

PRIMER NOTE

Characterization of tetranucleotide microsatellite loci in a Lake Victorian, haplochromine cichlid fish: a *Pundamilia pundamilia* × *Pundamilia nyererei* hybrid

MARTIN I. TAYLOR, FIONA MEARDON,* GEORGE TURNER,† OLE SEEHAUSEN,†‡
HILARY D. J. MROSSO†§ and CIRO RICO*

*School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, Norfolk, UK, †Department of Biological Sciences, University of Hull, HU6 7RX, UK, ‡Institute for Evolutionary and Ecological Sciences, University of Leiden, PO Box 9516, 2300 RA Leiden, the Netherlands, §Tanzania Fisheries Research Institute (TAFIRI), PO Box 475 Mwanza, Tanzania

Abstract

The haplochromine cichlid fishes inhabiting Lake Victoria in East Africa are of great interest to evolutionary biologists. We have isolated and optimized six tetranucleotide and a single dinucleotide locus from a hybrid of the haplochromine cichlid fishes, *Pundamilia pundamilia* and *P. nyererei*. Characterization in 18 individuals of *P. nyererei* from a single wild population revealed between six and 18 alleles per locus, with expected heterozygosity ranging from 0.68 to 0.94. These loci will prove useful for investigations of population structure, and elucidating relationships between closely related species. An additional 26 unoptimized loci have been deposited with GenBank and the MEN database.

Keywords: cichlid, haplochromine, microsatellites, *Pundamilia*

Received 1 April 2002; revision received 9 May 2002; accepted 30 May 2002

The cichlid fishes inhabiting Lake Victoria represent one of the most spectacular vertebrate radiations on the planet. As many as 500 species are thought to have evolved within the last 200 000 years (Meyer 1993) or possibly as little as 14 600 years (Owen *et al.* 1990; Johnson *et al.* 1996, 2000), although see Fryer (2001) and Seehausen (2002). Environmental deterioration and the introduction of the Nile perch (*Lates niloticus*) led to a catastrophic extinction of many of the endemic cichlid species, although the impact on the rock-dwelling species appears to have been less devastating (Seehausen *et al.* 1997). While numerous dinucleotide loci are available for use in African haplochromine cichlid species (van Oppen *et al.* 1997; Wu 1998) the majority are dinucleotide repeats, and none have been developed specifically for Lake Victorian haplochromine species. We present a suite of easily scored tetranucleotide loci for the rock-dwelling, haplochromine cichlids *Pundamilia pundamilia* and *P. nyererei*, both endemic to Lake Victoria. These loci will prove useful for both evolutionary research and conservation programmes focusing on the haplochromine cichlids of Lake Victoria.

Whole genomic DNA was extracted from a single F1 aquarium hybrid specimen of *P. pundamilia* × *Pundamilia nyererei* using a salt extraction protocol modified from (Aljanabi & Martinez 1997). Total DNA was cut simultaneously with *RsaI* restriction enzyme and ligated onto SNX oligonucleotide linkers (Hamilton *et al.* 1999) by incubating for 2 h at 37 °C. The mixture was amplified using polymerase chain reaction (PCR), and the product hybridized with biotin-labelled (GATA)₁₀ oligonucleotides. The mixture was then enriched using streptavidin MagneSphere particles (Promega, Southampton, UK), with three washes in Tris-buffered saline Tween-20 (TBST) (25 mM Tris/Tris-HCl, 0.15 M NaCl, pH 7.5, 0.05% (w/v) Tween-20), three washes in 0.2 × Sodium chloride/sodium citrate buffer (SSC) (30 mM NaCl, 3 mM sodium citrate, pH 7.0), the enriched DNA was eluted in 100 µL Tris low EDTA buffer (TLE) (10 mM Tris; 0.1 mM EDTA, pH 8.0). The enriched single-stranded DNA was immediately PCR recovered, PEG (polyethylene glycol) precipitated, and A-tailed by incubating 21.6 µL of PCR product at 72 °C for 30 min with 0.5 µL 10× PCR buffer (Bioline, London, UK), 0.4 µL MgCl₂ (25 mM), 1 µL dATP (10 mM), 0.2 µL *Taq* (Bioline), 2.2 µL H₂O. The A-tailed PCR product was then ligated into a pGEM-T Easy

