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TECHNICAL NOTE

Ten polymorphic microsatellite loci for the Atlantic Silverside, *Menidia menidia*

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Abstract Ten polymorphic microsatellite loci were isolated from the Atlantic Silverside (*Menidia menidia*) in order to test hypotheses regarding the role of adaptive phenotypic variation in structuring estuarine populations along coastal North America. Loci were amplified in three multiplex panels requiring a total of four individual PCRs. All loci were highly polymorphic in individuals screened from an estuary in Nova Scotia, and none exhibited significant departures from Hardy–Weinberg equilibrium. The results suggest that these loci will be sensitive to low levels of neutral divergence among populations across *M. menidia* populations.

Keywords Atherinidae · *Menidia menidia* · Microsatellites · Northwest Atlantic · Silverside

The Atlantic Silverside, *Menidia menidia*, is a small atherinid fish common to outer estuaries along the North American east coast from the Gulf of Saint Lawrence to northern Florida. Many aspects of the life history of the silverside are well known due to more than 30 years of field and laboratory studies, and it has served as a model organism for the study of population processes important for fisheries science (reviewed in Conover et al. 2005). Recently, *M. menidia* has received much attention for possessing clinal variation in various morphological traits that co-vary with environmental gradients along its range (see reviews in Conover 1998; Conover et al. 2005). Common garden experiments have confirmed the genetic basis of these traits (e.g., Billerbeck et al. 1997), invoking a role for natural selection in structuring the distribution of morphological variation in estuarine species. Adaptation in the wild, however, is difficult to prove since neutral processes, such as isolation by distance or secondary contact between historically isolated (and morphologically divergent) populations, can produce similar phenotypic patterns (Endler 1977; Schmidt et al. 2008). Therefore, attention must be paid to the potentially confounding role of gene flow in the formation and maintenance of phenotypic clines.

Understanding the balance between selection and gene flow is an important question in fisheries science and conservation management since spatially structured populations may respond differently to fishing pressures and climate change. Due to their rapid mutation rate and biparental mode of inheritance, microsatellite markers are useful tools to distinguish between neutral and adaptive variation in population structure. To assess the role of selection in structuring estuarine fish populations, we have characterized ten polymorphic microsatellite loci from *Menidia menidia*.

We isolated microsatellite markers from fin clips of a single *M. menidia* individual following a modified protocol from Hamilton et al. (1999) and also the "Microsatellite Easy Isolation 2000" protocol (http://www.uga.edu/srel/DNA_Lab/protocols.htm; subsequently refined in Glenn and Schable 2005). Briefly, whole genomic DNA was extracted from approximately 300 mg of muscle tissue using a phenol/chloroform/isoamyl extraction (Hillis et al. 1996) and was digested to completion with the restriction

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Table 1 Summary of details for ten polymorphic microsatellite loci from 20 *Menidia menidia* individuals from a Nova Scotia estuary

Multiplex panel	Locus	Primer sequence (5′–>3′)	5′ fluorescent tag	Repeat motif	Size range (bp)	*N*	N_A	H_O	H_E
SetA	Mm02	F: AATCATTAACTGTCCTCTTATCATAGC	6-FAM	$(GT)_7$	112–146	20	11	0.80000	0.87821
		R: GCAAGAATCCTTTGGGTCAA							
	Mm248	F: CTCTGACCTTTCTCCCTCTC	HEX	$(CA)_7$	166–180	20	6	0.75000	0.78718
		R: CACACTTCTTGATTACACCT							
	Mm251	F: ATTTGCGGTATTCTCAAGTG	6-FAM	$(GT)_{12}$	236–270	20	8	0.60000	0.74231
		R: GCGTGGAATCTGTTTTATTG							
	Mm272	F: AGTTAAACACAAGAAGCACA	HEX	$(TG)_{11}$	278–314	19	12	0.94737	0.89758
		R: GCTGCCCCCAATGAAGAGAC							
SetB	Mm09	F: CGGACTCAGGAGGGACAGTA	HEX	$(AC)_{10}$	136–148	20	6	0.75000	0.79103
		R: GAAGGCGTCCGGTACAGA							
	Mm108	F: AAGACCAACCAGTACAAACAG	6-FAM	$(TG)_{13}$	244–252	20	5	0.55000	0.51282
		R: CTGGGACAATGTAATCACTTC							
	Mm119	F: AAGTTTTTATTTCATTGTCA	HEX	$(TATC)_{12}$-CCATC-$(TATC)_{26}$	260–368	18	15	0.66667	0.91746
		R: GTTGGGTTGTAGTCAGTTCT							
	Mm202	F: AGAATGGGTAATGGGCTGAG	6-FAM	$(TG)_6$-AG-$(TG)_9$	374–398	18	6	0.72222	0.60476
		R: ACTTTCTCATTGTCGTGTTC							
SetC	Mm204	F: TGGATGTAACATGAGTGGTG	HEX	$(AC)_9$-TC-$(AC)_3$	170–186	20	6	0.75000	0.70769
		R: TTGGCTGTTCTGTCTTCTTT							
	Mm240	F: AACAAGTTAACAGCACAAAA	6-FAM	$(AC)_9$	220–232	20	7	0.85000	0.79872
		R: CCCACGTACACACACAATAG							

