Microsatellite-centromere mapping in the Japanese eel (*Anguilla japonica*) by half-tetrad analysis using induced triploid families

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Abstract

Genetic improvement of the Japanese eel (*Anguilla japonica*) can be achieved by artificially controlling its life cycle using recent advances in reproductive biology. In this study, we developed 43 microsatellite loci to confirm Mendelian inheritance at 10 of them as well at 16 previous reported in two full-sib families produced by artificial insemination. In order to establish a base for aquaculture genetics of this species in the near future, these microsatellite loci were mapped in relation to the centromere by half-tetrad analysis using four artificially induced triploid families. The second division segregation frequency (y) of the microsatellite loci ranged from 0.008 to 0.968 (mean ± SD = 0.645 ± 0.298). These results suggest the presence of strong chiasma interference in the eel. Significant differences were observed for the map distances of microsatellite loci between the two isolation procedures. Microsatellites isolated using the enrichment procedure were mapped to various sites starting from the centromere to the telomere, whereas those from the conventional size-selected library showed a tendency to be distributed in the telomeric region.

Keywords: *Anguilla japonica*, gene-centromere mapping, half-tetrad analysis, interference, microsatellite, triploid

1. Introduction

The Japanese eel (*Anguilla japonica*) is one of the most important species in aquaculture because of its high economic value, particularly in East Asia. Recently, we succeeded in obtaining artificially matured gametes by using hormonal treatment (Ohta et al., 1997). Viable leptocephali (Tanaka et al., 2001) and glass eels (Tanaka, 2003) have been successfully produced by the development of appropriate rearing techniques.

Genetic improvement will gain importance in eel aquaculture when mass production of artificially propagated glass eels is realized. Genetic mapping is one of the most effective approaches for understanding the genome of the target species. In aquacultural organisms, linkage mapping and its application to the effective selection of desirable traits by using comparative syntenic information of other species is a more practical approach than determining whole genome sequences for each species.

Microsatellite loci are useful markers for genetic mapping (see Reviews, Chamvers and MacAvoy, 2000). In the genus *Anguilla*, several authors have reported a number of microsatellite markers that have been mainly used for population studies (Daemen et al., 1997; Ishikawa et al., 2001; Tseng et al., 2001; Wirth and Bernatchez, 2001); however, the existent number of markers is still too small to construct a linkage map.

Recent advances in the artificial induction of maturation of the Japanese eel have made it possible to produce progeny for genetic studies by artificial fertilization of mature gametes in the laboratory. This enables the use of chromosome manipulation techniques on eel zygotes. In our previous study, we successfully induced triploid individuals for the first time in the Japanese eel by the heat shock treatment, which inhibited the second polar body release after normal fertilization (Nomura et al., 2003). The production of triploid families enables the application of half-tetrad analysis for

genetic mapping. Half-tetrad analysis can be performed if two of the four strands from a single meiosis can be recovered (Zhao and Speed, 1998). In fish species, triploids or gynogenetic diploids produced by the inhibition of the second polar body release provide a means to analyze meiosis II (MII) half-tetrads (Zhao and Speed, 1998). The recombination rate between the gene or marker and the centromere can be estimated from the frequency of recombinant heterozygous genotype in the half-tetrad progeny of the heterozygous mother. The proportion of heterozygous progeny is a measure of the frequency of second division segregation (y). Thus, the gene-centromere distance can be estimated by using an appropriate map function. The G-C map provides genetic information, such as the distribution of marker or gene loci along the chromosome, and it is the key to evaluating the success of the meiotic gynogenesis and the mitotic gynogenesis, which results from polar body inhibition and cleavage inhibition, respectively.

In this paper, we developed 43 microsatellite loci and, mapped 10 of them as well as 16 previously reported loci relative to the centromere of the eel chromosomes, by using four triploid families produced by inhibition of the second polar body release.

2. Materials and methods

Development of microsatellite markers

Microsatellite array of the Japanese eel was isolated using two methods: one from the partial genomic libraries that were selected for obtaining small insert size DNA and the other from the microsatellite (MS)-enriched genomic libraries.

The first procedure was performed in a manner similar to that used for the loach *Misgurnus anguillicaudatus* (Teleostei: Cobitidae)(Morishima et al., 2001) with the following modifications. The genomic DNA that was isolated from the blood of a cultured eel was digested with *Hae*III; the digested fragments were then electrophoresed on a 0.8% agarose gel in order to select fragments with sizes ranging between 300 and 600 bp. These size-selected fragments were ligated into the *Srf*I site of pPCR-Script™ Amp SK (+) vector, and transformed by using PCR-Script™ Amp Cloning kit according to the manufacturer's protocol (Stratagene, La Jolla, CA, USA).

The second method of microsatellite isolation was performed by using FIASCO (Fast Isolation by AFLP of Sequences Containing repeats) as described by Zane et al. (2002) along with some modifications. Briefly, the genomic DNA isolated from the blood of a cultured eel was digested with *Mse*I and ligated to *Mse*I AFLP adapters (5´-TACTCAGGACTCAT-3´/5´-GACGATGAGTCCTGAG-3´), or digested with *Hae*III and ligated to *Hae*III (blunt end) adapters (5´-CTCTTGCTTACGCGTGGACTA-3´/5´-pTAGTCCACGCGTAAGCAAGAGCACA-3´; Edwards et al., 1996). The digestion-ligation mixture was used as template for PCR, which was performed using an *Mse*I AFLP adapter-specific primer (5´-GATGAGTCCTGAGTAAN-3´) or a *Hae*III adapter-specific primer (5´-CTCTTGCTTACGCGTGGACTA-3´). The DNA was then hybridized with a biotinylated $(AC)_{17}$ probe and captured by streptavidin-coated beads. The

beads-probe-DNA complex was separated from the hybridization buffer by using a magnetic field. Nonspecific DNA was removed by three nonstringency washes and three stringency washes. The DNA was separated from the beads-probe complex by a denaturation step and used as template for PCR, which was performed using each adapter-specific primer. The PCR products were ligated to the pDrive Cloning Vector, and transformed by QIAGEN PCR Cloning kit following the manufacturer's protocol (QIAGEN, Hilden, Germany).

The resulting genomic libraries were screened using an alkaline phosphatase-labeled $(GT)_{10}$ probe. The positive colonies were suspended in distilled water and boiled for 10 min. The solution was used as a template for PCR employing T3 and T7 primers. The PCR products were purified and sequenced on an ABI prism 373A autosequencer using an FS cycle sequencing kit (Applied Biosystems, Foster City, CA, USA).

Forward and reverse primers were designed based on the unique flanking regions of each microsatellite repeat. In particular, the forward primers of each microsatellite locus isolated from the MS-enriched libraries were designed to include the 15-bp M13 sequence (5´-AGTCACGACGTTGTA-3´) at their 5´ end for the M13-tailed primer method described by Zhou et al. (2002). Newly developed microsatellite loci were serially named as *Ajp-1*, *Ajp-2*, and *Ajp-N*. Sequence data were deposited in DDBJ under the following accession numbers **AB194929-AB194936**, **AB233944-AB233978**.

