Microsatellite markers for the roman, *Chrysoblephus laticeps* (Teleostei: Sparidae), an overexploited seabream from South Africa

PETER R. TESKE,* PAUL D. COWLEY,† FABIEN R. G. FORGET‡ and LUCIANO B. BEHEREGARAY*
Molecular Ecology Laboratory, Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia, †South African Institute for Aquatic Biodiversity, Grahamstown 6140, South Africa, ‡Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown 6140, South Africa

Abstract

Eleven polymorphic microsatellite loci were developed from an enrichment library of the roman, *Chrysoblephus laticeps*, and characterized for 40 individuals collected in Africa's largest and oldest Marine Protected Area (MPA), the Tsitsikamma National Park. The number of alleles per locus ranged from three to 19, and heterozygosities ranged from 0.20 to 0.85. A significant departure from Hardy–Weinberg equilibrium was detected for one locus, and linkage disequilibrium was identified among three pairs of loci. The markers will be useful to detect whether populations resident in MPAs along the South African coast are genetically connected, and whether there is spillover of recruits into adjacent exploited areas.

Keywords: conservation, fishery, gene flow, Marine Protected Area, phylogeography, sparid, Tsitsikamma National Park

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The roman, *Chrysoblephus laticeps*, is one of several endemic South African sparids whose stocks are considered to have collapsed as a result of fishing pressure (Sauer *et al*. 2003). The roman is considered to be particularly vulnerable, because in addition to the slow growth rate, late maturation and age-related sex reversal characteristic of sparids, this species is highly philopatric (Kerwath *et al*. 2007) and therefore unlikely to actively colonize suitable habitats that are not in close proximity to their home ranges. The roman still occurs at high densities in several South African Marine Protected Areas (MPAs), most notably in the Tsitsikamma National Park. While the establishment of MPAs is considered to be highly successful in managing the remaining populations (Kerwath *et al*. 2007), little is known about levels of gene flow between MPAs, and to what extent adults or larvae seed adjacent exploited areas and in that way improve fishing. Eleven microsatellites loci were isolated using an enrichment technique (Fischer & Bachmann 1998) modified as described in Beheregaray *et al*. (2004) to facilitate the study of these and other management issues.

Genomic DNA was isolated using a modified cetyltrimethyl ammonium bromide protocol (Doyle & Doyle 1990) from fin clips of 40 individuals sampled in the Tsitsikamma National Park. The DNA was digested with *Rsa*I and *Hae*III (Promega), and fragments were then ligated to two oligo adaptors. Four biotinylated oligo probes (dGA_{10}, dCA_{10}, $dAAC_{10}$, $dACAT_{10}$) were hybridized to the digested DNA and selectively retained using Streptavidin MagneSphere Paramagnetic Particles (Promega). Polymerase chain reactions (PCRs) were then performed on the microsatellite-enriched eluate using one of the oligo adaptors as primer. The enriched library was purified using the UltraClean15 DNA Purification Kit (MO BIO Laboratories, Inc.), ligated into pCR 2.1-TOPO Vector (Invitrogen) and transformed into One Shot TOP 10 Chemically Competent Cells (Invitrogen). Plasmid DNA was PCR-amplified directly from the positive colonies using M13(-20) forward and M13(-40) reverse primers, and PCR products were directly sequenced by Macrogen Inc. Out of a total of 149 putatively positive clones sequenced, 32 contained microsatellite sequences with sufficient flanking regions to design primers. Primers were designed using default parameters in Primer 3 version 0.4.0 (Rozen & Skaletsky 2000) and one GC clamp was specified, whenever possible. An M13 universal sequence (5′-TGTAAAACGACGGCCAGT-3′) was appended to the 5′ end of each forward primer to facilitate subsequent fluorescent labelling.

Correspondence: Peter R. Teske, Fax: 61 (2) 9850 7972;
E-mail: peter.teske@bio.mg.edu.au

Table 1 Characteristics of 11 microsatellite loci isolated from *Chrysoblephus laticeps*. Number of alleles (N_A), size ranges of amplified fragments, observed (H_O) and expected (H_E) heterozygosity were calculated based on 40 individuals from a single population. Forward primers were tagged with a 5′-end M13 universal sequence (5′-TGTAAAACGACGGCCAGT-3′), but size ranges shown excluded this portion of the sequences

Locus	Repeat structure	Primer sequences (5′–3′)	N_A	Size range (bp)	H_O	H_E	GenBank Accession no.
Clat1	$(CA)_2AA(CA)_6AA(CA)_{10}$	F: ATGATCTCCTTCTGGGATGG	19	274–324	0.86	0.94	FJ554535
		R: AGATTGCCAAAATGCCTCTG					
Clat2	$(GT)_{14}$	F: CAACATGGAGGGAGGAGATG	12	215–251	0.92	0.84	FJ554536
		R: ATGGACCACGTCTCCAGAAC					
Clat3	$(CA)_{11}$	F: TGGGTGAACAGACAATGGAAG	14	202–238	0.90	0.91	FJ554537
		R: GCTGGGGTTTGTGGGTAAAG					
Clat4	$(CTTT)_{12}$	F: TTGTCTGTCTGCCTTTGCTC	13	173–225	0.88	0.88	FJ554538
		R: TGGTCTGTTATCCCTGTTAAATG					
Clat5	$(GT)_7$	F: CAACAAGGCTATCAGCAAAGG	3	180–184	0.20	0.18	FJ554539
		R: ACCCAGGCAGATTCTCAATG					
Clat6	$(GT)_{11}$	F: CCAGTTTGATGCAGTCCTTTC	11	210–234	0.83	0.78	FJ554540
		R: CAAGGAACATGCGATTTCTG					
Clat7	$(CA)_{13}$	F: GCAATGGCTCCTGCATTAAG	16	213–251	0.95	0.88	FJ554541
		R: GAGGACAGGGGACATCTTTTC					
*Clat8**	$(GATA)_{11}$	F: AGGGAGCACAGAAGAACCAA	11	180–228	0.83	0.89	FJ554542
		R: AGCGTCTTTCACTCCCAGTC					
Clat9	$(GATA)_{14}$	F: CATACCATCGCCTGACACTG	11	226–274	0.90	0.88	FJ554543
		R: GCCACACTTGAGCCTTTCTC					
Clat10	$(AAG)_7$	F: TGCAAATGAAACACCAGACC	6	178–202	0.25	0.31	FJ554544
		R: CGCTCCCTCAGTATGACTCC					
Clat11	$(GATA)_7GATG(GATA)_4$	F: CCATCGCTTGACACTAGCAC	11	222–270	0.90	0.88	FJ554545
		R: GCCACACTTGAGCCTTTCTC					