Correspondence: Martin I Taylor. E-mail: nitram8@hotmail.com

Table 1 Characterization of microsatellite loci isolated from a *Pundamilia pundamilia* × *P. nyererei* F₁ hybrid. AN is the GenBank accession no., T_A is the annealing temperature (Ppun5 and Ppun32 were amplified using a two-stage program). No. alleles is the number of alleles found in 18 individuals of *P. nyererei* (population Nyegezi North)

	Primer sequence (5'–3')	T _A (°C)	Repeat	Sequenced allele (bp)	Size range (bp)	No. alleles	Mg (mM)	H _O	H _E	AN
Ppun5	TGTTTGTGAGTCTTTTGTATCG* GCCCAATAAATACCAATGTGCAG	62/60	(GATA) ₄₃	269	199–267	15	2.0	0.89	0.94	AF491646
Ppun7	TGACCATCTGCACAAATAA† AGGCCTAAGTCCCCCTAACC	57	(GATA) ₃₀	239	189–299	17	2.0	0.89	0.92	AF491650
Ppun9	GAGTTGGGTTTCTGGTTG* TGCTGGAAATATTTCATGTCA†TC	57	(GATA) ₃₂	444	440–488	9	2.0	0.29	0.89	AF491647
Ppun17	TGTCCAAACTTTTGCATCC* CATCTCTCTCTCCACACC	57	(GATA) ₂₀	143	91–169	15	2.0	0.83	0.91	AF491649
Ppun20	AFTGCCCAFTTTCAGAAAG† TGGACATTTTCAGTAAGGAGAG	57	(GACA) ₈ (GATA) ₂₁	223	129–267	16	2.0	0.78	0.86	AF491652
Ppun21	GGTTGACAGCTGCAAAAAT† AGGCAGTGAACCTCTGCTCTC	57	(GATA) ₁₄ (GACA) ₇	339	311–389	18	2.0	0.78	0.94	AF491648
Ppun32	CCATTTAAATATACCTTCCACAC† CACAGGATGAGCAACAGAG	61/59	(GT) ₁₃ GG(GT) ₄	160	154–162	6	2.0	0.56	0.68	AF491651

*6-FAM-labelled primer; †HEX-labelled primer; ‡NED-labelled primer.

vector, and transformed into Epicurian coli® XL-10 Gold ultra competent cells (Stratagene, Ltd., Cambridge, UK). Recombinants were identified using blue/white selection. Synthetic biotin-labelled oligonucleotides (GATA)₁₀ were used to screen approximately 400 replated positive colonies which had been transferred to nylon membranes (Roche Diagnostics Ltd., Lewes, UK). The CDP-Star chemiluminescent detection kit (Sigma-Genosys Ltd., Cambridge, UK) was used to detect microsatellite-containing colonies. Glycerol stocks were prepared for positive colonies, 1 µL of which was used as a template in 50 µL PCRs using modified M13 primers (Bensasson *et al.* 2000). PCR products were cleaned using spin columns (Qiagen Ltd., Crawley, UK), sequenced using the Big-Dye kit (Applied Biosystems, Warrington, UK), and detected using the ABI 377 sequencer (Applied Biosystems). Forty-five of 79 positive clones contained microsatellite sequences, and 35 sets of primers were designed using the Primer3 web-based software package (Rosen & Skaletsky 1996).