Experimental diploid and triploid families

Cultured eels purchased from a commercial farm were acclimated to seawater at the National Research Institute of Aquaculture, Mie, Japan. Hormonal treatment was carried out for artificial maturation, as described previously (Ohta et al., 1996; Kagawa

et al., 1997). Four females (1-4) were repeatedly injected with salmon pituitary extract, followed by injection with 17α, 20β-dihydroxy-4-pregnen-3-one (SIGMA, St. Louis, MO, USA). Similarly, four males (1-4) were repeatedly injected with human chorionic gonadotropin (Teikoku hormone MFG Co. Tokyo, Japan). The gametes were obtained by gently stripping ovulating females and mature males. Thirty to fifty grams of the eggs were inseminated with 10 ml of prediluted milt (Ohta et al., 1996) and then divided into two groups; control groups to generate normal diploid families for testing Mendelian inheritance, and heat shock groups to generate triploid families for the half-tetrad analysis. Each group was stocked in a glass container containing filled with 500 ml of filtered seawater (pore size of filter, 0.2 μm) containing 100,000 IU/l of penicillin G potassium (Banyu Pharmaceutical, Tokyo, Japan) and 0.1 g/l of streptomycin sulfate (Meiji Seika, Tokyo, Japan). The water temperature was maintained at 23°C ± 1.0°C throughout the embryogenesis except for the period of heat shock treatment. The eggs of heat shock groups were immersed in the seawater kept at 37°C for 3 min, starting at 10 min after fertilization (m.a.f.) for families 1 and 2, and the eggs were treated from 5 to 8, 7 to 10, 10 to 13 m.a.f. for families 3 and 4. Damaged eggs and embryos, which were clouded and never hatched, were removed with a glass pipette in order to prevent a decline in the quality of seawater.

Ploidy determination and DNA extraction

The larvae were sampled from each group at two days post hatching (d.p.h), fixed with 70% ethanol, and stored at –20°C until analysis. A small piece of each larva was used to assess of the ploidy level by flow cytometry (FCM) using the PA type flow cytometer (Partec, Münster, Germany) as described previously (Nomura et al., 2003). The residue body of each larva was used for DNA extraction using Wizard SV Genomic DNA Purification System (Promega, Madison, WI, USA).

Microsatellite genotyping

Genotyping was carried out by the following two methods.

(1) The normal PCR were performed in a reaction mixture (10 μl) containing 50 ng template DNA, 40 μM dNTPs, 0.75 pmol of each primer, and 0.1 U of ExTaq polymerase (TaKaRa, Tokyo, Japan) under the following conditions: 30 cycles of denaturation for 1 min at 93°C, annealing for 30 s at 55°C-60°C, and extension for 30 s at 72°C. The PCR products were electrophoresed on 15% nondenatured polyacrylamide gels and visualized by SYBR Green I (TaKaRa, Tokyo, Japan) using UV transillumination. The microsatellite alleles were designated according to their molecular sizes (base pairs), which were estimated from a 20-bp DNA ladder (TaKaRa, Tokyo, Japan).

(2) The M13-tailed primer method and automated collection method were carried out as described previously by Zhou et al. (2002) with the following modifications. PCR was performed in a reaction mixture (10 μl) containing 50 ng template DNA, 40 μM dNTPs, 0.3 pmol M13-tailed forward primer, 3.0 pmol reverse primer, 3.0 pmol fluorescently labeled M13-tailed primer (5´-6FAM or PET or NED or VIC-CCCAGTCACGACGTTGTA-3´), and 0.025 U of TaKaRa Taq polymerase (TaKaRa, Tokyo, Japan) under the following conditions: denaturation for 1 min at 94°C, 30-40 cycles of denaturation for 15 s at 94°C, annealing for 15 s at 56°C, and extension for 30 s at 72°C, followed by final extension for 1 h at 72°C. Typical combinations of markers for capillary electrophoresis were prepared by combining PCR products for markers having alleles with a difference of at least 100 bp in size and different fluorescent labels. One microliter of each PCR product was added to 10 μl of HiDi formamide and 0.1 μl of ROX standard (Gene Scan 500 LIZ Size standard, Applied Biosystems, Tokyo, Japan) for genotyping and electrophoresis on an ABI PRISM 3130

Genetic Analyzer (Applied Biosystems, Tokyo, Japan). The genescan output files were analyzed using Gene Mapper 3.7 software (Applied Biosystems, Tokyo, Japan).

Microsatellite-centromere (M-C) mapping using triploids

Microsatellite genotypes were screened in all four-parent pairs used for triploidization. The M-C mapping by half-tetrad analysis can be carried out only at the locus for which the maternal genotype is heterozygous and the paternal alleles can be distinguished from the maternal alleles. The M-C recombination rate (second meiotic division segregation frequency = y) was estimated from the frequency of recombinant heterozygotes in triploid progeny at the locus that is genetically heterozygous in the mother eel. Assuming complete chiasma interference, the map distance in centimorgans (cM) will equal 100 (y/2).

3. Results

Microsatellite marker

Fifty microsatellite loci were isolated from the size-selected genomic library comprising approximately 4,300 clones (positive clone percent = 1.16%). Also, 72 microsatellite loci were isolated from the MS-enriched library comprising approximately 450 clones (positive clone percent = 16.00%). These clones were sequenced, and 28 primer sets (*Ajp-1~28*) selected from the size-selected library and 50 primer sets (*Ajp-29~78*) selected from the MS-enriched library were designed to amplify specific microsatellite loci. The remaining loci were not used for reasons such as proximity to the cloning site, degenerate repetitive sequences in one of the flanking regions, extremely small or extremely large microsatellites, and/or an unreadable sequence of the repeat region. Of the 28 loci selected from the size-selected library of the 50 loci selected from the MS-enriched library, 8 and 35 loci, respectively, yielded reproducible PCR products that corresponded to a single locus of the expected size (see Appendix A). Furthermore, cross-amplifications were obtained using the 7 primer sets from *A. anguilla* and *A. rostrata*. New primers were designed for loci that produced a smear or nonspecific amplification and tested again for amplification under various PCR conditions; however, the results were unsatisfactory and these loci were not used for further analysis. Sequences of primers and annealing temperatures of 68 microsatellite loci that used in this study are listed in Appendix A.

Triploidization

The rates of triploidy ranged from 0% to 5% in the control groups. The triploidy rates in families 1 and 2, which were subjected to heat shock starting at 10 m.a.f., were

6.9% and 6.2%, respectively; and families 3 and 4, which were subjected to heat shock starting at 5, 7, and 10 m.a.f., had triploid rates of 35.8% and 30.5%, respectively.

Families and microsatellite loci screening for M-C mapping

Of the 68 loci screened for the half-tetrad analysis, 26 loci in four families were heterozygous in the female parent and the male parent had alleles that could be distinguished from the maternal alleles. Of these loci, 18 (*Ajp-33, 45, 48, 49, 55, 58, 70, 76, 77, AjTR-5, 12, 22, 23, 24, 42, 43, 44,* and *Ang075*) were in family 1, 7 (*Ajp-7, AjTR-5, 12, 15, 22, 23,* and *Ang114*) were family 2, 12 (*Ajp-33, 49, 55, 58, 76, 77, AjTR-12, 23, 24, 44, 48,* and *Ang101*) were in family 3, and 14 *(Ajp-7, 33, 45, 48, 49, 55, 70, AjTR-11, 15, 17, 22, 43, 44,* and *45*) were in family 4.

Mendelian segregation

Genotypes of the 25 of the 26 loci examined in one or two full-sib families (family 1 and/or 2) showed Mendelian segregation and are suitable as genetic markers (Table 1). The exception, *Ajp-45* in family 1, showed significant segregation distortion (Chi-square test, $P < 0.005$).

