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Novel polymorphic microsatellites from Florida red tilapia and cross-species amplification in Mozambique and Nile tilapia

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Introduction

Tilapia is the common name for nearly a hundred species of cichlid fish which originated in Africa (El-Sayed 2006). It is one of the most important food fish genera in the world (El-Sayed 2006). A few red tilapia variants have been commonly seen in fish markets. While the genetic background of these varieties is not well documented, their derivation is generally attributed to the crossbreeding between mutant reddish-orange Mozambique tilapia (*Oreochromis mossambicus*) with other tilapia species like Nile tilapia (*O. niloticus*) and blue tilapia (*O. aureus*). Florida red tilapia is thought to be the result of blue tilapia mating with Mozambique tilapia (El-Sayed 2006). In Nile and Mozambique tilapia, microsatellites have been isolated (Kocher *et al.* 1998; Yue and Orban 2002) and applied in genetic studies (e.g. Kocher *et al*. 1998), whereas in Florida red tilapia, no microsatellites are available. Preliminary tests of cross-species amplification of 30 microsatellites isolated from Nile and Mozambique tilapia revealed that around 30% of tested microsatellites could not amplify products in red tilapia (Feng Liu and Gen Hua Yue, unpublished data). Therefore, to facilitate a better understanding of the genetic variations in tilapia species, we have isolated 19 polymorphic microsatellites from Florida red tilapia, and characterized them in Nile and Mozambique tilapia. These 19 microsatellites were all polymorphic in Florida red tilapia with an average allele number of 4.90 per locus, and average expected and observed heterozygosities of 0.62 and 0.74 respectively. Fourteen out of the 19 microsatellites amplified polymorphic products in Mozambique tilapia and all 19 amplified in Nile tilapia, respectively. Fourteen out of 19 and 13 out of 15 microsatellites were in Hardy-Weinberg equilibrium (HWE) in Nile and Mozambique tilapia, respectively. Most microsatellites are useable in all these three types of tilapia, which will facilitate genetic studies in these species.

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Materials and methods

We constructed a DNA library enriched for CA-repeats and GA-repeats using DNA isolated from a Florida red tilapia. For the enrichment of microsatellites, biotinylated $(CA)_{10}$ and $(GA)_{10}$ probes, as well as streptavidin-coated magnetic beads (Pierce, Rockford, USA) were used (Fischer and Bachmann 1998). DNA enriched with CA-repeats and GA-repeats was amplified using a 21-mer adaptor as primers (Li *et al.* 2007). PCR products were ligated to a pGEM-T vector (Promega, Madison, USA). The ligated DNA was transformed into XL-10 blue super competent cells and then plated on LB agar plates containing 60 mg/L IPTG, 50 mg/L ampicillin and 40 mg/L X-gal. White clones were sequenced using BigDye chemicals (Applied Biosystems, Foster City, USA), M13 and M13R primers with the sequencer AB13730xl. Sequences were aligned using Sequencher (GeneCodes, Ann Arbor, USA). Primers were designed for each microsatellite. One primer of each pair was labelled with either a 6FAM or a HEX fluorescent dye.

Twenty Nile tilapia, 20 Mozambique tilapia and eight Florida red tilapia were collected from a wild population in China, a wild population in Africa and a cultured population in France, respectively. DNA from each individual was isolated using a method developed previously (Yue and Orban 2005). PCR amplification of microsatellites was performed on T-100 thermal cyclers (Bio-Rad, Hercules, USA) in a 25 μL reaction volume, each containing 20 ng DNA, 1× PCR buffer with 1.5 mM $MgCl_2$, 200 nM of each primer, 50 μM of each dNTP and one unit of DNA polymerase (Finnzymes, Vantaa, Finland). PCR was carried out using the following program: one cycle of 94°C for

Table 1. Characterization of 17 microsatellites in Nile, Mozambique and Florida red tilapia.

