Cross-amplification of 10 new isolated polymorphic microsatellite loci for red mullet (Mullus barbatus) in striped red mullet (Mullus surmuletus)

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Abstract

Ten polymorphic dinucleotide microsatellite loci were isolated and characterized for the red mullet (Mullus barbatus). Allele variability was tested on both the red mullet and its congener the striped red mullet (Mullus surmuletus). Characterization of 30 individuals of both species from the western Mediterranean showed moderate to high allelic diversity ranging from two to 26 alleles per locus (mean 10.9). Three loci showed departures from Hardy–Weinberg proportions. No evidence of significant association between genotypes at pairs of loci was observed. These polymorphic loci could be suitable for population genetic assessments of both species.

Keywords: microsatellite, Mullidae, Mullus barbatus, Mullus surmuletus

Belonging to the family Mullidae, the red mullet (Mullus barbatus) and striped red mullet (Mullus surmuletus) are distributed across the eastern Atlantic Ocean and the Mediterranean and Black Seas (Hureau 1986). These demersal species are subject of commercial exploitation of bottom trawl multispecies fisheries throughout their geographical distribution range (Stergiou et al. 1997). Numerous studies have been carried out on various aspects of these specie's ecology, biology, taxonomic relationships and population structure (Labropoulou & Eleftheriou 1997; Mamuris et al. 1998, 1999; Özbilgin et al. 2004). However, despite their high economic value, no fishery genetic assessments exist for the striped red mullet and only one study has been performed on the red mullet within a small portion of its distribution range (Garoia et al. 2004). Here, we introduce a set of 10 polymorphic dinucleotide microsatellite markers developed for the red mullet and report their variability on both the red mullet and in the striped red mullet.

We developed an enriched genomic library as described in the protocol of Glenn et al. (2000), available at www.uga.edu/srel/DNA_Lab/protocols.htm. Particular modifications to the protocol were performed as follows. DNA extractions were carried out from lateral fin tissue out of 10 M. barbatus individuals from the western Mediterranean by phenol–chloroform method (Sambrook et al. 1989). Genomic DNA was simultaneously digested with RsaI restriction enzyme and ligated to double-stranded linker-adapted primers (Hamilton et al. 1999). Restricted-ligated DNA fragments were then amplified with single-stranded linker-adapted primers and hybridized with a biotinylated enriched probe mixture consisting of $(GT)_{10}$ and $(CT)_{10}$ at 10 μm each. DNA fragments with repetitive sequences were selectively captured by streptavidin-coated Dynabeads (Oxoid) and separated by a magnetic field. Enriched DNA was eluted in 200 μL dH_2O from the bead probes and concentrated by vacuum centrifugation to a final concentration of ~100 ng/μL. Subsequent ligation of enriched DNA into a cloning vector was carried out using a TOPO TA cloning kit (Invitrogen) following the manufacturer's protocol. More than 110 positive clones were obtained. All clones were sequenced and checked for inserts using ABI PRISM BigDye Terminator Cycle kit (Applied Biosystems) and resolved on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems).

Polymerase chain reaction (PCR) primers were designed using oligo 6.4 software. PCRs were performed in 25-μL

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Table 1 Characterization of 10 red mullet Mullus barbatus (N = 30 individuals) microsatellite loci and their variability on striped red mullet Mullus surmuletus (N = 30 individuals). H_O, observed heterozygosity; H_E, expected heterozygosity under Hardy–Weinberg equilibrium; F_{IS} inbreeding coefficient; *, $P < 0.05$

Locus/GenBank Accession no.	Locus	Repeat motif	Primer sequences (5′–3′)	Species	Number of alleles	Allele size (bp)	H_O	H_E	F_{IS}
DQ473548	Mbar3	$(CA)_{24}$	F: PET-GCTCCCCCGACACACTGTCT	M. barbartus	16	111–149	0.8793	0.9352	0.059
			R: ACCTTGGCCCTTCTTACGTC	M. surmuletus	8	115–145	0.5838	0.6480	0.179
DQ473549	Mbar11	$(GT)_{10}GC(GT)_{10}$	F: VIC-TGACTGTCAGCACTTGCATT	M. barbartus	9	156–176	0.5691	0.5847	0.204
			R: CTGAGGAGAGTCATGAGT	M. surmuletus	16	162–208	0.8571	0.9227	0.060
DQ473551	Mbar14	$(AC)_{48}AT(AC)_4AT(AC)_4$	F: FAM-GATAGCGAGCCTGAAACCAC	M. barbartus	26	195–265	0.9285	0.9603	0.034
			R: CCCTCTGCTTGATATTCCT	M. surmuletus	16	193–235	0.6000	0.9163	0.347*
DQ473555	Mbar28	$(GT)_{12}$	F: FAM-AAAGGGAGAATGAGGTGAAA	M. barbartus	2	156–164	0.1034	0.1324	−0.037
			R: AAGCGCTCGCAACAAAGTC	M. surmuletus	2	164–166	0.0333	0.0661	0.000
DQ473552	Mbar46	$(GT)_{12}$	F: NED-CCCGAGCAGCAGAAAAA	M. barbartus	7	250–262	0.7666	0.8310	0.079
			R:CTTGCCCTCTGCCTCTG	M. surmuletus	7	236–244	0.4285	0.5539	0.229*
DQ473547	Mbar55	$(CA)_7CG(CA)_3TA(CA)_6$	F: NED-TACACACAAACACTCACCCA	M. barbartus	12	146–176	0.8000	0.8717	0.074
			R: CGCAACCAATAGCACACTAC	M. surmuletus	7	142–166	0.5517	0.7489	0.267*
DQ473553	Mbar63	$(AC)_{10}AT(AC)_8$	F: VIC-AACCAGCAGGTCTCACA	M. barbartus	11	301–337	0.7900	0.8621	0.186
			R: TTCATGCTCCTTTTGTTCC	M. surmuletus	14	269–327	0.8200	0.8887	0.091
DQ473550	Mbar130	$(AC)_{10}$	F: NED-GAGGGTAGATTTGGTTGCAG	M. barbartus	8	185–209	0.7583	0.7787	0.071
			R: AGAGTATTGCATTTTTCGCC	M. surmuletus	11	185–217	0.7955	0.8473	0.079
DQ473556	Mbar132	$(GT)_{10}$	F: FAM-GGAGCAAGGAAGAGGAGA	M. barbartus	10	112–132	0.7896	0.8324	0.162
			R: CTCTGCAGACCTGCTCAA	M. surmuletus	9	118–136	0.7641	0.8451	0.145
DQ473554	Mbar133	$(CA)_{14}CG(CA)_5$	F: PET-CTCGGCACATCACAGAAAC	M. barbartus	11	226–266	0.7333	0.7847	0.065
			R: CCTCCCAAATTACACACATC	M. surmuletus	16	230–268	0.8247	0.8620	0.044

