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SHORT COMMUNICATION

DNA FINGERPRINTING OF WILDLIFE: A TECHNIQUE TO MEASURE THEIR GENETIC DISTANCE

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SUMMARY

The genetic relation between ten wildlife animals from three different vertebrate groups; the mammals, birds and reptiles was revealed by random amplified polymorphic DNA (RAPD) technique. The results revealed that the genomes of vertebrate were highly variable. Although mammals and birds are believed to evolve from reptiles, the mammals were genetically closer to reptiles than to birds. The degrees of polymorphism among animals within the same family were overall lower than those from different category.

Keywords: RAPD, relationships, similarity index, wildlife

INTRODUCTION

The use of random amplified polymorphic DNA (RAPD) analysis in genetic studies has increased enormously because the technique is simple, fast and inexpensive to perform. The DNA fingerprints produced by RAPD provide useful information for species identification, confirmation of biological relationships, detection of diversity and gene mapping. Many studies have been focused on microorganisms, plants, laboratory and domestic animals (Jeffreys and Morton, 1990; Welsh and McClelland, 1991; Welsh *et al.*, 1991; Baird *et al.*, 1992; Kemp and Teale, 1994;) but little attention was given to wildlife species. More data are needed on the molecular aspect of wild animals, especially those facing the treat of extinction such as the Malayan tiger and Malayan tapir. This is because their genetic information can be used to establish breeding programmes, to study and diagnose inherited diseases of valuable animals, as powerful forensic tool to identify animals involved in illegal hunting and other activities against the law (Deacon and Lah, 1989).

The purpose of this study was to reveal the genetic interrelationships among several wildlife species. This serves as a preliminary step towards building up a complete genetic record for future reference.

MATERIALS AND METHODS

Animals

Animals selected for the present study are listed in Table 1. Upon death, tissue samples were immediately collected in Hank's balance salt solution and kept at -20°C until use.

Table 1. Types of animal species used in this study

No	Common Name	Scientific Name	Abbrev.
1	False Gharial (crocodile)	*Tomistoma schlegeli*	FG
2	Asian Giant Turtle	*Orlitia borneansis*	AGT
3	Barking Deer	*Muntiacus muntjak*	BD
4	Springbok	*Antodorcas marsupialis*	SB
5	Large Mousedeer	*Trangulus napu*	LMD
6	Leopard Cat	*Felis bengalensis*	LC
7	Large Indian Civet	*Viverra zibetha*	LIC
8	Malayan Tiger	*Panther tigris corbetti*	TG
9	Malayan Tapir	*Tapirus indicus*	MT
10	Greater Flamingo	*Phoenicopterus roseus*	GF

Preparation of genomic DNA

The DNA was extracted using DNAzol® reagent (Medical Research Center, Inc) as described by the manufacturer. Briefly, the tissues were homogenised in DNAzol® reagent before the insoluble tissue fragments were removed by centrifugation at 10,000g at 4°C. Absolute ethanol was added to precipitate DNA from the homogenate. The DNA was then washed twice with

95% ethanol before re-dissolved in water. The aliquots of DNA samples were kept at –20°C until used.

Random amplified polymorphic DNA (RAPD) analysis

Amplification reactions were performed in volume of 50µL consisted of 31µL distilled water, 5µL 10x PCR buffer, 1µL dNTP mix (Prornega), 2µL random primer (Kit A, Operon Technologies), 2U Taq DNA polymerase (Promega) and 10µL template DNA.

Amplification was carried out in a programmable thermal controller (Perkin Elmer) started with 5 min of denaturation step at 94°C. In the following 35 cycles, denaturation, annealing and extension were carried out at 94°C for 1 min, 36°C for 1 min and 72°C for 2 min respectively. Finally, a 5 min prolong extension time was given. The amplified polymorphic DNA fragments were analysed in 1% agarose gel. The RAPD patterns were compared pair-wise and the similarity index between two animal species was calculated according to Lynch's (1990) formula:

$$S_{xy} = 2n_{xy} / (n_x + n_y)$$

where S_{xy} is the number of bands common to both species x and y while n_x and n_y are the numbers of bands in each species. After comparing each animal with all of the others in turn, the average similarities between all animals were determined. To investigate the genetic relation between the vertebrate group, a mean value of similarity indices between animals in the two groups involved were obtained and compared.

RESULTS AND DISCUSSIONS

The probability of band sharing between unrelated animals was extremely low since the similarity index was <0.5 (Tables 2 and 3). This suggested that the vertebrates have great variability.

An evolutionary trend was also observed. There was no great difference between the similarity index of reptile against bird (0.11) and reptile against mammal (0.22). These findings support the general believes that mammals and birds are descendants of reptiles, which underwent further adaptation to the terrestrial environment. However, the relationship between mammals and reptiles were closer when compared to birds. This is in agreement with Quinn and Mindell (1996) who reported that the reptiles (turtles and crocodiles) and mammals have the same mitochondrial gene order.

