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Utility of Microsatellite Markers for Eel Study

Abstract

The Japenese eel *Anguilla japonica* is generally assumed to be composed of a single population with wide distribution range, and some genetic studies using allozyme or mitochondrial DNA methods supported this population model. However, one genetic study suggested the existence of multiple populations in this species, and thus, more detailed studies on the population structure of this species is needed. Here, we characterized a total of twenty-one microsatellite markers of the Japanese eel. These will serve as powerful tools for detailed population study for the Japanese eel, though some of them showed the significant departure from the Hardy-Weinberg expectations.

Key words: Japanese eel *Anguilla japonica,* Microsatellite primers, Marine fish

The Japanese eel *Anguilla japonica,* is one of the most important fishery resources around East Asia. In recent years, the catch of glass eels of this species has decreased drastically(1). Because producing artificial seedlings is still unsuccessful(2), the wild glass eels are the exclusive source of eel aquaculture. Over-fishing as well as pollution and degradation of freshwater habitats are assumed as the reason for the decrease of glass eels(1). At any rate, it is important to clarify population structure of the Japanese eel for rational stock management and enhancement.

The Japenese eel is generally assumed to be composed of a single population. Three genetic studies supported the single population for this species(3-5). On the other hand, one genetic study's suggestion of existence of plural populations has been made on the basis of observation of geographical cline of allele frequency at some isozyme loci at the glass eel stage(6). Therefore, the possibility of existence of plural populations with different spawning timings would not be ruled out for the Japanese eel, and clarifying population structure would be difficult by allozyme and mitochondrial method. In this paper we describe the identification of highly variable microsatellite markers which provide of powerful tool for the population study of the Japanese eel.

Total genomic DNA was extracted using a modified phenol-chloroform extraction procedure following Ishikawa et al.(5) from a ethanol-preserved muscle tissue of an individual of *A. japoica,* and was digested with *Alu* I and *Hae* III, and size-selected (300 – 800 base pairs) by excision from an agarose gel. Fragments were cloned into a dephosphorylated pUC118 digested with *Hinc* II vector (Takara). Ligation products were subsequently transformed into

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competent *Escherichia coli* JM109 cells (TOYOBO). Approximately 30,000 recombinant colonies were grown on nitrocellulose membranes, and clones were hybridized with $(TG)_{10}$, $(TC)_{10}$, $(AGC)_6$, $(ACT)_7$, $(ACG)_6$, $(GTC)_6$, CT $(ATCT)_6$ and $(TGCA)_6$TG probes labeled with fluorescein-11-dUTP using Gene Images 3'-oligolabelling module (Amersham pharmacia), and were screened using Gene Images CDP-Star detection module (Amersham pharmacia). A total of 527 positive clones were isolated, and each one was amplified using polymearse chain reaction (PCR) with M 13 (-21) forward and reverse primers. A total of 342 of these clones were sequenced using an ABI 377 Sequencer following the supplier's Big Dye protocol (Applied Biosystems). Primers were designed on the basis of sequences flanking the repeat regions using the web cgi program Primer3[7] for 48 loci (Table 1).

Table 1. Characteristics of 21 Japanese eel microsatellites.

