

Microsatellite Markers for Red Drum, *Sciaenops ocellatus*

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Polymerase chain reaction (PCR) primers are reported for 68 nuclear-encoded microsatellites developed during the past several years from genomic libraries of red drum (*Sciaenops ocellatus*). All 68 microsatellites were tested for reproducibility and polymorphism on a sample of five to 12 red drum; 60 of the microsatellites were found to be polymorphic. Estimates of observed and expected heterozygosity (gene diversity) and tests of conformity of genotypes to Hardy–Weinberg equilibrium were carried out for a subset of 31 microsatellites on a larger sample of 45 adults provided by Texas Parks and Wildlife. Levels of allelic and gene diversity were average relative to values observed for marine and anadromous fishes. The set of genetic markers should be useful for a variety of studies, including monitoring and assessment of red drum stock enhancement.

The red drum, *Sciaenops ocellatus*, is an estuarine-dependent sciaenid fish found in the western Atlantic Ocean from Massachusetts to the Yucatan Peninsula, including the Gulf of Mexico (Pattillo et al., 1997). The species comprises an important recreational fishery in bays and estuaries of Gulf Coast states and along the Atlantic coast of the southeastern United States (Swingle, 1987; Van Voorhees et al., 1992). Because of significant declines in red drum abundance stemming from overfishing and habitat deterioration (Heffernan and Kemp, 1982; Swingle et al., 1984), recovery plans were implemented in nearly all Gulf Coast and Atlantic states. These recovery plans included assessment of red drum stock structure and in some states, principally Texas, stock enhancement with hatchery-raised fingerlings (McEachron et al., 1995).

Previous studies of red drum population genetics have used a variety of genetic markers and shown that 1) red drum in the Gulf comprise a different stock than red drum along the Atlantic coast (Bohlmeyer and Gold, 1991; Gold et al., 1993) and 2) population structure of red drum in the northern Gulf follows a modified one-dimensional, linear stepping-stone model (Gold et al., 2001). More recently, there has been an increasing interest for estimating the genetic component of traits important in red drum culture. This necessitates development of genetic markers that permit identification of kinship among offspring raised in the same environment from early life stages when physical tagging is impossible. For both types of studies, i.e., population structure and kinship analysis, deoxyribonucleic acid (DNA) microsatellites have proved to be a powerful tool. Briefly, microsatellites are short stretches of nuclear DNA composed of di-, tri-,

and tetranucleotide arrays that are embedded in unique (specific) DNA flanking regions, inherited in a codominant fashion (Wright and Bentzen, 1994), and distributed throughout euchromatic regions of chromosomes (Weber and May, 1989; Weber, 1990). Variants at microsatellite loci are thought to arise rapidly (Schug et al., 1998), meaning that 1) recently diverged subpopulations (stocks) may be detected more easily with microsatellites than with other, commonly used genetic markers (e.g., mitochondrial DNA) and 2) the degree of genetic identity in microsatellite alleles may be used to estimate degree of genetic relatedness. When available in large number, microsatellites in principle can be used to carry out large-scale “family printing” for use in identifying hatchery-raised juveniles released into the wild, enabling evaluation of long-term survival and ecological performance of these fish. Large numbers of microsatellites also can be used to generate genetic maps and, ultimately, to localize quantitative trait loci or QTLs (Georges et al., 1995) of interest for both wild and cultured populations.

In this note, we report on polymerase chain reaction (PCR) primers and optimized annealing temperatures for the 68 microsatellites developed during the past several years in our laboratory from red drum genomic libraries. Most of the PCR primer pairs are published (Turner et al., 1998; O'Malley et al., 2003), but assays (primarily, annealing temperature) for many of the microsatellites were not optimized nor were data on gene diversity based on sample sizes of more than a few individuals. We also report data on observed and expected heterozygosity (gene diversity) and results of tests of conformity of genotypes to expectations of Hardy–Weinberg equilibrium for 31 of the

TABLE 1. Summary data for 68 microsatellites developed from red drum (*Sciaenops ocellatus*) genomic libraries. The PCR primer sequences are forward (top) and reverse (bottom). Primers developed from a single clone are designated with the same letter as subscript.^a