At some microsatellite loci, inconsistent genotype segregation was observed between parents and offspring in Family 1 and/or 2. For example, the observed parental genotypes of *AjTR-48* in Family 2 were *122*/*122* in females and *132*/*126* in males. Although, the genotype is frequencies of offspring would be expected to be equal for *122*/*132* and *122*/*126*, individuals with only one allele, either *132* or *126,* were observed. The genotypes observed in the offspring -- *122*/*132*, *122*/*126*, *null*/*132*, and *null*/*126* -- can be explained in the female genotype was *122*/*null*. Similar distortions observed at 5 other loci; *AjTR-11* in Family 1, *AjTR-44* in Family 2, *Ang075* in Family 2, and *Ang101* in Families 1 and 2, can also be explained by the occurrence of a null allele.

Microsatellite-centromere recombination

The microsatellite-centromere recombination frequencies (second division segregation frequencies = *y*) for the 26 loci in one, two, or three triploid families are shown in Table 2. The frequency of *y* ranged from 0.008 at *Ajp-55* to 0.968 at *AjTR-12*. The mean *y* value was 0.645 for the 26 loci and the value of *y* at *AjTR-12* was approximately equal to one. This result indicates the presence of complete or near complete chiasma interference in the eel. Because the distribution of *y* values were uneven and biased toward a high value, we compared the distributions of *y* between two groups of loci, *Ajp-33~70* (7 loci) and *AjTR-5~48* (13 loci), which developed by different isolation procedures. The distribution of *y* differed significantly between *Ajp-33~70* obtained from an MS-enriched library (*Mse*I-digested inserts) and *AjTR-5~48* obtained from a size-selected library (300-600bp *Alu*I- and *Hae*III-digested inserts)(Fig. 1, Mann-Whitney test, $P < 0.01$).

Two non-recombinant maternal homozygotes occurred at almost equal frequencies. In most cases, the segregation of the paternal loci was in good agreement with the expected Mendelian segregation ratio. However, five paternal loci, namely, *Ajp-45* in triploid family 1, *Ajp-49* in triploid family 3, *AjTR-22* in triploid family 4, *AjTR-23* in triploid family 2, and *AjTR-44* in triploid family 3 showed significant segregation distortion ($P < 0.05$).

4. **Discussion**

Isolation efficiency of microsatellite loci

The percentage of clones containing dinucleotide repeats in the size-selected libraries was 1.16%. Previous studies of Japanese eel had success rates of 1.76% (Ishikawa et al., 2001) and 3.82% (Tseng et al., 2001). The mean frequency observed in 16 species of fish was 3.1% (Zane et al., 2002). In contrast, the percentage of positive clones in the MS-enriched libraries was 16.0%. The isolation frequency increased by approximately 10 fold as compared to that of the size-selected method; however, this frequency was quite low as compared to that (50%-95%) reported by Zane et al. (2002). The MS-enriched method is more efficient than the conventional size-selected procedure for isolating microsatellite loci because the costs and times required to build libraries is similar.

Inheritances of microsatellite loci

Inheritances of the 26 microsatellite loci, which were mapped in relation to the centromere, were examined by using two full-sib families. All loci showed Mendelian inheritance except *Ajp-45*, which also had significant segregation distortion for both full-sib family 1 and triploid family 1, which originated in the same parents. Segregation distortion was not observed at *Ajp-45* in the triploid family 4, which was the product of different parents. These results suggest that the segregation distortion observed at *Ajp-45* is not a locus-specific phenomenon but one that depends on the families involved. Possibly, *Ajp-45* is linked to the trait locus that affects survival of larvae. It is not likely that this segregation distortion generally affects the value of *Ajp-45* as a genetic marker.

In the analysis of triploid families, significant distortion was observed only in the

segregation of paternally derived alleles in the five loci. We have not determined the reason behind this interesting phenomenon.

Null alleles in microsatellite loci

We inferred the presence of null alleles from analyses of the full-sib families. Null alleles at microsatellite loci were reviewed in detail by Dakin and Avise (2004). The null alleles detected in the present study are probably a result of "poor primer annealing due to nucleotide sequence divergence," which is one of the three factors resulting in a null allele as classified by Dakin and Avise (2004). Such types of null alleles have also been reported in other fish species (Jones et al., 1998; Holm et al., 2001). These null alleles were confirmed by the fact that the allelic segregation law is contradicted in the full-sib families. If recognition of the existence of a null allele in the pedigree analysis is possible, such an allele can be treated as a null allele analogous to the "*O*" allele of the human ABO blood group system. In this study, the allelic segregation at the loci in which null alleles were assumed, followed the Mendelian laws of inheritance, and segregation distortion was not observed. However, when these markers are used for population genetics studies, it is necessary to apply a model that considers the existence of null alleles in order to prevent bias caused by typing errors due to the null alleles.

Distribution of y values and chiasma interference

In this study, the mean of *y* values at 26 microsatellite loci was 0.645, and the distribution of *y* was uneven and biased toward a high value. Distributions of *y* biased toward a high value have also been reported at 34 microsatellite loci in the pink salmon

(*Oncorhynchus gorbuscha*), 101 anonymous DNA loci flanked by paired interspersed nuclear elements (PINEs) (Lidner et al., 2000), 37 allozyme loci (Matsuoka et al., 2004), and at 10 microsatellite loci in the zebrafish (*Danio rerio*) (Kauffman et al., 1995). In contrast, a relatively even distribution of *y* has been reported at 168 amplified fragment length polymorphisms (AFLPs) in the pink salmon (Lidner et al., 2000), at 25 allozyme loci in the raibow trout (*Oncorhynchus mykiss*) (Allendorf et al., 1986), and at 15 microsatellite loci in the loach (*Misgurnus anguillicaudatus*) (Morishima et al., 2001).

The cause of the biased distribution of *y* toward the telomeric region observed in the present study may be related to: (1) distribution of the recombination spot along the chromosome arm, (2) distribution of the isolated microsatellite loci in the genome, and (3) the relationship between the strength of the chiasma interference and the chromosome length.

Generally, the frequencies and distributions of the recombination are not random. Recombination decreases in the neighborhood of the telomeric region and centromeric heterochromatin, while it does not occur in the centromeric heterochromatin (Hawley and Walker, 2003). Consequently, the distribution of *y* does not reflect the precise physical location of the marker loci, even if the distribution of marker loci is physically uniform.

It is likely that the bias of the genome fragments that are generated during the isolation procedure would influence the distribution of *y* rather than the actual distribution of the loci in the whole genome. The composition of the cloned fragments is probably more related to the processes used for genome fragmentation than to the ligation into the cloning vector. The distribution of *y* of microsatellite was different between the two types of isolation procedures (Fig. 1). These differences may be attributed to the differences in the recognition sites of the restriction enzymes used for the fragmentation of the genome. The recognition site for *Mse*I, which was used for the

isolation of *Ajp-33~70*, is TTAA and is biased toward A and T. On the other hand, the recognition sites for *Alu*I and *Hae*III, which were used for the isolation of *AjTR-5~48*, are AGCT and GGCC respectively, and are biased toward G and C. The recognition sites for the restriction enzyme are probably more abundant in the AT-rich region for *Mse*I and in the GC-rich region for *Alu*I and *Hae*III. Viñas et al. (1994) observed a difference in the G-band patterns of the chromosomes when *Mse*I and *Hae*III were used in the European eel (*Anguilla anguilla*). This result clearly suggests that the distributions of the recognition sites for these restriction enzymes may be different in the eel genome. Lidner et al. (2000) reported that in the pink salmon, the distribution of *y* at AFLP loci was different from the microsatellite loci and PINEs. Their results suggest that the distribution of *y* among different types of markers may depend on the restriction enzyme used. Hence, the choice of procedure used for isolating microsatellite loci is important. If uniformly distributed markers are to be produced, the genome should be randomly cut using a sonication technique, etc.