Locus, accession no.	Motif	Primer (5′–3′)	*A*	Size range (bp)	H_O	H_E	HWE (*P*)
OmRf001 JX156306	$(CA)_{15}$	F:GTACACGCTGGTCAGCAGGTGAT	5	304–341	0.75	0.74	0.40
		R: GAGGGGCACGTAAACAAGAATGAA	3		0.90	0.58	0.01
			5		0.50	0.74	0.06
OmRf004 JX156307	$(GA)_9$	F:CTGAATCGCAGCAGACAGAG	3	230–244	0.55	0.51	0.82
		R: TTTCTTGGGTTACCTTTCCTTC	5		0.40	0.58	0.24
			3		0.75	0.67	1.00
OmRf005 JX156308	$(GA)_{19}$	F:ACTGAGCCTGCATAACCCTGAAAG	5	352–379	0.95	0.67	0.08
		R: GAAGCCCTGCTCGATATGGTATGT	4		1.00	0.56	0.02
			6		1.00	0.83	0.24
OmRf006 JX156309	$(GT)_{12}$	F:AGACCCCGCTGTATCCAGGACT	6	361–375	0.75	0.84	0.00*
		R: ACTGTCCCCCTCCCCAGATTC	–		–	–	–
			4		1.00	0.74	0.53
OmRf007 JX156310	$(GT)_8$	F:GAGCTGGCAGAACAGTCCTCAT	6	260–296	0.30	0.74	0.00*
		R: ATTCAGCGGTTCTGTGGAGTCA	6		0.55	0.58	0.08
			5		0.50	0.73	0.32
OmRf009 JX156311	$(CA)_8$	F:ATGTGCACAAAGTCCTTTCTCTAA	5	302–402	0.74	0.74	0.27
		R: ACTCAGTGGTGCGGTTTACAAAT	–		–	–	–
			8		0.38	0.90	0.00*
OmRf0011 JX156312	$(CA)_7$	F:TGGCTGTGCTTTTATATGTCATCT	5	166–182	0.75	0.65	0.80
		R: AAGGGGCTTCATAAATACACCATC	1		0	0	–
			4		0.88	0.65	0.31
OmRf0012 JX156313	$(TG)_{11}$	F:TGCTCGCTGAAGACAGTTCTGACA	7	205–245	0.45	0.64	0.09
		R: TTTTGGGCTTCCAGTTCACTTCAA	7		0.85	0.85	0.22
			9		0.75	0.91	0.22
OmRf0013 JX156314	$(CA)_{11}$	F:ACAAAGCACAAAGACAGAACTCA	5	316–338	0.85	0.79	0.39
		R: GGAGACATGACAAGGCATCCT	4		0.40	0.35	1.00
			6		0.40	0.83	0.13
OmRf015 JX156315	$(CA)_{12}$	F:ATGGGAGGGGAGGAGGAGTCTT	4	325–341	0.15	0.42	0.00*
		R: GCGCGTGTTTGCAATTCACTAC	6		0.45	0.75	0.00*
			4		0.88	0.74	0.48
OmRf0016 JX156316	$(GT)_7$	F:GTGCGGGCCAGTGAGTGAGTC	2	365–373	0.90	0.50	0.00*
		R: CCTGCAATGCGTCCTGTCATAGAA	2		0.95	0.51	0.00*
			3		0.75	0.54	0.63
OmRf0018 JX156317	$(CA)_{12}$	F:CGCGGAAATACAGTGCAATGTTAT	4	334–348	0.60	063	0.43
		R: TTTGTCCTGTTCACTGTGCTTGAC	3		0.60	0.53	0.51
			4		0.62	0.72	0.52
OmRf0019 JX156318	$(GT)_9$	F:CTTCGCGGCTCAGATTACCACAT	4	261–277	0.65	0.60	0.77
		R: GTTGGCTGCGGGCACAGAC	3		0.50	0.53	0.62
			2		0.12	0.46	0.09
OmRf0020 JX156319	$(GT)_{12}$	F:GGCGTGTTTGTCTGAGCTGTTACC	8	387–417	0.95	0.81	0.12
		R: AACGGCTGAATAGGTTGGCTCTGT	4		0.50	0.42	0.64
			5		0.50	0.78	0.07
OmRf0022 JX156320	$(CA)_6$	F:CCTTTCCCACTTATCCTTCTGTTT	2	366–372	0.35	0.30	0.60
		R: CTCACTTTCCCCTGTCTACCACTT	1		0	0	1.00
			3		0.50	0.67	0.26
OmRf0023 JX156321	$(GT)_{12}$	F:GGGACTTGGGGCCTTTTGGTA	5	176–197	0.50	0.62	0.08
		R: TGCATATGGCAGGGGACTCTTT	4		0.30	0.54	0.07
			3		0.13	0.61	0.01
OmRf0024 JX156322	$(CT)_{22}$	F:CCCAAACACACTCACTGCCTCT	6	99–151	0.85	0.78	0.54
	$(CA)_{14}$	R: CTGATGCTATTGATACTGATTATCG	7		0.65	0.81	0.07
			6		0.63	0.80	0.37