total volume, which included 50 ng of DNA, 2 mm of $MgCl_2$, 0.75 μm of each primer, 200 μm dNTP's, 1× reaction buffer [75 mm Tris-HCl, 20 mm $(NH_4)_2SO_4$] and 0.5 U Taq polymerase (BIOTAQ). Reaction conditions were as follows: an initial denaturation step of 5 min at 95 °C, 30 cycles consisting of 30 s at 92 °C, 30 s at 56.5 °C annealing temperature, and 30 s at 72 °C.

Microsatellite variability was assessed in 30 individuals of both species from the western Mediterranean. Observed and expected heterozygosities were calculated using arlequin version 2.0 (Schneider et al. 2000). The number of alleles per locus, allele size range as well as deviations from Hardy–Weinberg expectations and linkage disequilibrium between pairs of loci were estimated using fstat version 2.9 (Goudet 1995). Locus polymorphism ranged from moderate to high. Allele variability and heterozygosity estimates are listed in Table 1. We found no evidence of significant association between genotypes at pairs of loci within each sample. Three loci showed departure from Hardy–Weinberg proportions for the striped red mullet (Mb11, Mb46, Mb55). This could be due to the presence of null alleles segregating at high frequencies. Nonetheless, the results suggest that most of these loci are suitable for population genetic assessments of both species.

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References

Garoia F, Guarniero I, Piccinetti C, Tinti F (2004) First microsatellite loci of red mullet (Mullus barbatus) and their application to genetic structure analysis of Adriatic shared stock. Marine Biotechnology, 6, 446–452.

Glenn TC, Cary T, Dust M et al. (2000) Microsatellite Isolation. http://www.uga.edu/srel/DNA_Lab/protocols.htm.

Goudet J (1995) fstat (2.9.3) a program for IBM compatible PCs to calculate Weir and Cockerman's (1984) estimators of F-statistics. Journal of Heredity, 86, 485–486.

Hamilton MB, Pincus EL, Di Fiore A, Flesher RC (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. BioTechniques, 27, 500–507.

Hureau JC (1986) Mullidae. In: Fishes of the North-eastern Atlantic and the Mediterranean (eds Whitehead PJP, Bauchot ML, Hureau JC, Nielsen J, Tortonese E), pp. 877–882. UNESCO, Paris.

Labropoulou M, Eleftheriou A (1997) The foraging ecology of two pairs of congeneric demersal fish species: importance of morphological characteristics in prey selection. Journal of Fish Biology, 50, 324–340.

Mamuris Z, Apostolidis AP, Theodorou AJ, Triantaphyllidis C (1998) Application of random amplified polymorphic DNA (RAPD) markers to evaluate intraspecifc genetic variation in red mullet (Mullus barbatus). Marine Biology, 132, 171–178.

Mamuris Z, Stamatis C, Bani M, Triantaphyllidis C (1999) Taxonomic relationships between four species of the Mullidae family revealed by three genetic methods: allozymes, random amplified polymorpic DNA and mitochondrial DNA. Journal of Fish Biology, 55, 572–587.

Özbilgin H, Tosunoglu Z, Bilecenoglu M, Tokaç A (2004) Population parameters of Mullus barbatus in Izmir Bay (Aegean Sea), using length frequency analysis. Journal of Applied Ichthyology, 20, 231–233.

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

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN version 2.0: A Software for Population Genetics Data Analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Stergiou KI, Christou ED, Georgopoulos D, Zenetos A, Souvermezoglou C (1997) The Hellenic seas: physics, chemistry, biology and fisheries. Oceanography and Marine Biology: an Annual Review, 35, 415–538.