According to the classical phylogeny of living reptiles, crocodile is paired with bird while turtle is placed at the bottom of the tree. Such arrangement, however, had been changed. Analysis carried out on mitochondrial DNA and the nuclear genes of these animals have rearranged crocodile with turtle (Hedges and Poling, 1999). Similar conclusion was reached by the RAPD patterns observed in this study. False gharial was found to resemble the giant turtle at approximately 50% while resembling flamingo at only 6%.

Higher level of polymorphism was detected between the unrelated species compared to those within the same family. High value of similarity among the large mousedeer, barking deer and springbok was expected because they are belonging to the same family, *Cervidae* (Table 4). Their relatedness in ascending order is as follow: large mousedeer < springbok < barking deer. Morphologically, the leopard cat and the tiger resemble each other (Table 5). Their RAPD fingerprints were also found to be related to a certain extent but not as close as the barking deer and springbok. Another member of this group, the large Indian civet, surprisingly produced low similarity index when paired with leopard cat and tiger (Table 5). The calculated similarity index revealed that this animal has closer relationship with flamingo. This finding is perhaps a consequence of poor band resolution. In RAPD technique, many bands with mixed intensity were generated. Thus, some fragments were too close to one another giving the appearance as one broad band while some were too faint to be captured by the camera. The close relationship between large Indian civet and flamingo, however, could be confirmed by performing another round of RAPD assay with new sets of primers and sequencing.

Table 2. Average similarity (S_{xy}) index between animal species

	GF	AGT	FG	LMD	BD	SB	LC	LIC	TG	MT
GF	-	0.15	0.06	0.14	0.00	0.14	0.48	0.11	0.06	0.06
AGT	0.15	-	0.50	0.28	0.06	0.32	0.31	0.39	0.28	0.08
FG	0.06	0.50	-	0.22	0.07	0.25	0.29	0.09	0.18	0.25
LMD	0.14	0.28	0.22	-	0.35	0.44	0.33	0.47	0.08	0.26
BD	0.00	0.06	0.07	0.35	-	0.52	0.13	0.15	0.13	0.06
SB	0.14	0.32	0.25	0.44	0.52	-	0.25	0.15	0.43	0.14
LC	0.48	0.31	0.29	0.33	0.13	0.25	-	0.20	0.36	0.21
LIC	0.11	0.39	0.09	0.47	0.15	0.15	0.20	-	0.10	0.12
TG	0.06	0.28	0.18	0.08	0.13	0.43	0.36	0.10	-	0.19
MT	0.06	0.08	0.25	0.26	0.06	0.14	0.21	0.12	0.19	-

Table 3. Average similarity index between vertebrate groups

	Average similarity index
Reptile/bird	0.11
Reptile/mammal	0.22
Mammal/bird	0.14

Table 4. Estimated similarity index for each primer between animals within same family *Cervidae*

Primer	LMD/BD	LMD/SB	BD/SB
OPA-03	0.00	0.00	0.50
OPA-04	1.00	1.00	1.00
OPA-09	0.40	0.67	0.25
OPA-10	0.33	0.55	0.50
OPA-20	0.00	0.00	0.33
Average	0.35	0.44	0.52

Table 5. Estimated similarity index for each primer between animals in the feline group

Primer	LC/TG	LC/LIC	LIC/TG
OPA-01	0.50	0.00	0.00
OPA-02	0.50	0.00	0.00
OPA-05	0.00	0.00	0.80
OPA-10	0.50	0.00	0.00
OPA-12	0.33	0.00	NA
OPA-18	0.29	0.80	0.00
OPA-19	0.75	0.13	0.00
OPA-20	0.00	0.67	0.00
Average	0.36	0.20	0.10

Determination of true genetic marker that can be used to differentiate animal species could not be done in the present study because the animals were not selected according to their species or sexes but subjected to the availability of tissue samples. Nevertheless, the 2kb fragment produced by primer OPA-11 is a potential RAPD marker for differentiating between springbok, mousedeer and barking deer.

Although RAPD method is simple to perform, reproducibility of the results is highly dependent on many factors. Slight changes in the procedure will significantly affect the banding patterns. In addition to proper standardise of all reaction components and conditions, reproducibility in this study was improved by using high quality template. DNAzol® reagent was used to isolate genomic DNA from the animal tissues instead of the conventional organic solvent extraction method to reduce fragmentation of the large genomic DNA, which consequently might result in loss of some large polymorphic fragments during the amplification.

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RINGKASAN

SIDIKJARI DNA HIDUPAN LIAR: SUATU TEKNIK UNTUK MENYUKAT JARAK GENETIK

Perkaitan genetik di antara sepuluh ekor haiwan hidupan liar daripada tiga kumpulan vertebrat berlainan; mamalia, unggas dan reptilia telah ditunjukkan melalui teknik DNA polimorfik terkuat rawak (RAPD). Hasil kajian menunjukkan yang genom vertebrat tinggi kepelbagaiannya. Walaupun mamalia dan unggas dipercayai terevolusi daripada reptilia, mamalia adalah secara genetik lebih dekat dengan reptilia daripada unggas. Tahap polimorfisme di kalangan haiwan dalam famili sama secara keseluruhan lebih rendah berbanding haiwan daripada kategori berbeza.