2 min, 57°C for 30 s, and 72°C for 2 min, followed by 36 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s with a final extension for 5 min at 72°C. One microlitre of each PCR product was separated using the sequencer ABI3730xl (Applied Biosystems, Foster City, USA). Sizes of PCR products were analysed using GeneMapper (Applied Biosystems, Foster City, USA). Allele number, observed and expected heterozygosities, HWE and linkage

Table 1. (*contd.*)

Locus, accession no.	Motif	Primer (5′–3′)	*A*	Size range (bp)	H_O	H_E	HWE (*P*)
OnRf0025 JX156323	$(CA)_{14}$	F:AGCCCAAGTCATTTCACGGATTT	9	204–238	0.85	0.84	0.35
		R: TTTCCCCTCTTTGTCTCCCATAGA	–		–	–	–
			6		0.63	0.85	0.27
OmRf0026 JX156324	$(GT)_{13}$	F:TGGCGTTAGAGCGCTGAGGATT	6	178–225	0.40	0.70	0.00*
		R: CAGGGCTTTGGCCTCCACTGTA	9		0.80	0.83	0.63
			7		0.75	0.83	0.13

In each row, the upper, middle and lower data of *A*, size, H_O and H_E are for Nile, Mozambique and Florida red tilapia, respectively. *A*, allele number in each population; H_O, observed heterozygosity; H_E, expected heterozygosity; HWE, Hardy–Weinberg equilibrium; –, no amplification. All microsatellites were amplified at annealing temperature 55°C. *Significant deviation from HWE after Bonferroni correction.

disequilibrium were analysed using GDA software (Lewis and Zaykin 2000).

Results and discussion

One hundred and ninety-two clones were sequenced. Sixty-eight unique microsatellites were identified. Primers were designed for 22 microsatellites containing enough flanking sequences. Nineteen out of 22 primer pairs amplified specific and polymorphic products. All 19 microsatellites were polymorphic in Florida red tilapia, with an average allele number of 4.9 per locus, ranging from 3 to 8 alleles (table 1). The expected and observed heterozygosities were 0.74 and 0.62, respectively. Among the 19 microsatellites, 19 and 15 amplified specific and polymorphic PCR products in Nile and Mozambique tilapia respectively, suggesting that Florida red tilapia is more closely related to Nile tilapia than Mozambique tilapia. In Nile and Mozambique tilapia, the average allele number of polymorphic markers was 5.1 per locus and 5.6 per locus, respectively. Fourteen out of 19 and 13 out of 15 microsatellites were in HWE in Nile and Mozambique tilapia, respectively. These results indicate that the majority of these novel microsatellites will be useful in studying genetic diversity of these tilapia species, which will facilitate selective breeding for genetic improvement for growth and disease resistance of these species. It is necessary to develop multiplex PCR to facilitate fast and cost-effective genotyping microsatellites in three tilapia species for genetic studies (Chamberlain *et al.* 1990).

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