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Primer Sequences and Cross-species Amplification for Parentage Discrimination of Tanganyikan Cichlid Fishes

Hiroyuki MUNEHARA[1], Satoshi AWATA[2], Rei KATOH[2], Masanori KOHDA[2] and Tomoki SUNOBE[3]

Abstract

It was done that isolation and cross-species amplification of mictosatellite loci for parentage analysis of the Tanganyikan cichlids. The sequences of the primers and the results of cross-species amplification were reported.

Key words: Cichlidae, Tanganyika, cross-species amplification, microsatellite loci

Since the late 19 th century, many ichthyologists have studied the radiation of cichlid fishes in Lake Tanganyika. There are more than 170 species of cichlids in Lake Tanganyika, most of which are endemic (Kohda et al., 1997), and Lake Malawi may contain even more species (Ribbink et al., 1983). We are studying the evolution of allopaternal care in the Tanganyikan cichlids, *Chalinochromis brichardi, Neolamprologus meeli, Julidochromis ornatus*, and *Telmatochromis temporalis*.

Microsatellite genotyping has proven to be a useful tool for phylogenetic (Lee and Kocher, 1996; Zardoya et al., 1996; Neff et al., 2000) and parentage analysis (e. g. Kellogg et al., 1995; Parker and Kornfield, 1996) of the cichlids. We designed six primer sets to amplify microsatellites in the four Tanganyikan species to search for genetic markers. Four primer sets (Pze1-4) from the Malawian cichlid fish, *Pseudotropheus zebra*, sequenced by van Oppen et al. (1997) were also tested in these four species. We described the sequences of the primers and the results of test amplifications.

Pieces of muscle from fish collected in Lake Tanganyika were fixed in 70% ethanol. The genomic DNA for the four cichlid species studied was isolated from this tissue. The DNA was extracted with a standard phenol-chloroform procedure after a reaction in lysis buffer. The procedures for the construction of the genomic libraries, isolation of plasmids containing $(GT/CA)_n$ regions, and sequencing the plasmids were the same manner as those described in Munehara and Takenaka (2000). Four loci containing perfect repeats of more than $(GT/CA)_{15}$ were screened from each genomic library of *C. brichardi* and *N. meeli*, and used to design the PCR primers (Table 1). The level of polymorphism for each locus was estimated using 10-36 individuals from different families in each species. Species and cross-species amplification with our 4 primer

Table 1. Primer sequences of useful microsatellite loci of two cichlid fishes.

Source	Code name	Primer sequence (5′-3′)	Accession number
Chalinochromis brichardi	Chb1	TTCTTTCAGGCTCTAGCTTTCC AAGCTCAGAGTCTGCATGTGC	AB012117
C. brichardi	Chb2	ATGTCAATTGAAGGTCCTAACT TCAGCAAACCTCAACTTAA	AB012118
Neolamprologus meeli	Nem1	TGGTGCAATTCAAACTTTTCTG CCTTTCCTAACTTCTTGGGG	AB012119
N. meeli	Nem2	CACAAACCAAGCAGAGGCTTA CTTAACGAGGTCAAATCTGTTG	AB012120

[1] *Usujiri Fisheries Station, Field Science Center for Northern Biosphere, Hokkaido University, Usujiri 152, Minamikayabe, Hokkaido 041-1613, Japan*
(北海道大学北方生物圏フィールド科学センター臼尻水産実験所)
[2] *Laboratory of Animal Sociology, Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka 558-0022, Japan*
(大阪市立大学理学部動物社会研究室)
[3] *Natural History Museum and Institute, Chiba, 955-2, Aoba-cho, Chiba 260-0852, Japan*
(千葉県立中央博物館)

Table 2. Polymorphism of some microsatellite loci for three cichlid fishes. Amplifications more than 0.50 in heterozygosity were showed in this table.

Chalinochromis brichardi

Code name	Annealing temp. (°C)	Length of PCR products (bp)	No. of alleles	Heterozygosity		Sample size
				observed	expected	
Chb1	58	88, 92, 110, 114, 116	5	0.60	0.56	30
Nem2	52	134, 136, 142	3	0.70	0.57	30

Neolamprologus meeli

Code name	Annealing temp. (°C)	Length of PCR products (bp)	No. of alleles	Heterozygosity		Sample size
				observed	expected	
Chb2	54	90, 96, 98, 100	4	0.55	0.66	30
Nem1	52	101, 111, 113, 115	4	0.57	0.65	30
Pze4	54	112, 114, 126, 128	4	0.70	0.62	30

Julidochromis ornatus

Code name	Annealing temp. (°C)	Length of PCR products (bp)	No. of alleles	Heterozygosity		Sample size
				observed	expected	
Chb1	61	range of 96-138	15	0.56	0.66	36
Chb2	54	range of 97-170	17	0.69	0.83	36
Nem1	50	range of 103-121	8	0.58	0.49	36
Pze3	50	range of 314-392	17	0.81	0.82	36

Telmatochromis temporalis

Code name	Annealing temp. (°C)	Length of PCR products (bp)	No. of alleles	Heterozygosity		Sample size
				observed	expected	
Pze2	46	range of 152-222	20	0.88	0.93	34
Pze4	54	range of 122-144	11	0.76	0.84	34

sets and cross-species amplification with the 4 primer sets from the Malawian cichlid (van Oppen et al., 1997) were performed in a thermocycler, following the manufacturer's recommendation (Perkin-Elmer). An initial denaturation at 95°C for 5 min was performed before the addition of *Taq* DNA polymerase (TaKaRa Co.). The reaction was carried out for 23 or 25 cycles of 30 s at 94°C, 50 s at the annealing temperature (Table 2), and 80 s at 72°C. The procedures described in Munehara and Takenaka (2000) were used to detect PCR products. The length of the PCR products was estimated with 10 bp or 20 bp ladder markers and PCR products from individuals used for primer design.

Cross-species amplification successfully identified heterozygous alleles of *C. brichardi, N. meeli, J. ornatus*, and *T. temporalis*, and useful primer sets varied with species (Table 2). Successful cross-species amplifications with Pze 2, 3 and 4 suggested that primers from Tanganyikan and Malawian cichlid fishes could provide useful information for parentage analysis.

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