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PRIMER NOTE

Isolation and characterization of tetranucleotide microsatellite markers in a mouth-brooding haplochromine cichlid fish (*Pseudocrenilabrus multicolor victoriae*) from Uganda

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Abstract

Eight tetranucleotide microsatellite loci were isolated from the haplochromine cichlid fish, *Pseudocrenilabrus multicolor victoriae*, an important model species for studies in respiratory ecology, conservation, and evolution. We surveyed variation at these loci in 23 individuals from western Uganda, finding four to 19 alleles per locus and an average expected heterozygosity of 0.8575. These microsatellite loci will be used to examine gene flow and population structure in Ugandan *P. m. victoriae*.

Keywords: African cichlid, enrichment, population genetics, primer design, sequencing, tetranucleotide

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The mouth-brooding haplochromine cichlid, *Pseudocrenilabrus multicolor victoriae*, is found throughout the Nile River system and Lake Victoria basin (Greenwood 1965; Schierwater & Mrowka 1987). This eurytopic species inhabits a diversity of selective environments, including well-oxygenated lakes and rivers and hypoxic swamps. Its widespread distribution, coupled with its ability to colonize diverse selective regimes, has resulted in its use as a model system for the study of ecology, evolution, and conservation (e.g. Chapman *et al*. 2000; Rosenberger & Chapman 2000; Chapman *et al*. 2002). Population genetic studies are necessary to better understand population structuring and gene flow among selective regimes within this species.

DNA was extracted from one individual fish from the Lwamunda Swamp, surrounding Lake Nabugabo (a satellite lake of Lake Victoria), in southern Uganda (figure 1 in Rosenberger & Chapman 2000). Extraction was performed using DNeasy Tissue Kits (QIAGEN). DNA was enriched twice using the methods of Glenn & Schable (2005; updates are available at http://baddna.srel.edu/). In brief, the DNA was digested using *Rsa*I restriction enzyme (New England Biolabs), and simultaneously ligated to superSNX linkers. This was followed by hybridization to the following biotinylated oligonucleotides: $(AG)_{12}$ $(TG)_{12}$ $(AAC)_6$ $(AAG)_8$ $(AAT)_{12}$ $(ACT)_{12}$ $(ATC)_8$; $(AAAC)_6$ $(AAAG)_6$ $(AATC)_6$ $(AATG)_6$ $(ACAG)_6$ $(ACCT)_6$ $(ACTC)_6$ $(ACTG)_6$; $(AAAT)_8$ $(AACT)_8$ $(AAGT)_8$ $(ACAT)_8$ $(AGAT)_8$. Biotinylated DNA was then captured using Dynabeads (Dynal). Enriched DNA was recovered using polymerase chain reaction (PCR; conditions are given in Glenn & Schable 2005). The amplified enriched DNA was subjected to a second round of enrichment and then ligated into a cloning vector, and plasmids were transformed, using TOPO TA Cloning Kits (Invitrogen). A total of 192 clones with inserts were isolated and 96 plasmids were sequenced with M13 forward and reverse primers using BigDye Terminator version 3.1 (Applied Biosystems) and an AB-3130xl capillary sequencer. Sequences were assembled and edited using SEQUENCHER version 4.1 (Genecodes). Seventy-six of the 96 sequenced clones contained microsatellites.

Primers were designed using FASTPCR version 3.6.31 (Pmv1; Kalendar 2007) and PRIMER 3 (all other loci; Rozen & Skaletsky 2000) (Table 1). Primers were designed for 12

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Table 1 Characterization of eight tetranucleotide microsatellite loci in 23 *Pseudocrenilabrus multicolor victoriae* from western Uganda

Locus	Repeat	Primer sequence (5′–3′)	Forward primer label	T_a (°C)	[$MgCl_2$] (mM)	*n*	No. of alleles	Size range (bp)	H_O	H_E	GenBank Accession no.
Pmv1	$(ATCT)_{34}$	F: ACCCATCCCTAGCACCAAGG R: GTCACTGGTCAAACCTGGCAC	FAM	55.5	1.5	22	17	254–335	0.7826	0.9345	EF490438
Pmv3	$(TATC)_{37}$	F: AGCTTGGTAGGAAGGCATCA R: GCTTATTTATCTGATCTGTTCGTGA	VIC	53	2.5	23	14	156–330	0.9130	0.9130	EF490439
Pmv4	$(TAGA)_{45}$	F: CGCATTTAACCAAATGAGCA R: GACCCAGTGTGGAGTTCGAT	VIC	53	2.5	23	16	193–280	0.8696	0.9275	EF490440
Pmv6	$(ACAT)_4(GTAT)_{15}$ $(GT)_2(GTAT)_2$	F: ATGCACAAGTTGTATACAGACACA R: CACTCAAGGACACTCGCACT	PET	56	1.5	22.5	4	161–194	0.4348	0.4596	EF490441
Pmv9	$(GATA)_{28}$	F: AAAAATCCCTGATGGTGGTG R: CCCCTTCACAATGTTTACCA	VIC	52	2.5	22.5	19	161–275	0.8261	0.9434	EF490442
Pmv13	$(ACTC)_{31}$	F: TCCAGGATGAAATGAATGAAA R: CACGCTTGACACCAAATTAAA	FAM	50	2.5	23	10	140–184	0.9130	0.8744	EF490443
Pmv15	$(TCTA)_{38}(TG)_8$	F: CAAAAAGGAGGACATGTAAAGTGA R: TCCCTCTGGTGGCAAAAG	NED	52	2.5	23	12	145–199	0.9130	0.8870	EF490444
Pmv17	$(TAGA)_{39}$	F: GCAGGATGATTGAAGACAGC R: CTCAGTGTGGAGTTCGATCTTT	FAM	53	2.5	22	14	174–253	0.7826	0.9207	EF490445