Micro-satellite	PCR primer sequence (5' → 3')	Repeat sequence of cloned allele	Size of cloned allele (base pair)	AT	N	N _A	Range in allele size (base pair)	H _o /H _e	P _{HW}
Soc 9	AACATTTCCATCACGTATTTATCT TCCACATGAACACCAGTGCAGTTC	(AT) ₂₇	233	58	12	4			
Soc 11	GCCGAGTCACGAAGGAACAGAGAA TGTCGTCTCATCTATCTCCATCTC	(GA) ₁₁	219	62	45	14	217-240	0.733/0.721	0.713
Soc 12	GCACCATCTTGCCACTGATGAATT GGGCTCTTACAACTCGTTTCAGAT	(GT) ₇	187	62	12	2			
Soc 19	GGGTACAACATAACAGACACAATA TTTGAAAATGTTCCCTGTGAATCAC	(GATA) ₁₆	229	58	45	21	195-267	0.844/0.898	0.019
Soc 34	TCTTTTCTGTCTTTCAGTAAGC AACCGTCTTCAACAAGGCTGTGAC	(GT) ₈	176	58	12	1			
Soc 35	TGTCCCATCAATCAAGCAGACTCT CTCTACCTCACACTCCTCAAAGTT	(CT) ₅ (CA) ₉	262	62	12	19			
Soc 44	GAGGGTGACGCTAACAGTTGA CACAGCTCCACTCTGATATG	(CA) ₂₂ (GT) ₅	230	62	43	6	211-271	0.907/0.930	0.363
Soc 49	GTTGCCTTCTGACAATACACTGTT CCGGCTCGCCTTGAAATGAATGAT	(CA) ₂₄	237	58	12	6			
Soc 50	CCCGTGATTTTAGGCTCAGATA CCTTAGAGTGCAGTAAGTGATTT	(GT) ₇	183	58	12	3			
Soc 60	TCTATTGAAGCCTGTAAGTTAGTT CAAGGAAGGAGTGGGAATGACAA	(AGG) ₈	155	56	45	6	151-163	0.511/0.570	0.752
Soc 77	TAGCCCTTTGCCTCTCAGAA ACCCATAATGGACCTATTTTC	(TG) ₂₂	147	58	12	1			
Soc 83	TGCTGTAATTGAAAAGCAGTGATAC AGCGGAAGTGAATGGTTTATA	(TG) ₁₉	130	56	45	6	114-142	0.867/0.826	0.682
Soc 85	TTTTGGACCTACACTAGAGTAGC CGTGGGAGACTAGCGATGTAGAT	(AC) ₁₇	104	58	45	5	80-122	0.822/0.869	0.105
Soc 86	TCTGCTTCTATATTTCCACTTTT TTACACGGTGCCGCTCACAG	(TGTC) ₉	135	56	12	1			
Soc 99	CACCCACTGACACACACATACAC GGAACCAATATGTCTGCCATGAT	(CA) ₂₉	185	62	45	1	131-209	0.933/0.923	0.662
Soc 105	TGGGAAGAAAAACAGGGAG AAACCCCTGCATCTCTCTAAAC	(AG) ₅	191	56	12	1			
Soc 125	CCGCCGCCACTCTGAGGACTCAT ACACTTGGCCTCATAAGTTAGCT	(TG) ₁₀	124	56	12	7			

TABLE 1. Continued.

