$[TG]_{10}$	56	0.88	0.70
Soc 129	GCGGCTGCAACACAAGAATT TGCACGGGGAAACAGAACG	137	$[TATC]_{11}$	58	1.00	0.95
Soc 133	CATTTGGACCATCGCTACTGCTG CTTGGCATTTCCAGACATCACTG	205	$[TGC]_8$	58	1.00	0.85
Soc 140	GGTGCAAACACAGCCATACAGT GCAAAATCGAAGACCGAGTTTAG	142	$[CTGT]_8$	58	0.72	0.67

Table 1 *Continued*

Locus	Primer sequences (5' – 3')	Length (bp)	Repeat sequence	Annealing temp. (°C)	H_O	H_E
Soc 156	CCTCTCCTTTCTCCATCAGTGC AGCCCGGCTGTCATCTCCTGTA	182	$[CCT]_6/[TCC]_4$	58	0.64	0.60
Soc 177	TCCAAGTATTTGACTGTTGTAGC AGATTACGAGTTTAGGTAGACAT	192	$[TAGA]_{10}$	58	0.71	0.89
Soc 201	GGAGGAACTGATGAGGGCAGTGT GCACAACACACCTCGCTATATC	229	$[CCT]_6$	58	1.00	0.70
Soc 204	ACAGCAGTACCTGCCCAAAACTG TCCCCTTCGTCTTCTTCCACTTC	193	$[CTG]_{12}$	58	1.00	0.84
Soc 232	AGGGCACAGTTGCATCTCTG CCCATCCTCAAGGCAGAAC	184	$[AGAC]_4$	56	0.00	0.00
Soc 243	GACGGGGATGCCATCTGC AATGCGAAAAAGACGAAACAGT	106	$[CCT]_9$	56	1.00	0.80
Soc 247	AGGCGCTGTTTCTGAATTTC TGGGAGTTTTTTATGGTGGT	210	$[TAT]_7$	58	0.86	0.79
Soc 252	GCTCCAATTAGTCCCCATTC GCGGGCTTTCTCTAGTCACA	114	$[CA]_{10}$	62	0.70	0.90
Soc 276	GGTGGGCGGGAACTTAAACTA CCGAAAGCACCTCTGCCTCTG	108	$[CA]_{13}$	56	1.00	0.84

Table 2 Number of alleles scored in cross-species amplification experiments using nine red drum loci. Seven to 11 individuals were examined for each species

Locus	Red drum	Spotted seatrout	Black drum	Atlantic croaker
Soc 012	1	5	6	5
Soc 035	6	3	5	11
Soc 044	11	1	*	1
Soc 049	12	1	4	7
Soc 050	2	5	2	5
Soc 085	9	5	4	11
Soc 133	3	5	5	4
Soc 140	4	1	4	6
Soc 201	4	3	1	7
Mean	5.8	3.0	3.9	6.3

* indicates no scorable product obtained.

References

Engelke DR, Krikos A, Bruck ME, Ginsburg D (1990) Purification of *Thermus aquaticus* DNA polymerase expressed in *Escherichia coli*. *Analytical Biochemistry*, **191**, 396–400.

Jones AG, Avise J (1997) Polygynandry in the dusky pipefish *Syngnathus floridae* revealed by microsatellite DNA markers. *Evolution*, **51**, 1611–1622.

Ruzzante DE, Taggart CT, Cook D, Goddard S (1996) Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: microsatellite DNA variation and antifreeze level. *Canadian Journal of Fisheries and Aquatic Science*, **53**, 634–645.

Sambrook J, Fritcsh EF, Maniatis T (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Microsatellites in reindeer, *Rangifer tarandus*, and their use in other cervids

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Reindeer and caribou (*Rangifer tarandus*) are distributed throughout the northern part of the Holarctic region. The species are separated into numerous different populations and subspecies and exist both as semidomestic and wild animals (Banfield 1961). The amount of differentiation and the evolution of the different populations are, however, unknown and open to speculation. Studies of the genetic