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Random amplified polymorphic DNA analysis of eel genome

QIU JIA JING, YI PING LI*
Shanghai Institute of Cell Biology Chinese Academy of Sciences, 320 Yueyang Road, Shanghai 200031, China

ABSTRACT

Eel family is a huge one, in which many kinds of eels especially some migratory eels, bear strong resemblance to each other, and are therefore difficult to be identified. In this study 29 random primers were used to make RAPD analysis for Japanese eel (Anguilla japonica), European eel (Anguilla anguilla) and Pike eel (Muraenesox cinereus). And totally 299 fragments were counted. Shared or specific fragments were counted and genetic similarity or genetic distance were calculated. The genetic similarity between Japanese eel and Pike eel is 0.68 and the genetic distance between them is 0.32; those between European eel and Pike eel are 0.72 and 0.28 respectively, and between Japanese eel and European eel are 0.74 and 0.25 respectively. The method has been shown to be suitable to molecular identification of eels. It provides an alternative approach to determine the relationship between species.

Key words: *Eel, RAPD, polymorphic fragments, genetic distance.*

Members of the eel order (Anguiliformes) have now reached to 600, divided into 19 families. Most of them live in the sea, and only a few take migration. The latter has a high economic value in Asia. The economic importance of eels to a number of countries in the world has been fully awared, and more and more individuals and organizations are showing an interest in this market. However so far little information of the background of the eel is available[1], [2]. Eels are usually classified by limited morphological characters such as patch of pigment, backbone number and so on. It is even difficult to distinguish one from another in some species, especially in larvae. So it is necessary to estab-

*Corresponding author, e-mail: liyp@sunm.shcnc.ac.cn

lish a quick, simple and more scientific method to identify different kinds of eel.

The RAPD (Random Amplified Polymorphic DNA) analysis was developed in 1990 by Williams, and also named as AP-PCR (Arbitrary Primed PCR)[3],[4]. It has been used to construct genetic maps in a variety of species. It is also suitable to the study of genetic variation, relationship between species and development of organism and so on. The RAPD assay is based on the amplification of target genomic DNA with single arbitrary chosen primers with nucleotide in length of about 10bp, the G+C content of which is between 30 % and 70 %. Arbitrary primers can pair to template with only two or more complementary sites and suitable inter-spaces. Because of the short length of primers, several fragments of different sizes and sequences can be amplified from more than one site on the template under relatively low annealing temperature. Based on different genomic DNA with different numbers of complementary sequence and different inter-spaces of them, DNA polymorphisms can be detected by analyzing the different amplified band patterns after electrophoresis. A single primer may probably be used to detect only a small portion of genomic DNA template, but the whole region of genomic DNA could be covered with a set of primers[3]. Williams et al. have proved that the set of arbitrary primers could be used for genomic DNA of various organisms, and that most of the RAPD markers are inherited following Mendel's principles.

The RAPD assay was proved to be useful to identify different species of eel and their larvae, particularly for the species with similar appearance. Thus their evolutionary relationship could be studied. In this paper the RAPD assay was used to analyze the polymorphism of genomic DNA of Anguilla anguilla, Anguilla japonica and Muraenesox cinereus. According to morphological classification the three species of eel all belong to Anguiliformes. Both Anguilla anguilla and Anguilla japonica belong to Anguillidae, while Muraenesox cinereus belongs to Muraenesocidae. Our results conform to the above classification and provide information to their genetic distance. The species specific bands can also be used as precise markers to identify different species.

MATERIALS AND METHODS

Material

Japanese eel (Aguilla japonica), European eel (Anguilla anguilla), Pike eel (Muraenesox cinereus) were obtained from Nantong Jiang Su province. The eels were killed and their livers were taken out immediately and stored at -70°C until use.

Extraction and purification of genomic DNA

The procedures of extraction and purification of genomic DNA were modified according to those described previously[6]. Briefly, 0.5 g liver of each sample was cut into pieces and grounded. After addition of 4.5 ml extracting solution (10 m*M* Tris.HCL, 10 m*M* EDTA, 10 m*M* NaCl, 1.0 % SDS, pH 8.0) and 1 mg Protease K (4 mg/ml), it was incubated under 56 °C for 3 h. Then it was centrifugalized at 5000 g under 4 °C for 5 min. The supernatant was extracted with equal volume of phenol for three times. After that, an equal volume of phenol-chloroform-isoamyl alcohol (25: 24:1) was added to extract the supernatant again. Then DNA was precipitated by 0.5 volume of

NH4Ac and double volume of anhydrous alcohol. It was washed twice with 70 % alcohol, dried naturally at room temperature and dissolved in TE buffer for further use.

Primers

Random primers were synthesized in Shanghai Institute of Cell Biology and a part of them were obtained from Sengon (Canada). Each primer is 10 bp long and their sequences are listed in Tab 1.

