

- Taylor AC, Sherwin WB, Wayne RK (1994) Genetic variation of microsatellite loci in a bottlenecked species: the Northern Hairy-nosed Wombat, *Lasiornhinus krefftii*. *Molecular Ecology*, **3**, 277–290.
- Ward RD, Woodwark M, Skibinski DOF (1994) A comparison of genetic diversity in marine, freshwater, and anadromous fishes. *Journal of Fish Biology*, **44**, 213–232.

Microsatellite characterization in the rainbow wrasse *Coris julis* (Pisces: Labridae)

T. GUILLEMAUD,*† R. STREIFF,*‡
R. SERRÃO SANTOS,§ P. AFONSO,§
T. MORATO§ and M. L. CANCELA*

*Universidade do Algarve, CCMar/UCTRA, P-8000 Faro, Portugal,
‡Universidade dos Açores, Departamento de Oceanografia e Pescas, P-9900,
Horta (Açores), Portugal

Keywords: Dinucleotide repeats, Labridae, tetranucleotide repeats

Received 23 October 1999; revision accepted 12 November 1999

Correspondence: Thomas Guillemaud. Present address: †USVE, INRA, BP 2078, 06606 Antibes cedex, France. Fax: (351) 289818353; E-mail: tguille@ualg.pt
Present address: ‡INRA, Laboratoire de Modélisation et de Biologie évolutive, 488 rue de la Croix-Lavit, 34000 Montpellier, France

The rainbow wrasse, *Coris julis* (Linnaeus 1758), is one of the most common fish in shallow coastal waters within its geographical distribution (e.g. Sanchez Delgado 1981; Lejeune 1987) that includes the Mediterranean, southern Black Sea and the North-Eastern Atlantic (Porteiro *et al.* 1996). Dispersion is apparently limited in the rainbow wrasse, giving that it shows sedentary habits and that migration movements have not been described to date in this species (Lejeune 1987). Thus, it constitutes a good model for investigating genetic drift and differentiation in marine fishes. In this study, we have developed microsatellite markers to analyse the genetic population structure of *Coris julis* throughout its distribution area.

A (CT)_n-microsatellite enriched partial genomic library was constructed following a modified protocol of Kijas *et al.* (1994). Fragments ranging from 300 to 600 bp of *Sau3A*-digested DNA were ligated into annealed *Sau3A* adaptors (AdapF: 5'-CTCTTGCTTACGCGTGGACTC-3' and AdapR: 5'-GATCGAGTCCACGCGTAAGCAAGAGCACA-3'). After denaturation, single stranded DNA was enriched for microsatellites through the hybridization with 5'-biotinylated, 3'-aminated (CT)₁₅ oligonucleotides bound to streptavidin-coated magnetic beads (MagneSphere, Promega, Madison, WI). The enriched DNA was eluted twice in 10 µL water for 20 min and then used as a template for a polymerase chain reaction (PCR) using AdapF as a primer to recover double stranded DNA fragments. The PCR product was directly ligated overnight at 4 °C into pGEM-T easy vector (Promega, Madison, WI) using a 1:1 insert:vector molar ratio. The ligation was performed in 10 µL using 50 ng vector, 8 ng purified PCR product, and three units of T4 ligase (Promega). One tenth of

the ligation was transformed into 50 µL supercompetant cells (X11-Blue MRF', Stratagene, La Jolla, CA). Eighty recombinant clones were screened for the presence of (CT)_n microsatellites and 16 for (GT)_n microsatellites using the PCR method described by Waldbieser (1995). Approximately 40% of the clones were positively scored for both the CT and GT motifs.