In meiosis, a recombination event interferes with additional; recombination in the adjacent area of the same chromosome. Chiasma interference normally decreases with increased distance and finally disappears. However, when an extremely strong chiasma interference exists generally within on organism or on a specific segment of a chromosome, recombination may occur only once per bivalent chromosome. In this study, the *y* values of several loci exceeded 0.667 and the maximum *y* value was 0.968. From these results, we can conclude: (1) there is a strong chiasma interference and (2) recombination does not occur between sister chromosomes or the chiasma interference does not interfere with the recombination between the homologous chromosomes even if recombination between sister chromosomes occurs.

The value of *y* exceeding 0.667 has also been reported in other fish species (Thorgaard et al., 1983; Streisinger et al., 1986; Arai et al., 1991; Kauffman et al., 1995;

Lindner et al., 2000; Morishima et al., 2001; Matsuoka et al., 2004); and this phenomenon appears to be common in fishes. Here, the *y* value is estimated under the following conditions: (1) the existence of strong chiasma interference, (2) a uniform physical distribution of markers, and (3) an even distribution of recombination spots on a chromosome. The *y* value linearly increases with the G-C distance near the centromeric region and exceeds 0.667 due to the influence of the chiasma interference. Subsequently, the *y* value stabilizes at 0.667. Therefore, the distribution of *y* is biased toward the telomeric region.

Perspective of aquaculture genetics in the eel

In this study, by using four induced triploid families, we mapped 26 microsatellite loci in relation to the centromere of the eel chromosomes. The loci included ones that we developed as well as those reported by other researchers. In the future, we intend to prepare a high-resolution linkage map with the location information of the centromere by using these microsatellite loci. Such integration of a gene-centromere map and linkage map will satisfy an essential requisite not only in elucidating syntenies among different species but also in identifying commercially important quantitative traits in the aquaculture species. To apply marker-assisted selection (MAS) to improvement of eel strains genetically for aquaculture, further genetic mapping must be conducted and new chromosome manipulation techniques must be developed.

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Figure caption

Figure 1. Relationship between isolation procedure of microsatellite loci and distribution of second division segregation frequency (*y*) of each microsatellite loci. Distribution of *y* were significantly different between *Ajp-33~70* originated from a MS-enriched library (*Mse*I-digested inserts) and *AjTR-5~48* originated from a size-selected library (300-600 bp *Alu*I-, *Hae*III-digested inserts) (Mann-Whitney test, $P < 0.01$). Bar (-) indicates the mean of *y* value.

Table 1.
The genotypic segregation in 26 microsatellite loci in two full-sib families of the Japanese eel.

Locus	Family	Genotypes (bp)						Total	d.f.	χ^2
		Female	Male	Progeny						
				Observed (Expected)						
Ajp-7	1	*146/140*	*140/140*	*146/140*	*140/140*			38	1	0.105
				20 (19)	18 (19)					
	2	*146/140*	*152/148*	*146/152*	*146/148*	*140/152*	*140/148*	37	3	1.000
				8 (9.25)	8 (9.25)	11 (9.25)	11 (9.25)			
Ajp-33	1	*135/131*	*127/119*	*135/127*	*135/119*	*131/127*	*131/119*	43	3	4.907
				11(10.75)	15(10.75)	5(10.75)	12(10.75)			
Ajp-45	1	*163/157*	*184/142*	*163/184*	*163/142*	*157/184*	*157/142*	44	3	13.273**
				3(11)	10(11)	11(11)	20(11)			
Ajp-48	1	*198/183*	*181/141*	*198/181*	*198/141*	*183/181*	*183/141*	41	3	4.756
				8(10.25)	7(10.25)	16(10.25)	10(10.25)			
Ajp-49	1	*226/222*	*216/212*	*226/216*	*226/212*	*222/216*	*222/212*	43	3	0.673
				12(10.75)	12(10.75)	9(10.75)	10(10.75)			
Ajp-55	1	*160/158*	*156/156*	*160/156*	*158/156*			44	1	3.273
