

Intra-specific relationships among Tibetan Eared-pheasants based on randomly amplified polymorphic DNA (RAPD) analysis

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Abstract The Tibetan Eared-pheasant *Crossoptilon harmani* is a rare species native to China. A captive population has been established in the Beijing Zoo since 1999. In order to determine the kinship of the offsprings in 2001, randomly amplified polymorphic DNA (RAPD) was used to examine the parenthood of seven Tibetan Eared-pheasants in the Beijing Zoo. To amplify the genomic DNA of each individual, 53 arbitrary primers were selected. The results of amplifications showed that 14 primers had clear and distinct RAPD patterns. Totally, 226 amplified fragments were generated by RAPD in this study. Cluster analysis of the seven Tibetan Eared-pheasants indicated that all the four young birds had the same father (No.5 male). This study provides a practical method to determine the relationship of offsprings whose parents are unknown in birds.

Keywords Tibetan Eared-pheasant, *Crossoptilon harmani*, genetic relationship, random amplified polymorphic DNA (RAPD)

1 Introduction

The Tibetan Eared-pheasant *Crossoptilon harmani* belongs to the family Phasianidae, order Galliformes. It is a rare and endemic species of pheasants distributed only in the south-east of Tibet, China (Zheng and Wang, 1998). In recent years, field work on the ecology and behavior of this species have been carried out (Lu et al., 1998). However, the captive population had not been established until 2000 when some young birds were first introduced into Beijing Zoo from their natural habitats in Tibet, China. With great

efforts, Beijing Zoo has set up the first captive population of the Tibetan Eared-pheasant in China. The captive population started to breed in 2001.

Four offsprings of Tibetan Eared-pheasants were kept in a large enclosure during the breeding season in 2001. The pedigree of the four young birds was not clear because there were two males and one female kept in the same enclosure. In order to avoid inbreeding between the offsprings and to maintain genetic diversity in the captive population, the parenthood of the population must be identified.

Several molecular techniques have been developed to identify the parenthood of wild animals, such as randomly amplified polymorphic DNA (RAPD), DNA fingerprinting, and analysis of microsatellite DNA (Zhou et al., 2001; Chang and Ke, 2002). RAPD is based on PCR, which uses random primers composed of oligodeoxynucleotides (ONDs). DNA sequence polymorphism is analyzed by comparing amplified products. For the simple manipulation and the efficiency in providing abundant and informative data on DNA sequence polymorphism of many loci, the technique of RAPD has been widely used in studying genetic diversity and pedigree of many species of animals (Haig et al., 1994; Smith et al., 1996; Chen and Zhang, 1997; Liu et al., 1999; Lu et al., 1999; Ren et al., 2001). RAPD is one of the powerful methods to study species whose genetic backgrounds are unknown.

We devised an experiment to examine the parenthood of the population of Tibetan Eared-pheasants bred in Beijing Zoo.

2 Materials and methods

2.1 Materials

The seven Tibetan Eared-pheasants used in this study were obtained from the Beijing Zoo. Three adult birds (N5, N6, one female, F) came from the natural habitat in Tibet, while

Translated from *Journal of Beijing Normal University (Natural Science)*, 2005, 41(5): 507–509 [译自: 北京师范大学学报 (自然科学版), 2005, 41(5): 507–509]

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the remaining four young birds were their offsprings born in 2001. DNA samples were taken from the humeral vein and anticoagulated using EDTA- Na_2 , and then stored at -20°C .

2.2 DNA Extraction

Total DNA was extracted from the blood samples using the Total Blood DNA Isolation Kit (Beijing Dingguo Biotechnology Developing Center).

2.3 Random primers screening

Four random template DNA were amplified by using all available primers. According to the numbers, definition and polymorphism of the bands, 14 primers were screened out. We then used the 14 primers to amplify the DNA from the seven birds. All DNA bands obtained using the 14 primers were used to analyze the genetic relationship of the seven Tibetan Eared-pheasants.

2.4 Random amplification

Random primers were synthesized by Operon Technologies (USA) and named OPG (01–20) and CPK143 (01–33). The total number of primers was 53. RAPD amplification was performed in 25 μL reaction mixtures, which contained 1.5 mmol/L MgCl_2 , 8 pmol random primer, 60 ng template DNA, 2.5 μL $10 \times$ buffer and 1.0 unit Taq DNA polymerase (Operon Technologies, USA). Template DNA was pre-denatured at 94°C for 3 min, then denatured, annealed and extended at 94°C for 1 min, 36°C for 1 min and 72°C for 2 min, respectively (45 cycles). A final extension was carried out at 72°C for 10 min. Amplification products were separated on 1.5% agarose gel with 0.5 ng ethidium bromide in $1 \times$ TBE buffer. Gels were viewed and photographed under UV light.