The primers were then optimized using the following conditions. PCR mixtures (10 µL volume) consisted of ~20 ng of genomic DNA, 0.2 mM each dNTP (Promega), 5 pmol each primer, 0.26 U *Taq* polymerase (Bioline), between 1.0 and 2.0 mM MgCl₂ (see Table 1) and 1 µL 10× Mg free reaction buffer (Bioline). PCR amplification was carried out using a Perkin Elmer 3700 thermal cycler using the following parameters: 3 min at 94 °C for 1 cycle, followed by 94 °C for 30 s, annealing temperature (see Table 1) for 30 s and 72 °C for 30 s for 30 cycles, followed by 1 cycle of 10 min at 72 °C. Two loci (Ppun5 and Ppun32) were amplified using a two-stage protocol. This consisted of five cycles at an initial high annealing temperature, followed by 30 cycles at a lower annealing temperature. The annealing temperatures for the two stages are detailed in Table 1. Amplification products were resolved on an ABI 377 sequencer.

Of the 35 sets of primers designed, seven loci have currently been optimized which give easily scorable and repeatable patterns. An estimate of the variability at each of the loci was determined by scoring a sample of 18 individuals of *P. nyererei* from Nyegezi North in the Mwanza Gulf of Lake Victoria, Tanzania, collected in December 2000 (Table 1). Information on 26 unoptimized loci has been deposited in the MEN primer database (GenBank accession nos AY101470–AY101495).

Acknowledgements

We thank Sylvester Wandera, Mhoja Kayeba and Jongo Machuma for help with sampling at Nyegezi North and Martine Maan for other help in the field. Fiona Meardon was supported by a NERC postgraduate studentship. Martin Taylor was supported on NERC grant no. NER/A/S/2000/01246: 'Testing for sympatric parallel speciation through sexual selection in lake Victoria cichlid fishes'. Hilary Mrosso was supported on WOTRO grant WB 82–250.

References

- Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, **25**, 4692–4693.
- Bensasson D, Zhang DX, Hewitt GM (2000) Frequent assimilation of mitochondrial DNA by grasshopper nuclear genomes. *Molecular Biology and Evolution*, **17**, 406–415.
- Fryer G (2001) On the age and origin of the species flock of haplochromine cichlid fishes of Lake Victoria. *Proceedings of the Royal Society of London Series B—Biological Sciences*, **268**, 1147–1152.
- Hamilton MB, Pincus EL, Di Fiore A, Fleischer RC (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. *Biotechniques*, **27**, 500–507.
- Johnson TC, Kelts K, Odada E (2000) The holocene history of Lake Victoria. *Ambio*, **29**, 2–11.
- Johnson TC, Scholz CA, Talbot MR *et al.* (1996) Late pleistocene desiccation of Lake Victoria and rapid evolution of cichlid fishes. *Science*, **273**, 1091–1093.
- Meyer A (1993) Phylogenetic relationships and evolutionary processes in East-African cichlid fishes. *Trends in Ecology and Evolution*, **8**, 279–284.
- van Oppen MJH, Rico C, Deutsch JC, Turner GF, Hewitt GM (1997) Isolation and characterization of microsatellite loci in the cichlid fish *Pseudotropheus zebra*. *Molecular Ecology*, **6**, 387–388.
- Owen RB, Crossley R, Johnson TC *et al.* (1990) Major low-levels of Lake Malawi and their implications for speciation rates in cichlid fishes. *Proceedings of the Royal Society of London Series B—Biological Sciences*, **240**, 519–553.
- Rosen S, Skaletsky HJ (1996) Primer3. http://www-genome.wi.mit.edu/genome_software/other/primer3.html
- Seehausen O (2002) Patterns in fish radiation are compatible with Pleistocene desiccation of Lake Victoria and 14600 year history for its cichlid species flock. *Proceedings of the Royal Society of London Series B—Biological Sciences*, **269**, 491–497.
- Seehausen O, Witte F, Katunzi EF, Smits J, Bouton N (1997) Patterns of the remnant cichlid fauna in southern Lake Victoria. *Conservation Biology*, **11**, 890–904.
- Wu L (1998) Isolation of microsatellites in *Astatoreochromis alluaudi* and their cross specific amplifications in other African cichlids. *Molecular Ecology*, **8**, 895–906.