				16(22)	28(22)					
Ajp-58	1	*148/138*	*144/142*	*148/144*	*148/142*	*138/144*	*138/142*	43	1	1.744
				12(10.75)	12(10.75)	7(10.75)	12(10.75)			
Ajp-70	1	*114/106*	*132/108*	*114/132*	*114/108*	*106/132*	*106/108*	39	3	1.513
				11(9.75)	7(9.75)	9(9.75)	12(9.75)			
Ajp-76	1	*154/146*	*148/144*	*154/148*	*154/144*	*146/148*	*146/144*	34	3	4.824
				10(8.5)	6(8.5)	13(8.5)	5(8.5)			
Ajp-77	1	*186/172*	*180/162*	*186/180*	*186/162*	*172/180*	*172/162*	39	3	1.513
				7(9.75)	9(9.75)	12(9.75)	11(9.75)			
AjTR-5	1	*194/170*	*189/175*	*194/189*	*194/175*	*170/189*	*170/175*	38	3	2.632
				13 (9.5)	10 (9.5)	9 (9.5)	6 (9.5)			
	2	*198/178*	*194/194*	*198/194*	*178/194*			38	1	0.105
				18 (19)	20 (19)					
AjTR-11	1	*109/null*	*109/105*	*109/109 or 109/null*		*109/105*	*null/105*	37	2	4.135
				21 (18.5)		12 (9.25)	4 (9.25)			
	2	*117/89*	*117/101*	*117/117*	*117/101*	*89/117*	*89/101*	38	3	6.211
				16 (9.5)	6 (9.5)	8 (9.5)	8 (9.5)			
AjTR-12	1	*137/133*	*175/139*	*137/175*	*137/139*	*133/175*	*133/139*	37	3	7.216
				16 (9.25)	6 (9.25)	6 (9.25)	9 (9.25)			
	2	*145/143*	*163/133*	*145/163*	*145/133*	*143/163*	*143/133*	38	3	0.947
				7 (9.5)	11 (9.5)	10 (9.5)	10 (9.5)			
AjTR-15	1	*145/127*	*159/null*	*145/159*	*145/null*	*127/159*	*127/null*	38	3	1.789
				8 (9.5)	11 (9.5)	7 (9.5)	12 (9.5)			
	2	*177/155*	*133/133*	*177/133*	*155/133*			38	1	0.947
				22 (19)	16 (19)					
AjTR-17	1	*89/67*	*89/85*	*89/89*	*89/85*	*67/89*	*67/85*	37	3	6.784
				8 (9.25)	4 (9.25)	15 (9.25)	10 (9.25)			
	2	*95/91*	*91/97*	*95/91*	*95/97*	*91/91*	*91/97*	38	3	1.368
				10 (9.5)	9 (9.5)	7 (9.5)	12 (9.5)			
AjTR-22	1	*102/94*	*98/88*	*102/98*	*102/88*	*94/98*	*94/88*	38	3	2.211
				12 (9.5)	9 (9.5)	6 (9.5)	11 (9.5)			
	2	*100/98*	*108/92*	*100/108*	*100/92*	*98/108*	*98/92*	38	3	5.368
				10 (9.5)	10 (9.5)	4 (9.5)	14 (9.5)			
AjTR-23	1	*121/105*	*101/101*	*121/101*	*105/101*			37	1	0.027
				18 (18.5)	19 (18.5)					
	2	*113/115*	*127/107*	*113/127*	*113/107*	*115/127*	*115/107*	38	3	0.947
				11 (9.5)	10 (9.5)	10 (9.5)	7 (9.5)			
AjTR-24	1	*213/171*	*142/124*	*213/142*	*213/124*	*171/142*	*171/124*	38	3	4.316
				12 (9.5)	13 (9.5)	5 (9.5)	8 (9.5)			
	2	*181/133*	*133/139*	*181/133*	*181/139*	*133/133*	*133/139*	37	3	2.243
				7 (9.25)	12 (9.25)	11 (9.25)	7 (9.25)			
AjTR-42	1	*133/125*	*135/129*	*133/135*	*133/129*	*125/135*	*125/129*	38	3	2.632
				7 (9.5)	12 (9.5)	7 (9.5)	12 (9.5)			
	2	*109/107*	*107/105*	*109/107*	*109/105*	*107/107*	*107/105*	38	3	0.526
				8 (9.5)	11 (9.5)	9 (9.5)	10 (9.5)			
AjTR-43	1	*182/166*	*178/172*	*182/178*	*182/172*	*166/178*	*166/172*	37	3	1.595
				8 (9.25)	10 (9.25)	7 (9.25)	12 (9.25)			
	2	*188/182*	*182/182*	*188/182*	*182/182*			36	1	0.000
				18 (18)	18 (18)					
AjTR-44	1	*122/106*	*154/138*	*122/154*	*122/138*	*106/154*	*106/138*	36	3	1.778
				11 (9)	11 (9)	7 (9)	7 (9)			
	2	*114/null*	*150/106*	*114/150*	*114/106*	*null/150*	*null/106*	37	3	1.811
				11 (9.25)	6 (9.25)	11 (9.25)	9 (9.25)			
AjTR-45	1	*137/131*	*131/135*	*137/131*	*137/135*	*131/131*	*131/135*	38	3	0.526
				10 (9.5)	11 (9.5)	9 (9.5)	8 (9.5)			
AjTR-48	1	*134/114*	*134/114*	*134/134*	*134/114*	*114/114*		37	2	3.486
				13 (9.25)	13 (18.5)	11 (9.25)				
	2	*122/null*	*132/126*	*122/132*	*122/126*	*null/132*	*null/126*	38	3	4.737
				13 (9.5)	4 (9.5)	10 (9.5)	11 (9.5)			
Ang075	1	*149/111*	*117/105*	*149/117*	*149/105*	*111/117*	*111/105*	37	3	2.676
				11 (9.25)	11 (9.25)	5 (9.25)	10 (9.25)			
	2	*133/null*	*125/117*	*133/125*	*133/117*	*null/125*	*null/117*	38	3	3.684
				13 (9.5)	11 (9.5)	9 (9.5)	5 (9.5)			
Ang101	1	*141/null*	*167/141*	*141/167*	*141/141 or null/141*		*null/167*	38	2	3.474
				10 (9.5)	14 (19)		14 (9.5)			
	2	*147/139*	*147/null*	*147/147 or 147/null*		*147/139*	*139/null*	38	2	0.474
				19 (19)		11 (9.5)	8 (9.5)			
Ang114	1	*111/107*	*115/103*	*111/115*	*111/103*	*107/115*	*107/103*	38	3	0.947
				12 (9.5)	9 (9.5)	8 (9.5)	9 (9.5)			
	2	*110/98*	*116/108*	*110/116*	*110/108*	*98/116*	*98/108*	37	3	2.459
				12 (9.25)	6 (9.25)	11 (9.25)	8 (9.25)			