2.5 Data analysis

DNA bands were scored for the presence (1) or absence (0) in each accession. The data matrices were fed into the SPSS/PC+ program. Data were analyzed using hierarchical clustering and by generating correlation similarity coefficient matrix. Correlation similarity coefficient matrix was used to construct the dendrogram (Nei, 1978; Welth and Mecllelland, 1990).

3 Results

From the two groups (OPG and CPK143), 14 primers were screened out. Using the 14 primers, we obtained 226 DNA bands from the seven Tibetan Eared-pheasants (Fig. 1 and

Table 1). From Table 1 we can find that the seven Tibetan Eared-pheasants are well distinguished by the 14 random primers. No individual shared the same RAPD products with others.

Table 1 Amplification results of the seven Tibetan Eared-pheasants using 14 random primers

	N1	N2	N3	N4	N5	N6	F
OPG02	1	1	1	1	1	1	1
OPG02	1	1	1	1	1	1	1
OPG02	1	1	1	1	0	1	0
OPG02	0	1	1	1	0	0	0
OPG02	1	1	1	0	0	0	0
OPG02	1	1	1	0	0	0	0
OPG03	0	1	0	0	0	0	0
OPG03	1	0	0	0	0	0	0
OPG03	1	1	1	1	1	0	1
OPG03	0	0	1	1	0	1	1
OPG03	1	1	0	0	0	0	0
OPG03	1	0	1	1	0	0	1
OPG03	1	0	1	1	0	0	1
OPG03	0	0	1	1	0	0	1
OPG06	0	0	0	0	0	0	0
OPG06	0	0	0	0	1	0	0
OPG06	1	1	1	0	1	0	0
OPG06	1	1	1	1	0	0	1
OPG09	0	1	1	1	0	0	0
OPG09	0	1	1	0	0	0	0
OPG09	1	1	1	0	0	1	1
OPG10	0	0	0	1	0	0	1
OPG10	0	1	0	0	0	0	0
OPG10	1	1	1	1	0	1	1
OPG10	1	1	0	1	0	0	1
OPG11	1	0	0	0	1	0	0
OPG11	0	0	0	0	0	1	0
OPG11	1	0	0	0	1	1	0
OPG11	0	0	1	0	0	0	0
OPG11	1	0	0	0	1	1	0
OPG11	0	0	0	0	0	1	0
OPG12	0	0	1	0	0	0	0
OPG12	1	0	1	1	1	0	0
OPG15	1	1	0	0	0	0	0
OPG15	0	0	1	0	0	0	0
OPG15	0	1	1	1	1	0	0
OPG15	1	1	1	0	0	0	0
OPG15	1	1	0	0	0	0	0
OPG17	1	1	0	1	0	0	1
OPG17	0	0	0	1	1	0	0

(continued)

	N1	N2	N3	N4	N5	N6	F
OPG17	1	0	1	1	0	0	1
OPG17	0	0	1	1	0	1	1
OPG17	0	0	1	1	0	0	1
OPG17	1	1	1	1	1	0	1
OPG17	0	1	1	1	0	0	0
OPG17	1	0	1	1	0	0	0
OPG17	1	0	1	0	0	0	0
OPG18	0	1	0	0	0	0	0
OPG18	0	1	0	0	0	0	0
OPG18	1	1	1	1	1	0	0
OPG18	1	1	0	1	0	1	0
OPG18	1	1	0	0	0	0	0
OPG19	0	0	1	0	0	0	0
OPG19	1	1	1	1	1	1	1
OPG19	1	1	1	1	1	0	1
OPG19	1	1	1	0	0	0	0
OPG19	1	1	1	1	1	0	0
CPK143(02)	0	1	0	0	0	0	1
CPK143(02)	1	1	0	0	0	1	0
CPK143(02)	0	1	0	0	0	0	0
CPK143(10)	1	1	1	1	1	1	1
CPK143(10)	1	1	1	1	1	1	1
CPK143(10)	0	1	1	1	0	0	0
CPK143(10)	0	0	1	1	0	1	0
CPK143(23)	1	1	0	1	0	0	1
CPK143(23)	1	1	0	1	1	0	1
CPK143(23)	1	1	1	1	0	0	0
CPK143(23)	0	0	1	1	0	0	0
CPK143(23)	1	0	1	1	0	0	1

Using cluster analysis of the RAPD products, the correlation coefficient matrix of the seven Tibetan Eared-pheasants was obtained (Table 2). Figure 1 shows the dendrogram obtained by distance cluster analysis of RAPD data from the seven Tibetan Eared-pheasants.

