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PRIMER NOTE

Isolation and characterization of nuclear microsatellite loci in the anadromous marine fish *Morone saxatilis*

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Abstract

Molecular genetic studies of population structure and gene flow are commonly employed in fish stock assessment and breeding population delineation. Genetically structured breeding populations may have different effective population sizes, distinct reproductive rates and variable susceptibility to harvesting or breeding habitat degradation. Nine microsatellite loci were isolated for *Morone saxatilis* (Moronidae), an anadromous fish inhabiting the mid-Atlantic. Microsatellite loci were isolated with a subtractive hybridization method and will be used to estimate population structure. The loci averaged 8.5 alleles each. Seven loci in the Choptank population and two loci in the Potomac population deviated from Hardy–Weinberg expected frequencies of heterozygotes.

Keywords: fisheries genetics, microsatellite, *Morone saxatilis*, Moronidae, population structure, striped bass

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Striped bass (*Morone saxatilis*) are a long-lived, marine fish found along the North American Atlantic coast from Florida to Canada. Mid-Atlantic populations between North Carolina and Maine are anadromous while smaller, often disjunct populations found south of Cape Hatteras and along the eastern Gulf of Mexico as well as North of Nova Scotia are thought to be largely estuarine to riverine. The mid-Atlantic stocks are recruited from major breeding populations in Roanoke River and Albemarle Sound, multiple tributaries in the Chesapeake Bay, the Delaware River and the Hudson River. Mid-Atlantic striped bass thus constitute a classical mixed stock fishery where adults are harvested as one stock that is potentially composed of fish from geographically discrete, genetically distinct breeding populations.

Striped bass are the basis of a large commercial and recreational fishery that represents a significant management challenge due to the various breeding populations that must be monitored. Genetically structured breeding populations may have different effective population sizes, distinct reproductive rates and variable susceptibility to harvesting or breeding habitat degradation. Therefore, estimating the contributions of individual breeding populations to a mixed stock with genetic markers is a useful tool for stock assessment and breeding population identification (reviewed by Waples 1998). By identifying breeding groups, it may be possible to gauge the reproductive contribution of spawning populations and monitor variation in the population-specific production of juveniles. Identification of population structure can assist in the design of biologically appropriate stocking programs by describing populations that may be partly genetically isolated in a given watershed or geographical region. Genetic stock studies also facilitate recognition and protection of genetically unique populations. Our results expand the number of *M. saxatilis* microsatellite loci available for genetic studies of stock composition. We were motivated to develop additional loci after preliminary data from a few of the eight loci developed by Roy *et al*. (2000) and the 13 loci (seven striped bass-specific and six heterospecific) reported by Han *et al*. (2000) appeared to have insufficient diversity for a fine scale study within and among Chesapeake Bay tributaries.

The loci described here were isolated following the subtractive hybridization method of Hamilton *et al*. (1999; see detailed protocol at bioserver.georgetown.edu/faculty/hamilton). Genomic DNA was obtained from frozen

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Table 1 Annealing temperature (T_a), number of PCR cycles, PCR 72 °C extension time, use of 1.25 M betaine and thermal cycler

Locus	T_a (°C)	Cycles	Extension time (s)	Betaine	Thermal cycle
AG25-1#1	56	28	30	x	RC
AT150-2#11	56	28	30	x	RC
AC25-1#2	56	28	30	x	RC
AT150-2#4	56	25	30	x	RC
AC25-6#12	58	25	15		RC
AT150-2#12	56	31	30	x	RC
AT150-2#10	59	25	15		AB
AT150-2#21	59	25	15		AB
AC25-6#10	58	26	30		AB

*Applied Biosystems GeneAmp 9700 (AB) or Stratagene Robocycler Gradient 96 (RC).

muscle tissue digested with proteinase K and extracted with either a DNeasy tissue kit (QiaGen) or Puregene DNA isolation kit (Gentra Systems) following the manufacturer's instructions. Genomic DNA was digested with *Hae*III, *Nhe*I and *Rsa*I (New England Biolabs) to obtain a majority of DNA in the 200–1200 base pair size range. After treating digests with mung beannuclease to create blunt ends and ligation of the SNX linker,lstreptavidin-bead subtractive hybridization used 30-mer biotinylated oligonucleotides with AT, AC, AG, CG, AAG, AGG, CAC and CCG repeat motifs in independent reactions. DNA recovered from each of the subtractive hybridizations was amplified by polymerase chain reaction (PCR) and all reactions showed some product. Therefore, all subtractive-hybridization products were ligated into pBS SK + vector (Stratagene, La Jolla, CA) and transformed into competent cells. Plates of colonies for each of the eight enrichment sequences were screened by colony lift hybridization. Hybridization-positive colonies were picked and the insert PCR amplified with T7 and T3 primers. PCR products were purified with QiaQuick spin columns (QiaGen), sequenced with both T7 and T3 primers in reactions containing 4.6 μL water, 2 μL template, 2.4 μL primer (1 μM) and 6 μL dRhodamine or BigDye terminator reaction ready mix (Applied Biosystems) and electrophoresed on a model 377 or 3100 sequencer (Applied Biosystems). The resulting sequences were aligned into contigs and edited using SEQUENCHER 3.1.1 or 4.1 (GeneCodes). Potential primers were identified manually and tested with AMPLIFY 1.2 (Engels 1993).

