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Isolation and characterization of polymorphic microsatellite markers in *Abudefduf luridus* (Pisces: Pomacentridae)

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The bluefin damselfish, *Abudefduf luridus* is commonly found in rocky shallow waters of the Macaronesian Islands. During the breeding season males prepare and defend nests in an open rock within their territories. Females deposit egg masses in single layers (clutches) and return to their territories after spawning. Males care for the eggs until the hatching of planktonic larvae. Behaviour observations have shown that deserted nests are generally reoccupied by other males who take care of present eggs and begin attracting females, similar to the redlip blenny (Santos 1995). Competition for territories is high and is a central factor of mating and reproductive success of the males.

A. luridus is, therefore, a promising case study species for the genetic analysis of reproductive strategies and male mating success. For this purpose, we present in this paper the development of microsatellite markers for further population analysis.

Genomic DNA from *A. luridus* muscle tissue was isolated using a phenol-chloroform extraction protocol (Sambrook *et al.* 1989). Approximately 10 µg of DNA was digested with *Sau3A* (Gibco) and size selected (300–800 bp) by 1.5% LMP (low melting point) agarose gel electrophoresis. Microsatellite enriched partial genomic library was performed according to the procedure of Kijas *et al.* (1994) with modifications. Size-selected fragments were ligated to adaptors (AdapF: 5' CTCTTGCTTACGCGTGGACTC-3' and AdapR: 5'GATC-GAGTCGACGCGTAAGCAAGAGCACA-3') and hybridized with 5'biotinylated, 3'aminated (CT)₁₅ oligonucleotides bound to streptavidin-coated magnetic spheres (magnisphere, Promega, Madison, WI). The hybridization was followed by several washing steps in 2× SSPE (0.3 M NaCl, 20 mM NaH₂PO₄·H₂O, 2 mM EDTA, pH 7.4) and the enriched DNA was then eluted in water. DNA fragments were polymerase chain reaction (PCR) amplified, using AdapF as a primer, and ligated into *Bam*HI digested and dephosphorylated pUC19 vector (Promega). The ligation was performed overnight cycling between 10 °C 30 s and 30 °C 30 s, in 10 µL using, 50 ng vector, 50 ng purified PCR product, 30 mM Tris-HCl pH 7.8; 10 mM MgCl₂; 10 mM DTT; 1 mM ATP and 10 U of T₄ ligase (Promega, madison, WI). One tenth of the ligation was trans-

formed into 50 µL *Escherichia coli* DH5α supercompetent cells (Gibco BRL) following Sambrook *et al.* (1989). A total of 400 colonies were screened according to the method described in Waldbieser (1995), based on PCR amplification with pUC19 forward or reverse universal primers and a (CT)₁₀ primer. Forty-six clones with a clear amplification were digested with *Pvu*II restriction enzyme (Gibco) to determine the insert size. Thirty positive clones were selected and sequenced using the dideoxy chain termination method (Sanger *et al.* 1977). The sequencing reactions were performed manually (T7 sequencing Kit, Pharmacia), using α35S dATP and forward/reverse M13 universal primers. Sequencing products were then separated by electrophoresis on standard acrylamide denaturing gels. Among them, seven sequences allowed the definition of primers (Table 1). Clear amplification was obtained with the following conditions: 20 µL total volume containing 20–50 ng of genomic DNA, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.5–1.5 mM MgCl₂ (Table 1), 60 µM of dCTP, dGTP, and dTTP, 12 µM dATP, 0.16 µL α35S dATP (12.5 mCi/mL, 1250 Ci/mmol), 2 µM each primer, 1 U *Taq* DNA polymerase (Gibco Brl Life Technologies, Gaithersburg, MD). The PCR was performed in a Robocycler (Stratagene Cloning Systems, La Jolla, CA) and consisted of an initial denaturation of 4 min at 94 °C followed by 30 cycles for 60 s at 94 °C, 60 s at annealing temperature (Table 1), and 60 s at 72 °C, with a final elongation of 10 min at 72 °C. Microsatellite polymorphism was analysed in 25 adult individuals from three populations from the islands of Açores and Madeira. Parameters and tests were computed using the GENPOP software version 3.1b (Raymond & Rousset 1995). The seven loci show a relatively high polymorphism, with 2–13 alleles per locus. Both expected and observed heterozygosity were very similar over all populations (Table 1) for each locus, except for locus 5. Hardy-Weinberg equilibrium could not be rejected in each population, except for the same locus. A genotypic disequilibrium was found between locus 3 and 7 in population 2 ($P < 0.05$) and over all populations ($P < 0.05$). No genotypic nor genic differentiation was found between populations. This could be due to the small sample sizes ($n = 11, 9$ and 5).

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Table 1 Characteristics of seven microsatellite loci in *Abudefduf luridus*. Primer sequences, repeat motifs, annealing temperatures (T_a), PCR final concentrations of $MgCl_2$, number of alleles (for $n = 25$ individuals), observed (H_O) and expected (H_E) heterozygosities are shown

Locus	Primer sequences 5'-3'	Repeat motif	T_a °C	$MgCl_2$ (mM)	Sizes (bp)	No. of alleles	H_E	H_O	Accession no.
2AL2	F: CAAGTCAGACTCTGAACTCG R: TCAATGACTGAACCAGACAC	(CT) ₂₉	52	1	243	13	0.90	0.92	AF208493
2AL5	F: TTCTGTACCATCGGTTGCGT R: TGTTCCTACTTCGGGTTCCCTC	(AG) ₄ C(GA) ₁₀ GGA(GGTA) ₃	50	1	190	5	0.59	0.64	AF209036
2AL10	F: TAGTCTACCCAGGGGAGAA R: CCATAATCCACACTCCTGTGTC	(GA) ₁₉	52	1	151	9	0.84	0.88	AF209037
2AL13	F: GTCCTCTGAGCCACAGTGTGTA R: TGTCTGTAAGTCCGCTGATG	(CT) ₁₀ ...(CT) ₈	52	1	235	9	0.87	0.88	AF209038
2AL15	F: GACGCTGCTTCCTGTTGGC R: CCTCTGCGTGGTCATTTCTC	(AC) ₃ ...(AAG) ₈ G ₅ A(AG) ₄	50	0.5	127	8	0.76	0.56	AF209039
2AL24	F: ATGACACGCATGGCTAACCT R: CATAGACGCACATTGACTGG	(GGGA) ₂ ...(AGG) ₆	52	1	168	2	0.46	0.44	AF209040
AL34	F: ATCCTGCCCGCCTTCAGTA R: TGTTCAGAGTGTCTGCCCT	(GT) ₁₇	51	1.5	131	3	0.34	0.32	AF209041

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