Table 2 Correlation similarity coefficient matrix according to RAPD data from the seven Tibetan Eared-pheasants

	N1	N2	N3	N4	N5	N6	F
N1							
N2	0.729						
N3	0.667	0.612					
N4	0.650	0.617	0.750				
F1	0.508	0.469	0.413	0.508			
F2	0.386	0.310	0.316	0.340	0.333		
M	0.537	0.471	0.537	0.730	0.391	0.400	

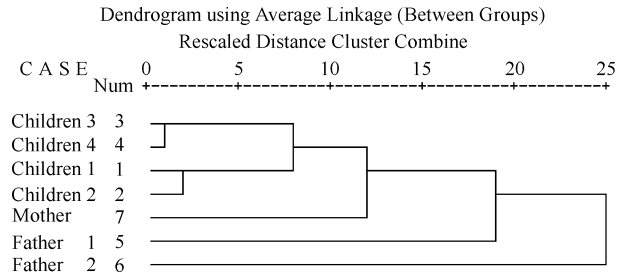


Fig. 1 Dendrogram constructed using distance cluster analysis from RAPD data of the seven Tibetan Eared-pheasants

4 Discussion

In the natural habitat, Tibetan Eared-pheasants live in pairs during the breeding period (Lu et al., 1998; Zheng and Wang, 1998). It is understandable to put several birds into one cage for saving space or increasing the breeding success in the zoo. Beijing Zoo and Beijing Normal University cooperated to establish the first captive population of Tibetan Eared-pheasants in 1999 when some eggs and chicks from different nests were collected nearby the Xiongse Temple of Lahsa, Tibet, and transferred to the Beijing Zoo for incubation. Eight birds survived and reached maturity successfully. In 2001, the eight adults were divided into three breeding groups and put into different enclosures. Two groups of adults that lived in pairs laid unfertilized eggs, while the third group living in a larger enclosure laid fertilized eggs, and four young birds from this group grew successfully. The kinship of these individuals was unclear as three adults were kept in the same enclosure. By using cluster analysis of the results of RAPD, the similarity coefficient matrix and dendrogram of the seven Tibetan Eared-pheasants were obtained, and the relationships among them successfully identified. We found that all the four offsprings came from the female and the No.1 male.

Filial generations may have genes inherited from both their fathers and mothers (Welth and Mecllland, 1990). It is suggested that when matrilineal and patrilineal genomes are tightly linked, parental genetic polymorphisms in filial generations will appear or disappear together. On the contrary, when matrilineal and patrilineal genomes link loosely, filial generations can inherit parental genes (Nei, 1978). So, DNA bands of the filial generation will include bands indicative of their origin either from the father or the mother. In this study, the cluster graph shows that No.3 has close relationship with No. 4, while No. 2 has close relationship with No. 1. Genetic distances between the offsprings and their mother are different, while differences between them and their fathers are smaller. That is to say, relationships between parents and the young do not synchronize. It indicated that the relationship between the mother and the fathers are relatively far. As the distance between N5 and the four offsprings is closer than that between N6 and the offsprings, we deduce that N5 is the father of the four offsprings.

Frequent inbreeding in small colonies will reduce survival and the reproductive potential of offsprings, and lead to the loss of genetic diversity (Liu et al., 1999). These can have a crucial impact on the long-term survival of the population. In addition, genetic drift leads to the loss of $1/(2N_e)$ genetic diversity (N_e is effective population size) in each generation in one population, and the genetic mutation rate is several quantitative grades lower than the genetic loss rate (Nei, 1978). Captive breeding is an important way to save endangered species of wildlife. It is essential to manage properly the captive populations in order to avoid inbreeding. RAPD is a useful technique to identify pedigree relationship of individuals that live together and when their genetic background remains unknown (Smith et al., 1996; Liu et al., 1999; Chang and Ke, 2002). In this study, we have applied this technique to determine the kinship of the seven Tibetan Eared-pheasants bred in captive at the Beijing Zoo. We suggest that birds whose genetic relatedness is far should be selected to mate in order to produce high quality offsprings.

Acknowledgements This study was funded by the Key Grant of Chinese Ministry of Education (No. 03012).

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