Locus	*Repeat motif*	*Primer sequences (5'-3')*	*Product size (range) (bp)*	*No. of alleles*	*Ta (°C)*	*N*	*He*	*Ho*	*DDBJ accession number*
AjTR-01	$(AGC)_3CT(GC)_4(AG)_3$	CTGTGTCAGGCTGTTTAACG CAGGTCCCTGATAACTCACTG	157(151-157)	4	50	24	0.593	0.167*	AB051080
AjTR-04	$(GT)_{15}$	CACCCTTGCCCTATTTTGATA GCTGAGTCATGATCACCTGT	207(167-217)	17	55	22	0.918	0.591*	AB051081
AjTR-05	$(CA)_{18}TA(CA)_3$	GGAGCAGTATGGAATAACATGA CATGTATTTACATAGGGGATGA	195(177-205)	14	50	22	0.854	0.818	AB051082
AjTR-11	$(TGC)_3(ATGC)_4N_6(TCAC)_{11}$	TGCAAGGTCAATGTCAAGG TAATGACACCCACTTAGATC	197(180-200)	6	55	20	0.764	0.400*	AB051083
AjTR-12	$(AG)_{14}$	AACGTTAGTCCCTAGGTTCC TAAGGGTGTTATATGTTCAG	157(154-214)	17	55	16	0.893	0.875	AB051084
AjTR-15	$(AC)_5GTG(CA)_{12}A_3(CA)_7$	GCCATATGATCGAACAGAT AGGAGTGACTGGGGTTTC	154(143-179)	15	60	21	0.942	0.619*	AB051085
AjTR-17	(AC)10	GTTATGCACTCACGCTAA ATCACCATTATTCTTTCTGA	151(139-169)	13	55	22	0.871	0.500*	AB051086
AjTR-22	$(TC)_7(AC)_{15}AAG(CA)_3$	CTATTCCCTCAACTTCACCGGC GAATTACCTGTCATTCCAA	123(103-129)	10	50	22	0.710	0.500*	AB051087
AjTR-23	$(TC)_{11}(AC)_{11}AA(AC)_3$	GGATAGAGAACAAACGCAGT GGACATGAACTTCTTACACAGA	260(238-276)	15	50	21	0.915	0.857	AB051088
AjTR-24	$(AC)_{13}N_{15}(AC)_{13}$	AAACAACATACACCAATACC ATGATCCCTCTGAATGATA	140(120-186)	14	50	21	0.818	0.810	AB051089
AjTR-25	$(ACAT)_2(AC)_6CA(AC)_7A_3G_2(CA)_9$	GCATACACGATTACATGCAC ACATAAAGGTGACCGGAAC	219(145-289)	24	60	21	0.931	0.762	AB051090
AjTR-26	$(AC)_3GCA(TC)_2(AC)_5C2(AC)_{14}$	TGCAGGTGCAGTATTAACAG ATTGTGAGGACAAATGATGG	192(182-222)	9	60	20	0.803	0.900*	AB051091
AjTR-27	$(TC)_8(CT)_3(CCT)_2$	GTCCTCCAGCCATCATTTGT CTTTGGCATTCTTACGCTCA	161(157-167)	6	60	22	0.624	0.500	AB051092
AjTR-35	$T_4(TG)_{14}TT(TG)_6CC(TG)_6$	AATGTGTGCATTGAAATAAGAG TAAGCATCCACACTTCAGACT	273(246-294)	16	60	21	0.913	0.524*	AB051093
AjTR-37	$(TG)_{14}$	AGACCTTATGTCACCTTATGCT AAGATGTTAAATTCAATTGTGC	200(188-202)	8	55	14	0.842	0.857	AB051094
AjTR-40	$(TG)_4(GA)_3N_{13}(CA)_5$	ATTCAGCATCACATTACTTTAGAA TTCAGTCCCTATTCTGAGATTATTA	202(184-202)	9	55	17	0.846	0.588*	AB051095
AjTR-42	$(TG)_{12}$	GTGAACATTGATCCTATTCATAATC ATAACTAGCCCTACTAACTGTTTTG	245(241-251)	6	60	24	0.738	0.458	AB051096
AjTR-43	$(ATTT)_9$	ATTCAGTGAAATGGAAAGTAATAAT CAGATTTAATCAGTTGAAGTCTTTG	190(178-208)	15	55	21	0.884	0.381*	AB051097
AjTR-44	$(CT)_{14}CC(CT)_2TTC(CA)_6$	GAACCTCTCTTGACTGATTG AGATATTATTACTTTCCATTTCACT	167(153-187)	16	50	21	0.908	0.524*	AB051098
AjTR-45	$(CT)_3T(TC)_2C(CT)_6$	GCGCATGGAGAACTCTAAT CAATAGAGTGAGGACAGTAGA	149(141-165)	12	60	22	0.871	0.864	AB051099
AjTR-48	$(GT)_7TA(GT)_{16}$	GTTCAACTGTAGTGTATCATCAG GGCAGGAAGATAAGACCT	178(166-202)	13	55	17	0.886	0.471*	AB051100

N: The number of individuals examined; Ta: Annealing temperature used; Ho: Observed mean heterozygosity; He: Expected heterozygosity.

*: Indicates the significant departure from HW expectations.

Twenty-one primer sets produced bright resolvable products and were polymorphic within 14 - 24 individuals of glass eels of *A. japonica* preserved in ethanol. Genomic DNAs of these individuals were extracted from muscle tissue using a modified phenol-chloroform extraction procedure mentioned above. PCR amplification was carried out using ABI GeneAmp system 9700 (Applied Biosystems) in 15μL of reaction mixture containing 100ng of template DNA, 1xPCR buffer (Takara, 10mM Tris-HCl (pH8.3), 50 mM KCl), 0.2 mM dNTP, 2 mM $MgCl_2$, 5 – 20 μM fluorescent dCTP (Applied Biosystems), 0.5μM of each primer and 0.2 units of Taq Polymerase (Takara). Following an initial denaturation step of 3 min at 94°C, the reactions underwent 35 thermal cycles (94°C for 30 s, 30 s at the annealing temperature (Table 1) and 60 s at 68°C). PCR products were visualized on an ABI 310 and the size of amplification was determined using HD 400 [ROX] size standards and the GENESCAN 3.1 software (Applied Biosystems).

The number of alleles ranged from 4 to 24 (average = 12.3) per locus. Indices of variability of each locus were calculated using genepop 3.2a[8]. Observed heterozygosity (Ho) ranged from 0.17 to 0.90 (average = 0.62), and expected heterozygosity (He) ranged from 0.59 to 0.94 (average = 0.83). The lack of correspondence between Ho and He is not unexpected considering the disparity between the large number of alleles per locus and the relatively small number of individuals examined. The significant departure from the Hardy-Weinberg expectations was observed at twelve (AjTR-01, AjTR-04, AjTR-11, AjTR-15, AjTR-17, AjTR-22, AjTR-26, AjTR - 35, AjTR - 40, AjTR-43, AjTR - 44, AjTR - 48) of the twenty-one polymorphic loci. Such departure might be ascribed to the presence of null alleles because significant deficiencies of heterozygotes were observed ($P<0.05$). Allelic variability at these loci may be ascertained using revised new primers. However, other nine loci will be useful for population study of *A. japonica.*

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