T_a (°C), annealing temperature; [$MgCl_2$] (mM), final concentrations of $MgCl_2$ used in the study; *n*, number of successfully genotyped individuals per locus (0.5 indicates one missing allele in a genotype); H_O, observed heterozygosity; H_E, expected heterozygosity.

loci, of which eight were suitable after optimization and genotyping. Primers were tested using 23 individuals from the Mpanga River in western Uganda (figure 1 in Chapman *et al*. 1999). PCR amplification was performed using 8 μL reaction volumes containing 20 ng DNA, 1× PCR buffer [QIAGEN; contains Tris-Cl, KCl $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, and has a pH of 8.7 at 20 °C], 0.15 U/μL HotStart *Taq* (QIAGEN), 0.1 mM dNTP (GE Healthcare), and 0.12 μM of each primer. Final concentrations of $MgCl_2$ are listed in Table 1. PCR was performed using an ABI GeneAmp 9700, with the following conditions: initial denaturation at 95 °C for 10 min, followed by 45 cycles of 95 °C for 30 s, specific T_a (Table 1) for 40 s, and 72 °C for 40 s, followed by a final extension at 72 °C for 10 min. A reading mixture was prepared using 2 μL of PCR products, 0.15 μL of GENESCAN 500 Liz size standard (Applied Biosystems) and 8.5 μL of Hi-Di Formamide (Applied Biosystems). Electrophoresis was performed on an Applied Biosystems 3730xl DNA Analyser. The genotypes were analysed with GENEMAPPER version 3.7 (Applied Biosystems).

Linkage equilibrium and Hardy–Weinberg equilibrium were tested using GENEPOP version. 3.4 (Raymond & Rousset 1995; default settings for the Markov chain), and heterozygosities, number of alleles, and allele size ranges were determined using MICROSATELLITE ANALYSER version 4.05 (Dieringer & Schlötterer 2003). The presence of null alleles, large allele drop-out, and stuttering were determined using MICRO-CHECKER version 2.2.3 (van Oosterhout *et al*. 2004). No loci were in linkage disequilibrium ($P > 0.109$). Only one locus showed evidence for Hardy–Weinberg disequilibrium (Pmv1; $P = 0.0291$). Heterozygosities, the number of alleles, and allele size range per locus are listed in Table 1. No null alleles, allele drop-outs, or stuttering were detected. We conclude that these eight new loci are suitable for population genetic studies in *P. m. victoriae*.

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References

Chapman LJ, Chapman CA, Brazeau DA, McLaughlin B, Jordan M (1999) Papyrus swamps, hypoxia, and faunal diversification: variation among populations of *Barbus neumayeri*. *Journal of Fish Biology*, **54**, 310–327.

Chapman LJ, Galis F, Shinn J (2000) Phenotypic plasticity and the possible role of genetic assimilation: hypoxia-induced trade-offs in the morphological traits of an African cichlid. *Ecology Letters*, **3**, 387–393.

Chapman LJ, Nordlie FG, Seifert A (2002) Respiratory oxygen consumption among groups of *Pseudocrenilabrus multicolor victoriae* subjected to different oxygen concentrations during development. *Journal of Fish Biology*, **61**, 242–251.

Dieringer D, Schlötterer C (2003) MICROSATELLITE ANALYZER (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes*, **3**, 167–169.

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. *Methods in Enzymology*, **395**, 202–222.

Greenwood PH (1965) The cichlid fishes of Lake Nabugabo, Uganda. *Bulletin of the British Museum of Natural History (Zoology)*, **12**, 315–357.

Kalendar R (2007) FASTPCR: a PCR primer design and repeat sequence searching software with additional tools for the manipulation and analysis of DNA and protein. (www.biocenter.helsinki.fi/bi/programs/fastpcr.htm).

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.

Raymond M, Rousset F (1995) GENEPOP version 1.2.: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rosenberger AE, Chapman LJ (2000) Respiratory characters of three species of haplochromine cichlids: implications for use of wetland refugia. *Journal of Fish Biology*, **57**, 483–501.

Rozen S, Skaletsky HJ (2000) PRIMER 3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365–386. Humana Press, Totowa, New Jersey.

Schierwater B, Mrowka W (1987) Factors influencing oxygen consumption in the cichlid fish *Pseudocrenilabrus multicolor*. *Zoologischer Anzeiger*, **219**, 305–312.