Micro-satellite	PCR primer sequence (5' → 3')	Repeat sequence of cloned allele	Size of cloned allele (base pair)	AT	N	N _A	Range in allele size (base pair)	H _o /H _e	P _{HW}
<i>Soc</i> 133	CATTTGGACCATCGCTACTGCTG CTTGGCATTTCAGACATCACTG	(TGC) ₁₀	205	56	12	3			
<i>Soc</i> 137	AGGATCAGTCTCCGTTTGT ACAGACAGATTACAGCCAGAC	(TGTC) ₈	223	58	12	1			
<i>Soc</i> 138	CTGGAGCTTTCCCTTCTGT TGGGAGGAGAAGGCAGGAAGG	(TGTC) ₆	91	58	45	8	77–123	0.889/0.820	0.203
<i>Soc</i> 140	GGTGCAAACACAGCCATACAGT GCAAATCGAAGACCGAGTTTAG	(CTGT) ₈	142	56	45	5	132–144	0.778/0.623	0.280
<i>Soc</i> 156	CCTCTCCTTTCTCCATCAGTGC AGCCCGGCTGTCATCTCCTGTA	(CCT) ₆ (TCC) ₄	182	58	45	12		0.533/0.453	0.018
<i>Soc</i> 177	TCCAAGTATTGACTGTTGTAGC AGATTACGAGTTTAGGTAGACAT	(TAGA) ₁₀	192	58	12	12			
<i>Soc</i> 201	GGAGAACTGATGAGGGCAGTGT GCACAACACACCTCGCTATATC	(CCT) ₆	229	58	45	4	224–243	0.600/0.678	0.310
<i>Soc</i> 204	ACAGCAGTACCTGCCAAAACTG TCCCCTTCGTCTTCTCCACTTC	(CTG) ₁₂	193	58	12	14			
<i>Soc</i> 206	GTTTCCACATCCCCAACC AGTTTGGTCGCTTTAAAGGC	(GCAC) ₅	257	58	45	4	249–265	0.578/0.548	0.028
<i>Soc</i> 232	AGGGCACAGTTGCATCTCTG CCCATCCTCAAGGCAGAAC	(AGAC) ₄	184	56	12	1			
<i>Soc</i> 243	GACGGGATGCCATCTGC AATGCGAAAAAGACGAAACAGT	(CCT) ₉	106	56	45	6	94–106	0.733/0.753	0.243
<i>Soc</i> 247	AGGCGCTGTTCTGAATTC TGGGAGTTTTTATGGTGGT	(TAT) ₇	210	56	12	2			
<i>Soc</i> 252	GCTCCAATTAGTCCCCATTC GCGGGCTTCTCTAGTCACA	(CA) ₁₀	114	62	12	19			
<i>Soc</i> 400	TGCCATTGTCATTCTACAGAGC TTATAGTGGGTGAGTGTGGA	(CA) ₁₉	253	52	45	7	245–266	0.622/0.719	0.688
<i>Soc</i> 401	ACGTCTTAATCGGTCTCTGTCC ATCTCTGTGTGAAAGGAAAACA	(TG) ₁₄	174	52	45	6	174–206	0.733/0.848	0.463
<i>Soc</i> 402	CATATTTAACGAGCGACATAGC AAACAGATGAAGCACCTGGACT	(CA) ₂₀	149	52	44	5	134–164	0.818/0.878	0.262
<i>Soc</i> 403 _A	AGGGAAATGGTTGGTGAAGTAG GTCTGGACCTGTTTGTGAGAG	(TG) ₃₆	272	58	6	11	272–310		

TABLE 1. Continued.

Micro-satellite	PCR primer sequence (5' → 3')	Repeat sequence of cloned allele	Size of cloned allele (base pair)	AT	N	N _A	Range in allele size (base pair)	H _o /H _e	P _{HW}
<i>Soc</i> 404 _A	AGACCCTTTTGGTTGATTTTCATA ATGACTGCACCATTTCAAAAAG	(TG) ₂₃	168	52	45	9	150–212	0.778/0.904	0.018
<i>Soc</i> 405	CTTAGCCTTTTGGTTAGTTTCC CACACTCATGGTCACTCCTCTC	(CA) ₁₂	189	56	8	5	189–217		
<i>Soc</i> 406	TAGGGGGTAAGGTAGGATGATG GAAGAGCAGTGACGCTATCAAT	(TG) ₁₀	165	52	8	1			
<i>Soc</i> 407	AAAGTCTGCCTCTTACAGCTTC GAGTTAAAGCGTGTGCTAGTCC	(CA) ₁₃	147	56	43	6	139–157	0.907/0.843	0.341
<i>Soc</i> 409 _B	TTTATCTGCTCTGTGTGGAAGT ATCTATTTGTCGGTTTCTCTGC	(TG) ₁₁	323	52	8	7	323–367		
<i>Soc</i> 410 _B	GTACCAAGTCAGCCAGTGTGAC TCTCTGTGTCCCTCTGTGTTG	(TG) ₁₇	318	56	43	7	306–344	0.721/0.810	0.169
<i>Soc</i> 411	TCTGCCTCTTACAGCTTCAAGG CTTGTGAGTTAAAGCGTGTGC	(AC) ₁₃	149	54	7	6	147–163		
<i>Soc</i> 412	CACAGAAACTCAGCTCGAGACC AGGAAGAATGTACAAGGTGTTT	(AC) ₁₃	114	49	44	6	102–168	0.818/0.906	0.003
<i>Soc</i> 415 _C	CTCAGCACCTCAGACATATGG CACAAAGTTAAGTGGTATCGAGT	(TG) ₁₅	193	52	45	6	187–235	0.667/0.709	0.583
<i>Soc</i> 416 _C	CTCGATAACCACTTAACCTGT ATCGACATAATCTGGCACCA	(GA) ₃₈	159	49	45	6	141–181	0.733/0.851	0.368
<i>Soc</i> 417 _C	CTTACGTGATAAAGTGTGGGTGA ATATGCCAGTAATCCACCGAAG	(AC) ₂₄	96	49	45	4	86–112	0.778/0.756	0.278
<i>Soc</i> 418	GTTTTTCTGGCATTATGGATG TGAGGTTATCAAACACCTGCCCACT	(TG) ₂₄	288	52	8	22	272–294		
<i>Soc</i> 419	ATTTAGCCAACTGCTCCGCTCA GAGTGCCTGGTGTAGGGGGTA	(AC) ₂₀	246	56	42	6	238–260	0.929/0.847	0.909
<i>Soc</i> 421	CTCACTGCTCCCTCGTCACACG CTGTGACAGGATGCGGCTTTTC	(TG) ₃₄	172	56	8	10	138–188		
<i>Soc</i> 422	CTGAAGGGATGGCAATGTTGATTGG ATTCTCTGGGTTTATGGGATGT	(TG) ₃₄	372	56	7	6	360–374		
<i>Soc</i> 423	GTCACCGCACCATGATGGAGAT TACCACTTACACTCAGCAGGTG	(CA) ₂₆	202	54	45	6	172–208	0.889/0.881	0.126
<i>Soc</i> 424	CACTCTTCACTCCCTCACTCGTC TTCGATGGGTGACAGCGTCAGG	(CA) ₁₅	208	56	45	9	204–230	0.844/0.840	0.333