** $P < 0.005$

Table 2.
Microsatellite-centromere recombination frequencies (*y*) and map distances (cM) of 26 microsatellite loci examined in four triploid families.

Locus	Family	Parental genotype (bp)		Genotypes of triploid larvae						Total	Recombination frequency (*y*)	Microsatellite-Centromere distance (cM)	χ^2	
		Female (*a*/*b*)	Male (*c*/*d*)	*a*/*b*/*c*	*a*/*b*/*d*	*a*/*a*/*c*	*a*/*a*/*d*	*b*/*b*/*c*	*b*/*b*/*d*				*a*/*a*:*b*/*b*= 1:1	*c*:*d* =1:1
Ajp-7	2	*146/140*	*152/158*	7	9	1	1	1	2	21	0.762	38.1	0.200	0.429
	4	*148/142*	*154/144*	20	20	1	3	2	1	47	0.894	44.7	0.143	0.021
											0.828	**41.4**		
Ajp-33	1	*135/131*	*127/119*	2	0	5	8	9	5	29	0.069	3.4	0.037	0.310
	3	*133/131*	*127/119*	3	2	5	9	8	8	35	0.143	7.1	0.133	0.257
	4	*129/125*	*127/119*	0	1	6	9	15	12	43	0.023	1.2	3.429	0.023
											0.078	**3.9**		
Ajp-45	1	*163/157*	*184/142*	1	4	1	7	3	6	22	0.227	11.4	0.059	6.545*
	4	*170/142*	*188/148*	3	1	5	13	11	10	43	0.093	4.7	0.231	0.581
											0.160	**8.0**		
Ajp-48	1	*198/183*	*181/141*	7	9	1	0	1	2	20	0.800	40.0	1.000	0.200
	4	*194/183*	*185/185*	32	-	4	-	4	-	40	0.800	40.0	0.000	-
											0.800	**40.0**		
Ajp-49	1	*226/222*	*216/212*	4	5	5	3	3	5	25	0.360	18.0	0.000	0.040
	3	*231/218*	*224/211*	4	9	6	8	2	8	37	0.351	17.6	0.667	4.568*
	4	*218/206*	*224/211*	5	10	9	3	9	7	43	0.349	17.4	0.571	0.209
											0.353	**17.7**		
Ajp-55	1	*160/158*	*156/156*	0	-	11	-	11	-	22	0.000	0.0	0.000	-
	3	*165/158*	*167/149*	0	1	10	10	13	6	40	0.025	1.3	0.026	0.900
	4	*175/165*	*167/149*	0	0	12	11	13	8	44	0.000	0.0	0.091	0.818
											0.008	**0.4**		
Ajp-58	1	*148/138*	*144/142*	15	5	1	4	2	2	29	0.690	34.5	0.111	1.690
	3	*158/131*	*148/144*	7	13	7	2	6	5	40	0.500	25.0	0.200	0.000
											0.595	**29.7**		
Ajp-70	1	*114/106*	*132/108*	3	1	5	5	6	7	27	0.148	7.4	0.391	0.037
	4	*122/102*	*112/100*	2	3	7	7	10	7	36	0.139	6.9	0.290	0.111
											0.144	**7.2**		
Ajp-76	1	*154/146*	*148/144*	6	3	3	3	3	2	20	0.450	22.5	0.091	0.800
	3	*158/148*	*146/144*	1	2	4	1	4	3	15	0.200	10.0	0.333	0.600
											0.325	**16.3**		
Ajp-77	1	*186/172*	*180/162*	10	11	1	2	0	1	25	0.840	42.0	1.000	0.360
	3	*180/176*	*186/182*	18	12	0	1	0	0	31	0.968	48.4	1.000	0.806
											0.904	**45.2**		
AjTR-5	1	*178/160*	*174/164*	14	10	2	4	0	3	33	0.727	36.4	1.000	0.030
	2	*203/177*	*193/193*	17	-	2	-	2	-	21	0.810	40.5	0.000	-
											0.768	**38.4**		
AjTR-11	4	*119/111*	*103/100*	5	13	0	0	2	1	21	**0.857**	**42.9**	3.000	2.333
AjTR-12	1	*137/133*	*175/139*	10	22	2	0	0	0	34	0.939	47.0	2.000	2.941
	2	*145/141*	*163/133*	7	14	0	0	0	0	21	1.000	50.0	-	2.333
	3	*162/148*	*176/140*	27	26	1	0	0	1	55	0.964	48.2	0.000	0.018
											0.968	**48.4**		
AjTR-15	2	*177/155*	*133/133*	18	0	1	0	1	0	20	0.900	45.0	0.000	-
	4	*136/126*	*147/133*	10	12	9	3	5	3	42	0.524	26.2	0.800	0.857
											0.712	**35.6**		
AjTR-17	4	*95/93*	*86/84*	27	18	0	0	2	1	48	**0.938**	**46.9**	3.000	2.083
AjTR-22	1	*102/94*	*98/88*	18	13	1	1	1	0	34	0.912	45.6	0.333	1.059
	2	*101/93*	*110/98*	8	8	2	0	1	2	21	0.762	38.1	0.200	0.048
	4	*108/106*	*104/100*	13	21	2	4	0	7	47	0.723	36.2	0.077	6.149*
											0.799	**40.0**		
AjTR-23	1	*125/109*	*107/107*	34	-	0	-	0	-	34	1.000	50.0	0.000	-
	2	*113/107*	*129/115*	14	3	3	0	1	0	21	0.810	40.5	1.000	10.714**
	3	*102/98*	*112/112*	51	-	1	-	2	-	54	0.944	47.2	-	-
											0.918	**45.9**		
AjTR-24	1	*202/162*	*136/120*	11	13	2	3	0	4	33	0.706	35.3	0.111	1.485
	3	*173/131*	*203/127*	24	19	2	4	2	3	54	0.796	39.8	0.091	0.074
											0.751	**37.5**		
AjTR-42	1	*110/104*	*114/106*	15	16	1	2	1	0	35	**0.886**	**44.3**	1.000	0.029
AjTR-43	1	*182/166*	*178/172*	16	16	0	0	0	2	34	0.941	47.1	2.000	0.118
	4	*221/181*	*193/183*	19	19	3	2	0	4	47	0.826	41.3	0.111	0.191
											0.884	**44.2**		
AjTR-44	1	*120/106*	*148/136*	16	16	2	0	0	0	34	0.941	47.1	2.000	0.118
	3	*125/103*	*115/101*	14	27	1	5	4	3	54	0.759	38.0	0.077	4.741*
	4	*125/95*	*113/99*	21	20	2	3	2	0	48	0.854	42.7	1.286	0.083
											0.851	**42.6**		
AjTR-45	4	*131/121*	*144/138*	9	4	10	11	6	6	46	**0.283**	**14.1**	2.455	0.348
AjTR-48	3	*134/130*	*140/126*	18	25	3	1	2	6	55	**0.782**	**39.1**	1.333	1.473
Ang075	1	*150/122*	*127/113*	14	10	1	2	4	2	33	**0.727**	**36.4**	1.000	0.758
Ang101	3	*151/143*	*149/133*	26	17	4	3	3	2	55	**0.782**	**39.1**	0.333	2.200
Ang114	2	*122/100*	*126/108*	8	6	0	1	5	1	21	**0.667**	**33.3**	3.571	1.190
										Mean	**0.645**	**32.2**		

* $P < 0.05$, **$P < 0.005$

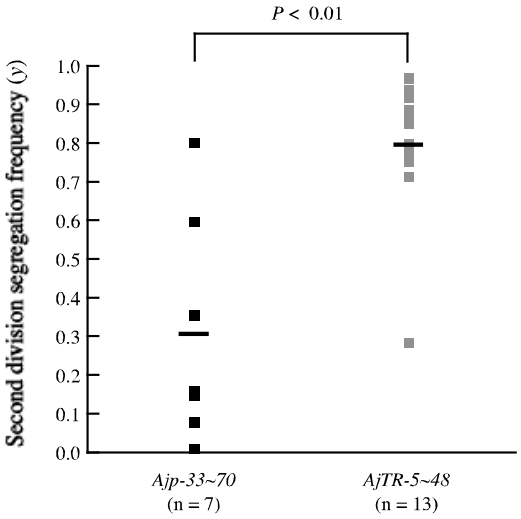

Fig. 1. Nomura et al.

Appendix A.

Microsatellite loci developed in Anguilla species, showing core sequences, sequences of primer sets for amplification, annealing temperatures, references, accession numbers in DDBJ, restriction enzyme for fragmentation, type of genomic library for isolation, and the manner of genotyping.