GenBank accession number for clone sequences are JF326837—JF326846. *N* number of individuals, N_A number of alleles, H_O observed heterozygosity, H_E expected heterozygosity

enzymes AluI and XmnI (New England Biolabs) to create blunt-ended fragments for linker ligation to SNX adapters (Hamilton et al. 1999). Repeat-enrichment was performed using the following probes: AC_{13}, $AGAT_8$, $ATCC_5$, and $ACAT_8$. All other steps through Dyna-bead enrichment, TA-cloning, amplification and sequencing of insert-containing colonies, and primer design were performed as described in Jones and Barber (2005). Specifically, 285 repeat-containing clones were identified by blue-white screening and sequenced. Primers flanking di- and tetranucleotide repeats were designed from 34 unique clones and loci were initially screened for size variation across eight *M. menidia* individuals from Nova Scotia and South Carolina. A total of 10 polymorphic loci were identified and one primer of each pair was fluorescently labeled with HEX or 6-FAM (Table 1) for further fragment analysis on an ABI 377 automated DNA sequencer.

To characterize each locus, 20 *M. menidia* individuals were collected from an estuary near Joggins, Nova Scotia, Canada for genotyping. Genomic DNA was extracted from fin clip tissue using a Chelex (BioRad) protocol (Walsh et al. 1991) and the 10 new markers were amplified in a combination of two multiplex panels containing four primer pairs each plus two additional PCRs containing a single primer pair each (Table 1). All PCR's were conducted in 10 µl reaction volumes and contained 1.0 µl DNA template, 0.25 U Amplitaq Gold DNA Polymerase (Applied Biosystems), 1× GeneAmp PCR Gold Buffer (Applied Biosystems), 200 µM each dNTP, 2.0 mM $MgCl_2$, and 0.5 µM each primer. Cycling parameters were 94°C for 10 min, 35 cycles of 94°C for 40 s, 59°C for 40 s, and 72°C for 1 min, and a final extension at 72°C for 1 h. Fragment analysis was performed on an ABI 377 with an MRK400 ROX-labeled internal size standard (Gel Company). The individually amplified loci (Set C, Table 1) were pooled for electrophoresis so that all 10 loci could be run in three lanes per individual. Allele sizes were scored using GENESCAN 3.1.2 (Applied Biosystems) and STRAND 2.2 (UC Davis' Veterinary Genetics Lab; http://www.vgl.ucdavis.edu/informatics/STRand/). Expected and observed heterozygosities were compared by exact tests using a Markov chain (1,000 dememorization steps, 100,000 steps in chain) in ARLEQUIN 3.11 (Excoffier et al. 2005; Guo and Thompson 1992). Pairwise genetic disequilibrium between all pairs of loci was calculated in FSTAT 2.932 (Goudet 1995, 2001).

All loci exhibited a high degree of polymorphism, with the number of alleles ranging from 5 to to 15 within the sampled population. The size ranges, number of alleles, and observed and expected heterozygosities for each locus are presented in Table 1. Exact tests at each locus found no significant departure from Hardy–Weinberg equilibrium after Bonferroni correction (Rice 1989), and linkage disequilibrium was not detected between any pair of loci. These microsatellite loci should serve as robust markers to measure the relative influence of selection and isolation in structuring populations of the Atlantic Silverside.

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