Tab 1. Random primers and their sequences

Primer	Sequence 5'-3'	Primer	Sequence 5'-3'
S1	GTTTCGCTCC	S18	CCACAGCAGT
S2	TGATCCCTGG	S20	GGACCCTTAC
S3	CATCCCCCTG	S23	AGTCAGCCAC
S4	GGACTGGAGT	S24	AATCGGGCTG
S5	TGCGCCCTTC	S25	AGGGGTCTTG
S6	TGCTCTGCCC	OPB12	CCTTGACGCA
S7	GGTGACGCAG	OPC2	GTGAGGCGTC
S8	GTCCACACGG	OPC15G	ACGCATCAG
S9	TGGGGGACTC	OPD5	TGAGCGGACA
S10	CTGCTGGGA	COPE4	CCAGATGCAC
S11	GTAGACCCGT	OPE6	AAGACCCCTC
S12	CCTTGACGCA	OPG2	GGCACTGAGG
S15	GGAGGGTGTT	OPG12	CAGCTCACGA
S16	TTTGCCCGGA	OPG17	ACGACCGACA
S17	AGGGAACGAG		

PCR

Each 10 μl of reacting mixture contains 2.55 m*M* $MgCl_2$, 2.0 μl 5 $\times$ buffer (50 mM Tris.HCl, 250 m*M* KCl, 0.005 % gelatin), dNTPs (0.1 m*M* each), 0.4 μ*M* primer, 1.5 - 2U Taq DNA polymerase (from Seng gong), and 25 ng template. After addition of 20 μl of liquid paraffin and centrifugation, it was amplified in MJ-PTC-100DNA amplifier. The reaction was first denatured at 94 °C for 5 min, followed by 40 cycles of denaturation for 1 min at 94 °C, annealing for 2 min at 35°C, and extension for 2 min at 72 °C. For the last cycle the extension was prolonged by 10 min at 72 °C. Amplification products were analyzed by electrophoresis in 1.2 % agarose gels and detected by staining with ethidium bromide.

TE was added instead of DNA template for control. Each reaction was repeated for 2-3 times.

Data analysis

Genetic similarity(F) is calculated according to F=2Nxy/(Nx+Ny),
(Nxy: number of bands shared by both individuals x, y
Nx: number of bands displayed by individual x
Ny: number of bands displayed by individual y),
Genetic distance (P) calculated according to P=1-F

RESULTS

Twenty nine arbitrary primers were used to amplify DNA fragments. Eight individuals of Anguilla anguilla, fourteen individuals of Aguilla japonica and eight individuals of Muraenesox cinereus were analyzed. The intraspecies similarity are 93.2 %, 91.2 % and

98.5 % respectively (Tab 2). Only those bands shared by all individuals of one species were considered to be common bands of this species and were counted.

Since RAPD markers are amplified from arbitrary primers, there were weak and unreproducible bands detected, which were amplified from sites that could not bind fast to the primers. These bands were negated. Only those bands that were clear, bright and reproducible were counted.

Notable differences in band patterns between species could be found in Fig 1(1-3). The bands that were shared by two individuals of one species but were specific in comparison with another species indicated inter-species disparity. They can be used as markers to identify different species.

Fig 1(4) showed a set of DNA fragments amplified with primer OPG12 from three species of eels, the electrophoresis pattern of which are absolutely consistent with each other. This implies the genetic similarity among the three species of eels.

Inter-species polymorphic fragments and common fragments were counted, and the ratio of amplified fragments was calculated (Tab 3) The genetic distance(P) and genetic similarity(F) were got according to the formula: F=2Nxy/(Nx+Ny); P=1-F (Tab 4).

Tab 2. The intraspecies disparity, similarity of three species of eels

species	individuals analyzed	common bands	intraspecie disparity	intraspecie similarity
Anguilla japonica	14	104	8.8 %	91.2 %
Muraenesox cinereus	8	114	1.5 %	98.5 %
Anguilla anguilla	8	81	6.8 %	93.2 %

Tab 3. The distribution of random amplified polymorphic DNA fragments in three species of eels

species compared	inter-species polymorphic fragments	inter-species shared fragments	the ratio between polymorphic and total fragments
Anguilla japonica~ *Muraenesox cinereus*	70	74	32 %
Muraenesox cinereus~ *Anguilla anguilla*	55	70	28 %
Anguilla anguilla~ *Anguilla japonica*	47	69	25 %

Tab 4. The genetic distance(P) and similarity(F) between three species of eels

	Anguilla japonica~ *Muraenesox cinereus*	*Anguilla anguilla* ~ *Muraenesox cinereus*	*Anguilla japonica*~ *Anguilla anguilla*
F	0.68	0.72	0.74
P	0.32	0.28	0.25