TABLE 1. Continued.

Micro-satellite	PCR primer sequence (5' → 3')	Repeat sequence of cloned allele	Size of cloned allele (base pair)	AT	N	N _A	Range in allele size (base pair)	H _o /H _e	P _{HW}
<i>Soc</i> 425	ACACCGCATTGCCACCAGGAA CGAGTTTATCCTTCACGCTTG	(CA) ₁₄	150	54	8	2	149–150		
<i>Soc</i> 426	GAGAGGACGTGAGCTGCTGA TGAGAAACAGAAACAGAAGGT	(CA) ₁₁	142	52	8	4	138–152		
<i>Soc</i> 428	GACATCGCATTTGTCTACAGAGTCG AACTCCCAGTCATAATATCCCTTT	(TG) ₃₈	229	53	45	8	172–242	0.956/0.946	0.256
<i>Soc</i> 429	AAAAATCTGCCTGCCTGTG TTAAGAGCAACCTCCGTCTC	(TG) ₁₂	128	52	8	4	124–132		
<i>Soc</i> 430	TAACAGTCCCTAAACAGGTT GTTTCTCCTCCCTTTCTC	(TG) ₂₃	277	52	8	10	265–339		
<i>Soc</i> 431	GACACGCTGTGGTAGATGAAAACG TGTATATTAGTTGGCAAGGCAGAG	(TG) ₂₉	172	53	8	8	151–180		
<i>Soc</i> 432 _D	TTTAGGCTACGTCTGGAGGCACA GTGTGTTGAGGGTCAGCGTAC	(AC) ₁₆	108	52	45	5	98–118	0.867/0.808	0.868
<i>Soc</i> 433 _D	AGTACGCTGACCCTCAAACACA TTCTCTTTGCGCTCCTTTTCCCTGA	(TG) ₁₆	100	52	45	6	84–102	0.867/0.828	0.194
<i>Soc</i> 434	GACACTCCAGATATGCTGA TCCTTGTTTATCTGGTGCTGT	(CA) ₂₃	197	52	7	7	169–219		
<i>Soc</i> 435	AACTGGAGCCTGACTCACTGC GTGAATACTCTCTTTTCTTGTG	(AC) ₂₂	179	49	8	1	179		
<i>Soc</i> 437 _E	CTACTTTCTAGTCTTTGCTCCACT GTCAAACGCTATTTTTTCCAGT	(TG) ₃₆	296	54	5	7	296–330		
<i>Soc</i> 438 _E	AATACAGCTAACTCGAAA ACTGCACCATTTCAAAAACGCCTCT	(TG) ₂₄	144	49	7	6	132–154		
<i>Soc</i> 439	ACTCTCGTCCCACTTACCACA TATGTTTGCATATAAGCTCA	(TG) ₁₇	103	49	6	4	91–105		
<i>Soc</i> 442	TTTGTGGCAATAAACTGCGAGA TTCTTAATACGTGCCCGACT	(TG) ₃₀	195	52	8	8	179–199		
<i>Soc</i> 443	CACAGGAGGAGTTTGTCCAAT ATGTTTCGGTTTTCGTTTGCTC	(TG) ₁₅	202	52	7	11	206–242		
<i>Soc</i> 444 _F	TGAACTAATCCAGCCACAGATG CACAGCCGATTAAAGAGAGGGAAT	(TG) ₁₇	161	52	45	3	161–165	0.600/0.504	0.526
<i>Soc</i> 445 _F	ATACAAAGGGACTCTCATACTCTC TTTTAATCCCAATTACAGCTTT	(TCC) ₁₀	156	52	45	7	134–166	0.778/0.805	0.129