Locus	Species	Primer sets Forward (5'-3') Reverse (5'-3')	Core sequence	Annealing temperature (°C)	Reference	DDBJ accession number	Isolation procedure		Genotyping
							Fragmentation	Library	
Ajp-1	*A.Japonica*	AATCCATACACATGTACACATG TAAACAGTGACAGATAACTAGG	$(CA)_8AA(CA)_8$	60	Present study	AB194929	*Hae*III	Size-selected	Nomal PCR and PAGE
Ajp-4	*A.Japonica*	ACACGGATACTGTTTTCCACAA ATTCCACCACAGTTGTGCAT	$(GT)_{20}$	52	Present study	AB194930	*Hae*III	Size-selected	Nomal PCR and PAGE
Ajp-5	*A.Japonica*	GACCCTAAGGGACCTGTTTT CAAGCCGAAGCCTCTGAACG	$(CA)_4(CG)_2$	60	Present study	AB194931	*Hae*III	Size-selected	Nomal PCR and PAGE
Ajp-7	*A.Japonica*	TCCTGAACCGAATGCTCTAT ATATTCACACCCTGCCGAAT	$(GT)_3GA(GT)_4C(TG)_{10}$	60	Present study	AB194932	*Hae*III	Size-selected	Nomal PCR and PAGE
Ajp-11	*A.Japonica*	GCACTGAATACACGCACAAT ATTAGGATCTGATCCTGCTG	$(CA)_7$	55	Present study	AB194933	*Hae*III	Size-selected	Nomal PCR and PAGE
Ajp-15	*A.Japonica*	CCTCGGTCAATTCTTAACAGTA GCACATTGATAGGCATCAGA	$(CA)_3$	55	Present study	AB194934	*Hae*III	Size-selected	Nomal PCR and PAGE
Ajp-16	*A.Japonica*	GCGAGACACCAACAGAGAAA ATGACGAAGCTGAAGTCTGG	$(GA)_4GG(GA)_{12}$	52	Present study	AB194935	*Hae*III	Size-selected	Nomal PCR and PAGE
Ajp-22	*A.Japonica*	TTCCCTCTCCACACCTCTGT CTCACCCCCAGATTCCCATC	$(TG)_{25}$	50	Present study	AB194936	*Hae*III	Size-selected	Nomal PCR and PAGE
Ajp-30	*A.Japonica*	AGTCACGACGTTGTATGTGCTTATGAATGCTGCCA TGATGATGCAAAATGCAGCC	$(GT)_5TT(GT)_8$	56	Present study	AB233944	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-32	*A.Japonica*	AGTCACGACGTTGTATGACAATATACGTTGGGATGC AATGTGTGTATGCGTGTGCGT	$(AC)_2A_3(AC)_{19}$	56	Present study	AB233945	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-33	*A.Japonica*	AGTCACGACGTTGTAACTTCCTTCTATGGTGAGCGT TGGCATTGTTCACACGATTG	$(CA)_{10}$	56	Present study	AB233946	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-34	*A.Japonica*	AGTCACGACGTTGTACTTTCCTAGTGGGACAAACA CCAGTGCGAGACACACAAAGA	$(CT)_7(GTCT)_3$	56	Present study	AB233947	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-35	*A.Japonica*	AGTCACGACGTTGTACATGCACAGAGTCTTACATGCA CACATTTTCTGAGTTTAGGTG	$(CA)_{36}GA(CA)_8$	56	Present study	AB233948	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-38	*A.Japonica*	AGTCACGACGTTGTACCTAAACACACACACACAAAGA TGTAGCGCGAATAAAACGGT	$(CA)_3CCTG(CA)_8$	56	Present study	AB233949	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-39	*A.Japonica*	AGTCACGACGTTGTAGCATGGCTCATAGACAGGTCA CCCGATCTGAATCCAATTGA	$(AC)_7AT(AC)_{11}$	56	Present study	AB233950	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-41	*A.Japonica*	AGTCACGACGTTGTAGCCTGCTGATACACATAACGA TGGGAACATATAGCGTGTGTG	$(AC)_{13}$	56	Present study	AB233951	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-42	*A.Japonica*	AGTCACGACGTTGTATGGCATTCATTCTGAAAGCAC GGATCTTCAGATTGCGTATATA	$(CA)_{16}$	56	Present study	AB233952	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-43	*A.Japonica*	AGTCACGACGTTGTAGCTTCATGGCTTTATGGAGA AGCGAAGACAAGCACAACAA	$(GT)_6(GCGT)_2(GT)_5$	56	Present study	AB233953	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-44	*A.Japonica*	AGTCACGACGTTGTAAAGGGAGGCATAGAGCTTTCA ATGAAGTGTGTGTGCTCAGGT	$(CA)_9$	56	Present study	AB233954	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-45	*A.Japonica*	AGTCACGACGTTGTAAATGAGTCCCTGTTTGGCTCT TGAAGCAGCTCCAAGTGTTT	$(AC)_4CT(CA)_{15}(CA)_6$	56	Present study	AB233955	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-47	*A.Japonica*	AGTCACGACGTTGTATTGTTTAGCTTCATGGGCTGG CCGACTCATTTTGCGTGTAT	$(CA)_8CG(CA)_8$	56	Present study	AB233956	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-48	*A.Japonica*	AGTCACGACGTTGTATGAAGGGCAGACAAGTAGAGA TGACCTGAGACAGCATAAGTG	$(AC)_3GC(AC)_8$	56	Present study	AB233957	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-49	*A.Japonica*	AGTCACGACGTTGTAATTCAGCATTAGGGGTGTGG ATTTCAGGCGGTATTCAGGA	$(GT)_{11}$	56	Present study	AB233958	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-50	*A.Japonica*	AGTCACGACGTTGTATTCCTCAGTCACAGACAGGGA ATGACCGCTGAAAAGGACAGT	$(GT)_6GAGTGTGA(GT)_9$	56	Present study	AB233959	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-51	*A.Japonica*	AGTCACGACGTTGTATGCATGTGTGTTTGAGTGCA TGTAGCGATCGGTCAATCAA	$(GT)_{14}$	56	Present study	AB233960	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-52	*A.Japonica*	AGTCACGACGTTGTACCTTTGGTTTTTCATTCACA TGGAACCTGAATTGTTGGGA	$(CA)_{13}$	56	Present study	AB233961	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-53	*A.Japonica*	AGTCACGACGTTGTATGATTTACGTGCGTGCTTG AGAATGAGAGGGGAGGACAGA	$(GT)_6GC(GT)_4GC(GC)_3$	56	Present study	AB233962	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-54	*A.Japonica*	AGTCACGACGTTGTACCTCACACATTCTGAATGGAA TGCATGTGTATGTCTGTGGAA	$(CA)_4CG(CA)_{20}$	56	Present study	AB233963	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-55	*A.Japonica*	AGTCACGACGTTGTATGGAGCACCTCAACTGTTATT ATTTGTGAGCAAGGCTTGCA	$(GT)_{16}$	56	Present study	AB233964	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-56	*A.Japonica*	AGTCACGACGTTGTACAACCCTAAGCTTACAGTCAATTT CCTGATGTGTATGCATGATGTG	$(AT)_8AC(AT)_8(AC)_{17}$	56	Present study	AB233965	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-57	*A.Japonica*	AGTCACGACGTTGTATCCTCCCAGTTCTTTCATCTT TGAAATGCTGAGCAGTGTGTG	$(CA)_{14}$	56	Present study	AB233966	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-58	*A.Japonica*	AGTCACGACGTTGTAAAAACCGGATTTGCTAGCCA GGCAGCGTTCTAAGAATCAA	$(CA)_{17}$	56	Present study	AB233967	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-59	*A.Japonica*	AGTCACGACGTTGTATTTCCCTGAATCCCTTCTGA CGCATGTGTGGGTGCTTACA	$(CA)_{18}CGCA(CG)_2(CA)_9$	56	Present study	AB233968	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-61	*A.Japonica*	AGTCACGACGTTGTAAGAGGGATTTTTACACTGCCA ATGCTTGATTTGAGGTAGCCT	$(CA)_{16}$	56	Present study	AB233969	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-63	*A.Japonica*	AGTCACGACGTTGTATTGAGCACCTGCACACACCTA ATGCAGCATGCGTATACACA	$(AC)_{11}GT(AC)_7$	56	Present study	AB233970	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-66	*A.Japonica*	AGTCACGACGTTGTATGCACACACAATCTCCTTCTC TCAGCAGAAGGGACACTGCA	$(CA)_{14}CG(CA)_5$	56	Present study	AB233971	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-67	*A.Japonica*	AGTCACGACGTTGTATATTGGGAAAGAGACGCAGA TTCAAAGCTCTATGCCAACG	$(GT)_5CG(GT)_8$	56	Present study	AB233972	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-68	*A.Japonica*	AGTCACGACGTTGTAATTCTGCTGCATTAGCCGTGT ACTTCCTGCACAGACTCAGCG	$(GT)_{14}$	56	Present study	AB233973	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-69	*A.Japonica*	AGTCACGACGTTGTATTTTTCGAGCAGGGACACAA TGAAGGCGGGGAATGTCT	$(CA)_8G(CA)_5$	56	Present study	AB233974	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection

Appendix A.