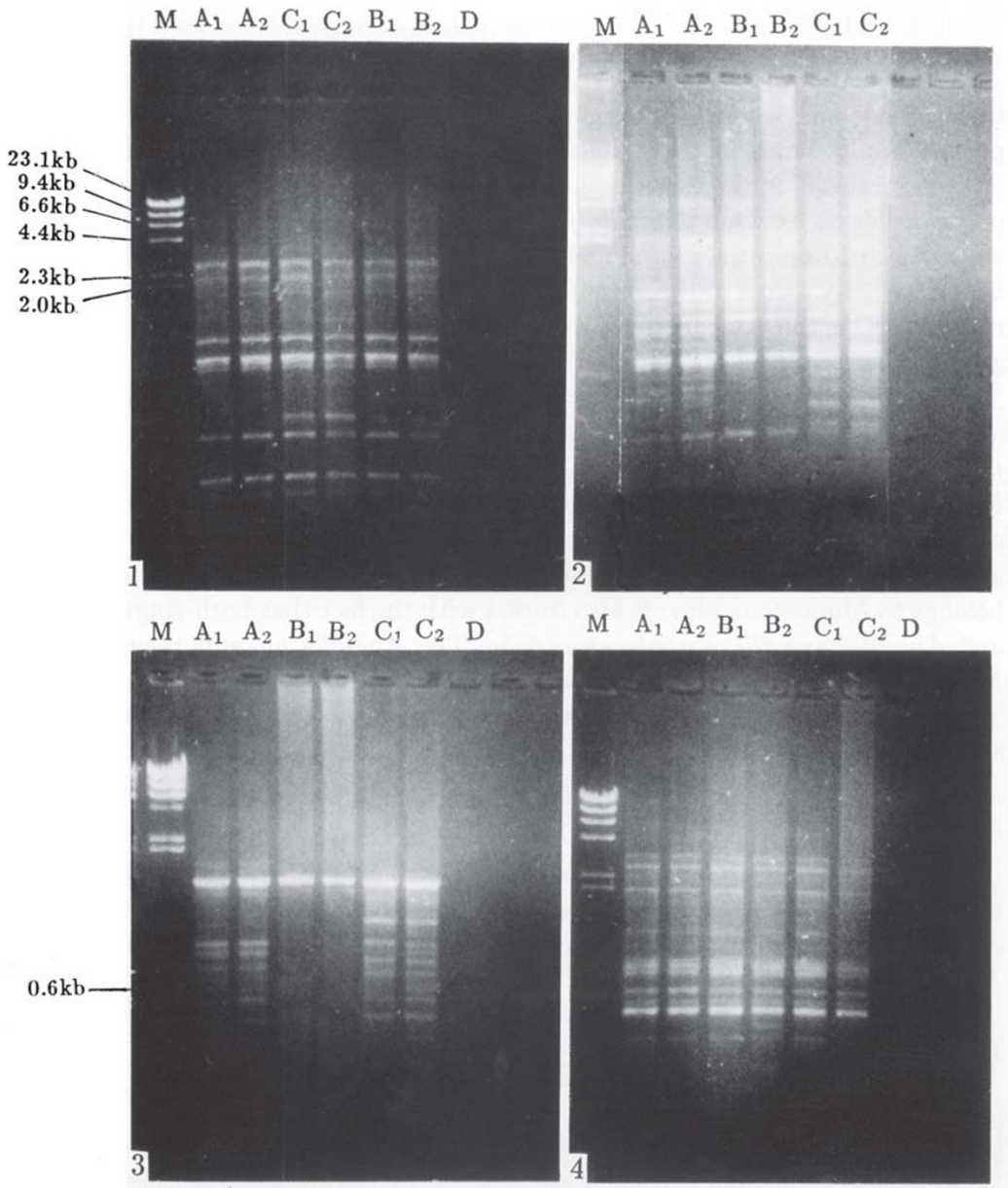

Fig 1.
Electrophoresis pattern of random amplified products
A:Muraenesox cinereus
B: Anguilla anguilla
C: Anguilla japonica
D: Template was omitted in control reaction
M: Molecular weight marker (λDNA-EcoR I /Hind III)
(1) primer S1 (2) primer S17 (3) primer OPE6 (4) primer OPB12
(A1 and A2, B1 and B2, C1 and C2 refer to two individuals of Muraenesox cinereus, Anguilla anguilla and Anguilla japonica)

DISCUSSION

RAPD employs single arbitrarily chosen primers and the polymerase chain reaction (PCR), and is useful in fingerprinting complex genomes. By RAPD a set of primers can generate multiple genetic markers to distinguish closely related species or even strains[8],[9] . In this study, 29 random primers were applied to measure the polymorphisms between Anguilla japonica, Anguilla anguilla and Muraenesox cinersus. Each 10 bp primer amplified 2-11 fragments in length of 300-2300 bp, with a high specificity. Most of the amplified signals are inherited following the Mendelian rule. RAPD is thus an effective approach to distinguish eel species and analyze their genetic relationship.

Our data indicated that the intra-species disparities of the three species of eel are between 1.5-8.8 %. The inter-species disparity is the biggest between Anguilla japonica and Muraenesox cinereus, while the relationship between Anguilla japonica and Anguilla anguilla is the closest. Our results conform to the morphological classification that the three species of eel all belong to Anguiliformes. Anguilla anguilla and Anguilla japonica both belong to Anguillidae, while Muraenesox cinereus belongs to Muraenesocidae. It also consist with the fact that both Anguilla japonica and Anguilla anguilla are migratory fish. The Anguilla anguilla may be closer to Muraenesox cinereus than to Anguilla japonica. The former lives in the sea for life. The species specific bands can also be used as precise markers to identify different species.

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