From 35 clones sequenced, using the dideoxy chain termination method (Sanger *et al.* 1977), 28 contained at least one microsatellite and three sequences were identical. Nineteen clones contained a CT-motif, four a GT-motif, three both CT- and GT-motifs, one an AAAC-microsatellite, and one an ACG-microsatellite. The two last clones were screened positively for the GT motif. These results seem to indicate that the enrichment procedure and the PCR screening are not highly specific to the motif used. Due to the positions of the microsatellites within the inserts, we could define primer pairs for 21 different loci. Seventeen loci were successfully amplified using the following conditions: a total volume of 20 µL contained 10 ng DNA, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, between 0.75 and 2.5 mM MgCl₂, 100 µM dATP, 60 µM each of the other dNTPs, 2.4 µM each primer, one unit of *Taq* DNA polymerase (Gibco BRL, Life Technologies Inc., Gaithersburg, MD), and 0.16 µL [α-³²S]dATP (12.5 mCi/mL, 1250 Ci/mmol). PCR was performed in a Stratagene Robocycler (Stratagene Cloning Systems, La Jolla, CA) and consisted of a first denaturation step at 94 °C for 4 min, followed by 30 cycles of 45 s at 94 °C, 45 s at 47 or 50 °C depending on the locus, and 45 s at 72 °C, with a final extension of 10 min at 72 °C. Ten loci were polymorphic and showed a number of alleles ranging from 2 to 23. Eight of them were easily scorable with the conditions showed in Table 1.

These eight microsatellites were scored for 20 individuals from Farol Island, Portugal. None of the loci showed a significant departure from Hardy–Weinberg equilibrium (GENEPOP 3.1b; Raymond & Rousset 1995), and no linkage disequilibrium was detected between each pair of locus. Therefore, these eight loci seem highly informative for populations studies.

Acknowledgements

We thank Pedro Neves for helping in sample collection from Portugal, and Jacques Lagnel for technical advice. This work was funded in part by project PRAXIS EMG 1957/95 (CLIFE). T.G. was the recipient of postdoctoral fellowship (PRAXIS XXI/BPD/4470/96).

References

- Kijas JMH, Fowler JCS, Garbett CA, Thomas MR (1994) Enrichment of microsatellites from citrus genome using biotinylated oligonucleotide sequences bound to streptavidin-coated magnetic particles. *Biotechniques*, **16**, 657–662.
- Lejeune P (1987) The effect of local stock density on social behaviour and sex change in the Mediterranean labrid *Coris julis*. *Environmental Biology of Fishes*, **18**, 135–141.
- Porteiro FM, Barreiros JP, Santos RS (1996) Wrasse (Teleostei: Labridae) of the Azores. *Arquipélago*, **14**, 23–40.
- Raymond M, Rousset F (1995) genepop (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Table 1 Microsatellite loci from *Coris julis*. Number of repeats, repeat motif and size of the amplification product from the clones are indicated. Annealing temperatures and MgCl₂ concentrations, number of alleles obtained and expected (H_E) heterozygosities are also indicated. Number of alleles and heterozygosities are based on a sample of 20 individuals from Farol Island. GeneBank accession numbers for the cloned sequences are AF190802–AF190809

Locus	Repeats	Primer sequence (5'–3')	Size (bp)	Anneal. temp. (°C)	MgCl ₂ (mM)	No. of alleles	H_E
D-11	(CAA) ₂ TAAA(CAA) ₇	F: GGACACTTCGACCACGAACC R: GTCACCTCTCTGAGCTAACTGTGC	176	50	1.25	6	0.74
D-3-3	(CT) ₆	F: GAAGCACCTTTCCAGGGGAT R: CAGAGGATTCCTGTGGTAAAC	85	47	0.75	3	0.55
6-1	(TG) ₃ TAA(GT) ₃ (GA) ₂ AAG(AC) ₆ CCTC(AC) ₄	F: GGTCACCGTTGTTGTTTGTTC R: GATCCCGTATGCCAAACAC	159	47	1	2	0.18
A-1	(CT) ₈	F: GTGCTGCTGAGACACTGCGA R: TGTCTTCAGCTCATGCCTCC	230	50	0.75	4	0.65
F-7	(TG) ₃ C(GT) ₉	F: GCTCAGTGAAGTAAACGGAGAGG R: TATCAGGAAGCGGCAGTGTG	243	50	0.75	6	0.67
G-2-2	(CT) ₇ (TC) ₂ CC(TC) ₅	F: AACATCCTTGTGAACACACG R: AGAACCTGCTGCCTCTCTGC	122	50	0.5	5	0.32
F-2-4	(GA) ₁₉ ...(TC) ₁₃ ...?(TC) ₅ ...(TC) ₅ ...(TC) ₁₂ *	F: AGGAGAACCAGAACATTACG R: CTGAACTCAAATAAAGTCG	~ 250**	47	2	4	0.64
E-4	(CT) ₁₇	F: ACTTCCTTGCACTCACACTC R: GGCTCAGACTTGAGGCTTAG	232	50	0.75	15	0.81