We purchased primers for each locus with a regular pattern of at least 10 repeats(Operon Technologies) and experimented with PCR constituents and cycling parameters in an attempt to amplify a single band in the expected size range. Clone sequencing templates diluted between 1000:1 and 10 000:1 were used as positive control templates to verify PCR product molecular weight. PCR reactions contained 1–3 μL of DNA template (DNA concentration was not determined), 2 μL of 10 × Thermopol buffer (containing 2 mM $MgSO_4$), 0.2 mM each dNTP, 0.2–0.4 μm of each primer, 1.25 M betaine (Aldrich) for some loci and 0.4 units of Vent exo-polymerase (New England Biolabs) in a total volume of 20 μL. The thermal cycling profiles were 5 min at 96 °C followed by cycles of 96 °C for 45 s, annealing temperature for 45 s and 72 °C extension for 15–30 s. Table 1 providesocus-specific details on PCR cycling and inclusion of betaine.

Loci were initially tested for polymorphism by running PCR products from 10 to 12 individuals on 4% Metaphor gels (BioWhittaker, Rockland, ME). For those loci that showed multiple alleles, HPLC purified primers sets were ordered with a forward primer label of 6-FAM, HEX (Operon Technologies), NED, PET or VIC (Applied Biosystems). To score genotypes, PCR products were electrophoresed along with GENESCAN ROX 400 or LIZ 500 molecular marker on a 3100 sequencer and sized using GENESCAN 3.7 (all from Applied Biosystems). Genotypes for each locus were determined for 96 young of the year individuals(length = 125 mm) collected by beach seine in the Potomac (n = 46) and Choptank (n = 50) rivers in 2000.

CERVUS 2.0 (Marshall *et al*. 1998) was used to summarize the allele and genotype data (Table 2). Loci showed between two and 26 alleles with an average of 8.6 alleles per locus. GENEPOP web version 3.1c (http://wbiomed.curtin.edu.au/genepop) (Raymond & Rousset 1995) was used to test individual loci for deviation from Hardy–Weinberg expected heterozygote frequency. The probability test was employed (option three, equivalent to a two-tailed hypothesis test) using default values for the Markov chain parameters. The two populations were tested separately, since pooling samples from differentiated populations will produce a deficit of heterozygotes compared to the number of heterozygotes expected under Hardy–Weinberg in the pooled sample due to the Wahlund effect (see Hartl & Clark 1997) even in the complete absence of null alleles. Two loci in the Potomac and seven loci in the Choptank

Table 2 Locus name, primer sequences, repeat motif, observed size range, number of individuals genotyped (N), number of alleles (k), observed heterozygosity (H_O) and expected heterozygosity (H_E) in the total sample, and GenBank accession number

Locus	Primer sequences (5′–3′)	Repeat	Size range (bp)	N	k	H_O	H_E	Accession
AG25-1#1	F: GCT TCC GCA AGT TTA GTT GC	CTTT	154–308	93	26	0.817†	0.887	AY248735
	R: AAC GCA GAA TCC TGC CTG C							
AT150-2#11	F: GTG CAG TTA TTA AGT CAC CTG	GT	208–212	94	2	0.245†	0.343	AY248736
	R: TTC ACC ACT TCC AAC AGA GG							
AC25-1#2	F: GTC AGA CTG TAA TAA AGG CTC	AC	93–97	94	2	0.585*	0.478	AY248737
	R: TGT CTG ACA CAG CTC AAC AG							
AT150-2#4	F: TAT GAC GCC ATG TGT TGG CAC	GT	147–159	89	5	0.528	0.585	AY248732
	R: ATG TAT GAG TTG ATA GCA TGA GG							
AC25-6#12	F: ACC CAG TGG TCC AAT CAT GG	TG	161–209	90	18	0.700†	0.706	AY248734
	R: GTA TCA GAT CAT TTC CAA GTC C							
AT150-2#12	F: TTC CAG CTT GTG AAG TGA GC	GT	117–127	90	5	0.511†	0.479	AY248733
	R: TTC TGT CTA TTG CAC AGA CTC							
AT150-2#10	F: TCC TCT CTT GTT CAG TCT CC	TG	197–199	94	2	0.000†	0.082	AY248738
	R: CTA AAC CAT CTG CAC ATT CC							
AT150-2#21	F: GGG TGC CTC TCC TAA GTG C	GT	168–196	94	13	0.638†	0.799	AY248739
	R: CTC TGC TTG TAT TGC TGT TGG							
AC25-6#10	F: AGA GTC CCA CTA ATA ACA CG	AT/GT	218–224	94	4	0.394†*	0.424	AY248740
	R: TCC AGC ATG TTG ACA TAT TGC							

*Potomac observed and Hardy–Weinberg expected homozygote frequencies different ($P < 0.05$).
†Choptank observed and Hardy–Weinberg expected homozygote frequencies different ($P < 0.05$).

did not meet Hardy–Weinberg expectations with one Potomac locus and six Choptank loci showing significant deficits of heterozygous genotypes. The large number of Choptank loci deviating from Hardy–Weinberg expected genotype frequencies is consistent with population structure within the sample as well as with the presence of null alleles. These loci will be of great utility for estimating population structure and breeding patterns in *M. saxatilis*.

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