* AT = annealing temperature; N = number of individuals assayed; N_A = number of alleles detected; H_o/H_e = observed/expected heterozygosity; and P_{HW} = probability of conformity to Hardy-Weinberg equilibrium.

polymorphic microsatellites based on a sample of 45 wild-caught adults. We anticipate that the microsatellite markers will be useful for a variety of studies, including monitoring and assessment of red drum stock enhancement.

Details on genomic library construction, ligation of size-selected (200–1,200 base pair) fragments into cloning vectors, and transformation into *Escherichia coli* competent cells may be found in Turner et al. (1998) and O'Malley et al. (2003). A total of 14,080 clones were hybridized with cocktails of oligonucleotide probes, and 393 positive clones have been sequenced. Thus far, 204 clones that contained microsatellite motifs have been isolated. The PCR primers were designed from sequences flanking the microsatellites by using the program Oligo[®] (Macintosh version 4.0, National Biosciences), and optimization of PCR protocols was carried out on a panel of DNAs from 12 individuals. The PCR was performed in a 10- μ l volume containing 1 μ l (100 ng) of DNA, 1 μ l of 10 \times reaction buffer [500 mM KCl, 200 mM Tris-HCl (pH 8.4)], 1.5 mM MgCl₂, 2.5 mM of each deoxynucleoside triphosphates, 5 pmol of each primer, and 0.5 units *Taq* DNA polymerase (GibcoBRL). The PCR thermal cycling consisted of an initial denaturation at 95 C for 5 min, followed by 30 cycles consisting of 45 sec at 95 C, 45 sec at the optimized annealing temperature (Table 1), 1 min at 72 C, and a final extension of 10 min at 72 C.

The PCR primer sequences, repeat sequence and size (in base pairs) of cloned alleles, optimal annealing temperature, number of individuals assayed, and number of alleles detected for all 68 microsatellites are given in Table 1. The range in allele size is given for a subset of 48 microsatellites. The entire set of 68 microsatellites includes 51 di-, six tri-, and eight tetranucleotide repeat motifs; three microsatellites are complex repeats (i.e., a combination of different repeat motifs). Sixty of the microsatellites were found to be polymorphic; the average number of alleles per (polymorphic) microsatellite was 7.5 (range = 2–22 alleles). Genotypes for a subset of 31 microsatellites were acquired from 45 adults sampled from offshore waters along the Texas coast and held by Texas Parks and Wildlife (TPW) as broodstock for the TPW stock enhancement program. Estimates of observed and expected heterozygosity (gene diversity) and results of tests for conformity of genotype proportions to Hardy-Weinberg expectations for these 31 microsatellites also are given in Table 1. Estimates of observed and expected heterozygosity were computed using GENETIX v. 4.05 (Belkhir et

al., 1996–2002); probability of departure from Hardy-Weinberg equilibrium (P_{HW}) was assessed using a Markov chain method (Guo and Thompson, 1992), as implemented in GENEPOP v. 3.3 (Raymond and Rousset, 1995) and using 5,000 dememorizations, 500 batches, and 5,000 iterations per batch. The average number of alleles for these 31 microsatellites was 12.6, and the average expected heterozygosity (gene diversity) was 0.784. These values are intermediate between those typically found in marine and anadromous fish (DeWoody and Avise, 2000). Following (sequential) Bonferroni correction for multiple tests performed simultaneously (Rice, 1989), genotypes at all 31 microsatellites did not differ significantly from Hardy-Weinberg equilibrium expectations. Tests that were significant before Bonferroni correction included microsatellites *Soc* 19, *Soc* 404, and *Soc* 412 (heterozygote deficiency) and *Soc* 156 and *Soc* 206 (heterozygote excess). This set of microsatellites should prove to be an extremely powerful tool for future studies of red drum population structure and for parental assignment (kinship) in studies monitoring the results of red drum stock enhancement.

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