*The question mark corresponds to an unknown sequence of about 25 bp. **Approximate size as revealed from an agarose gel electrophoresis.

Sanchez Delgado F (1981) Contribution al conocimiento de los labridos (familia Labridae) de las costas ibericas. Parte I: Descripcion de las especies. *Boletin del Instituto Español Oceanografico*, **6**, 19–57.

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the USA*, **74**, 5463–5467.

Waldbieser GC (1995) PCR-based identification of AT-rich tri- and tetranucleotide repeat loci in an enriched plasmid library. *Biotechniques*, **19**, 742–744.

Characterization of microsatellite loci developed for the wattled curassow, *Crax globulosa*

COLIN R. HUGHES* and ERIK D. LARSON

*Department of Biology, University of Miami, Box 249118, Coral Gables, FL 33124–0421, USA

Keywords: Aves, Cracidae, curassow, microsatellite, polymorphic, primers

Received 13 November 1999; revision accepted 18 November 1999

Correspondence: C. R. Hughes. Fax: +1 305 284 3039; E-mail: hughes@fig.cox.miami.edu

The family Cracidae comprises 50 species of curassows, guans, and chachalacas (Monroe & Sibley 1993). All are large bodied Neotropical birds, and most are forest dwelling frugivores (Delacour & Amadon 1973). These characteristics

mean that species face double jeopardy; they are extensively hunted in addition to suffering from habitat destruction. As a consequence, 13 species are considered threatened species, including the wattled curassow (Collar *et al.* 1992). We developed and characterized six microsatellite loci in the wattled curassow as part of an effort to breed the birds in captivity. Additionally, we examined the polymorphism of these loci in six other members of the Cracidae.

DNA was extracted from blood or tissue using a proteinase K, phenol–chloroform extraction procedure modified from Müllenbach (1989). Size selected genomic DNA digested with *DpnII* was cloned into Lambda Zap Express, Stratagene, La Jolla, CA (Hughes *et al.* 1998). We screened ~ 125 000 clones with the oligo (AAT)₁₀ (Hughes & Queller 1993), sequenced 31 positives (Sequenase ver 2.0 or Thermosequenase, USB), and developed primers for 10 clones containing ≥ 6 uninterrupted repeats of the sequence AAT.

Polymerase chain reactions (PCRs) (5 µL) contained ~ 5 ng of DNA, 50 mM KCl, 10 mM Tris/Cl pH 8.3, 1.5% mM MgCl₂, 0.1% NP40, 100 mM each dNTP, 0.25 U *Taq* DNA polymerase (Perkin Elmer), 2.5 pmol each primer, and 0.05 µL ³⁵S dATP. Reactions were cycled using the 'tube-control' function of a Hybaid thermal cycler: 90 s at 92 °C, then 5 s at a suitable annealing temperature for each primer pair (Table 1), 5 s at 72 °C, 5 s at 92 °C, 30 times, and finally 90 s at 72 °C. Amplified fragments were resolved using 6% denaturing polyacrylamide gels. Allele length was determined by comparison to the sequencing products of M13.

Six loci tested were found to be polymorphic in the wattled curassow (Table 1). Original clones of these had