*Significant deviation from Hardy–Weinberg equilibrium, $P < 0.05$.

Amplification of microsatellite loci followed the protocol described by Schuelke (2000), in which PCR products are fluorescently labelled through the inclusion of a labelled M13 primer. PCRs were performed in 10 μL volumes and contained 1.2 μL of 5× GoTaq Buffer (Promega), 2.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.2 U of GoTaq DNA polymerase (Promega), 0.05 mM of forward primer, 0.2 mM of reverse primer, 0.2 mM of fluorescent M13 primer (FAM, NED, VIC or PET), and 1 μL of DNA template. The PCR amplification profile consisted of an initial denaturing stage (94 °C for 3 min), a touchdown phase (94 °C for 30 s, five cycles of annealing from 63 to 55 °C in increments of 2 °C, and 72 °C for 1 min), 32 cycles of 94 °C for 20 s, 55 °C for 45 s and 72 °C for 1 min, and a final extension stage (72 °C for 5 min). Of the 32 primer pairs tested, 11 amplified consistently and were used for genotyping.

Amplification products were mixed with Hi-Di formamide and LIZ 500 size standard (Applied Biosystems) before electrophoresis on a 3130xl Genetic Analyser (Applied Biosystems). Microsatellite profiles were examined using GeneMapper 4.0 (Applied Biosystems) and peaks were scored manually. Tests for departures from Hardy–Weinberg equilibrium and for linkage disequilibrium, as well as estimates of expected and observed heterozygosities, were performed in Arlequin version 3.1 (Excoffier *et al*. 2005) using default settings. Bonferroni corrections (Rice 1989) were applied when conducting multiple statistical tests.

All 11 loci were polymorphic, and between three and 19 alleles per locus were identified, with a mean of 11.5 alleles per locus (Table 1). A single locus (*Clat8*) was not in Hardy–Weinberg equilibrium, possibly due to the presence of null alleles, and three loci showed consistent linkage disequilibrium among themselves (*Clat8*/*Clat9*, *Clat8*/*Clat11* and *Clat9*/*Clat11*).

With the possible exception of loci *Clat8*, *Clat9* and *Clat11*, we expect these microsatellites to contribute significantly towards improving our present understanding of population structure and gene flow of protected and exploited populations of roman, and in that way improve management practices for this threatened species.

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Microsatellite loci isolation in the Canarian common chaffinch (*Fringilla coelebs*) and their utility in other Canarian finches

N. M. SUÁREZ, E. BETANCOR and J. J. PESTANO

Departamento de Genética, Facultad de Medicina, Universidad de Las Palmas de Gran Canaria, Las Palmas, 35080 Gran Canaria, Spain

Abstract

The taxonomic classification of the common chaffinch (*Fringilla coelebs*) that inhabits the Canary Islands has been under debate for decades, mainly due to the absence of nuclear DNA analyses. In this study we describe the isolation and characterization of ten microsatellite loci (AAAG, AAAT and GT) from a La Palma specimen using an enrichment protocol. Two loci were monomorphic in the populations analysed (La Gomera and La Palma), but the remaining ones presented 2 or more alleles, with an average of 11.63 alleles per locus and an average observed heterozygosity of 0.735 (n = 44). All loci were tested for their utility in other Canarian populations and other finch species.

Keywords: Canarian finches, *Fringilla coelebs*, microsatellite

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The common chaffinch (*Fringilla coelebs*) is widely distributed throughout Europe, northern Africa and the Atlantic Islands (Azores, Madeira and Canaries). Particularly, this species occurs in five (Gran Canaria, Tenerife, La Gomera, La Palma and El Hierro) of the seven islands that constitute the Canary archipelago. The taxonomic classification of these Canarian specimens has been subject to debate for decades. According to morphological traits, three subspecies are recognized (Martín & Lorenzo 2001): one in El Hierro (*Fringilla coelebs ombriosa*), one in La Palma (*Fringilla coelebs palmae*) and another in Tenerife, La Gomera and Gran Canaria (*Fringilla coelebs canariensis*). However, other studies based on the electrophoretic mobility of different proteins (Baker *et al*. 1990) suggested the presence of two subspecies: one in El Hierro and La Palma, and another in La Gomera, Tenerife and Gran Canaria, corroborating the conclusion of previous morphometric studies (Grant 1979). It would be of interest to use nuclear codominant markers such as microsatellites to complement previous studies based on mitochondrial DNA markers (Marshall & Baker 1999). Five microsatellite loci isolated in *Fringilla teydea* species (Suárez *et al*. 2008) have demonstrated their utility in *F. coelebs* specimens; however, more microsatellite markers would be necessary to further investigate the differentiation and gene flow of the Canarian common chaffinch populations.

Correspondence: J.J. Pestano, Fax: +(34) 928 454379,
E-mail: jpestano@dbbf.ulpgc.es