Microsatellite loci developed in Anguilla species, showing core sequences, sequences of primer sets for amplification, annealing temperatures, references, accession numbers in DDBJ, restriction enzyme for fragmentation, type of genomic library for isola

Locus	Species	Primer sets Forward (5'-3') Reverse (5'-3')	Core sequence	Annealing temperature (ºC)	Reference	DDBJ accession number	Isolation procedure		Genotyping
							Fragmentation	Library	
Ajp-70	*A.Japonica*	AGTCACGACGTTGTATACACGTGTGCTCACACAGG TCTCTGGGCAAGCTCAACAA	$(CA)_{14}$	56	Present study	AB233975	*Mse*I	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-76	*A.Japonica*	AGTCACGACGTTGTAAGCGGGCGTTCACACTTCTG GTCCGGTCTTCATGCACACCT	$(GT)_{10}$	56	Present study	AB233976	*Hae*III	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-77	*A.Japonica*	AGTCACGACGTTGTACCAGGCTGATCAAAGATGG GTCCAGTACAACACAGTCAG	$(CA)_{10}$	56	Present study	AB233977	*Hae*III	MS-enrichment	Tailed-primer PCR and automated collection
Ajp-78	*A.Japonica*	AGTCACGACGTTGTATCAAGCTCACCAGTTTGGACT ACCTCCCAAGGACAAGAATTT	$(CA)_{11}$	56	Present study	AB233978	*Hae*III	MS-enrichment	Tailed-primer PCR and automated collection
AjTR-5	*A.Japonica*	GGAGCAGTATGGAATAACATGA GTATTTACATAGGGGATGACCA	$(CA)_{18}TA(CA)_3$	60	Isikawa *et al.* (2001)	AB051082	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-11	*A.Japonica*	GGAAGTTGCTGACTTTTAGA GGCAGTTCTATGTGTGCTTA	$(CTCA)_{11}$	55	Isikawa *et al.* (2001)	AB051083	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-12	*A.Japonica*	AACGTTAGTCCCTAGGTTCC TATATGTTCAGTGGAGGGAG	$(GA)_{13}$	55	Isikawa *et al.* (2001)	AB051084	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-15	*A.Japonica*	GCCATATGATCGAACAGATG CGGATGGGGAAAAACTTCAT	$(AC)_5GTG(CA)_{12}AA(AC)_7$	55	Isikawa *et al.* (2001)	AB051085	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-17	*A.Japonica*	CAGTTATGCACTCACGCTAA AGGTATGTTGGAGAGTAAGG	$(CA)_{10}$	55	Isikawa *et al.* (2001)	AB051086	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-22	*A.Japonica*	CTCAACTTCACCGGCTTTTC TTACCTGTCATTCCAATGGA	$(TC)_7(AC)_{15}$	52	Isikawa *et al.* (2001)	AB051087	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-23	*A.Japonica*	GCAGCATCGGTGTATCTTTC AGTGTTCAGTTGGCTACTAC	$(TC)_{11}(AC)_{11}$	60	Isikawa *et al.* (2001)	AB051088	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-24	*A.Japonica*	CAACATACACCAATACCAGC GATCCCTCTGAATGATATGG	$(AC)_{13}$	60	Isikawa *et al.* (2001)	AB051089	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-25	*A.Japonica*	CGAGTGAGAACAAACACCAA TGTAACATAAAGGTGACCGG	$(CA)_7AAGG(CA)_9$	55	Isikawa *et al.* (2001)	AB051090	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-26	*A.Japonica*	TCTTTGTGAAGTGTGCATGA TTGTGAGGACAAATGATGGC	$(CA)_2CC(CA)_{15}$	55	Isikawa *et al.* (2001)	AB051091	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-27	*A.Japonica*	CGCCTGAATTCCAACTCTTG GGTCCTTTGGCATTCTTACG	$(TC)_8(CT)_3$	60	Isikawa *et al.* (2001)	AB051092	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-37	*A.Japonica*	CTAGATTTACTGCTCAGGGA AAATTCAATTGTGCCCTCCG	$(TG)_{14}$	60	Isikawa *et al.* (2001)	AB051094	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-42	*A.Japonica*	TGAAGCAGAACTGTCATGCTAT TATAACTAGCCCTACTAACTGT	$(TG)_{12}$	55	Isikawa *et al.* (2001)	AB051097	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-43	*A.Japonica*	CCAGGAATAAGCATCTGACACA CCGGATCCTGTGGTGTATTG	$(TTTA)_{10}$	55	Isikawa *et al.* (2001)	AB051098	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-44	*A.Japonica*	TGACTGATTGAAGTGGTATGTG GCTTATTCCTGGGAAGATCT	$(CT)_{14}$	55	Isikawa *et al.* (2001)	AB051099	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-45	*A.Japonica*	ACTCTAATGGGAGCCACATT TAGAGTGAGGACAGTAGAGG	$(CT)_7T(TC)_2C(CT)_6$	55	Isikawa *et al.* (2001)	AB051100	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AjTR-48	*A.Japonica*	ATTATAACCCCTGCATGGCA GAAGATAAGACCTGGTCGCA	$(TG)_7TTA(GT)_{16}$	60	Isikawa *et al.* (2001)	AB051101	*Alu*I and *Hae*III	Size-selected	Nomal PCR and PAGE
AJMS-3	*A.Japonica*	GGTATGAATGCAGGCGTTTATG CTGGAGATCAAATCGGTTGC	$(GT)_{10}$	52	Tseng *et al.* (2001)	AJ297601	*Alu*I, *Hae*III, and *Rsa*I	Size-selected	Nomal PCR and PAGE
Aan05	*A. Anguilla*	GCTTGTATGCATATGTATGTTCATGC TCTAGTAGACTGCTTCAGGCCATGCTG	$(TG)_{18}$	63	Daemen *et al.* (1997)	AY028638	*Hae*III, *Pvu*II and *Eco*RV	Size-selected	Nomal PCR and PAGE
Ang101	*A. Anguilla*	ACAATCGGGTACCACAGTAA ATGGAGCCACTCAATGAAGA	$(CA)_{17}$	60	Wirth and Bernatchez (2001)	AF237900	No description	No description	Nomal PCR and PAGE
Ang151	*A. Anguilla*	GATGTTGGTTTGGTCTGTCG TAGCATGCCTAGAACTGGAC	$(TG)_{16}$	60	Wirth and Bernatchez (2001)	AF237901	No description	No description	Nomal PCR and PAGE
Ang114	*A. Anguilla*	TCCTGTGAATCCAACAGGTG CTGGAGCAGTCACACCAATT	$(CT)_{27}$	60	Wirth and Bernatchez (2001)	AF237902	No description	No description	Nomal PCR and PAGE
Ang075	*A. Anguilla*	TATCAGGAACTCGATACGCC ACGCATCACCAGCCCTTGC	$(GA)_{15}$	60	Wirth and Bernatchez (2001)	AF237903	No description	No description	Nomal PCR and PAGE
Aro054	*A. Rostrata*	CTCAACTCCAGCACACTGGA ACAAAATAGCTCCGTAACAC	$(CA)_{14}$	64	Wirth and Bernatchez (2001)	AF237896	No description	No description	Nomal PCR and PAGE
Aro063	*A. Rostrata*	CAGATACCTTGACAACGGC TCAAGAGCTTCCTGACCCTC	$(GA)_{12}$	62	Wirth and Bernatchez (2001)	AF237899	No description	No description	Nomal PCR and PAGE