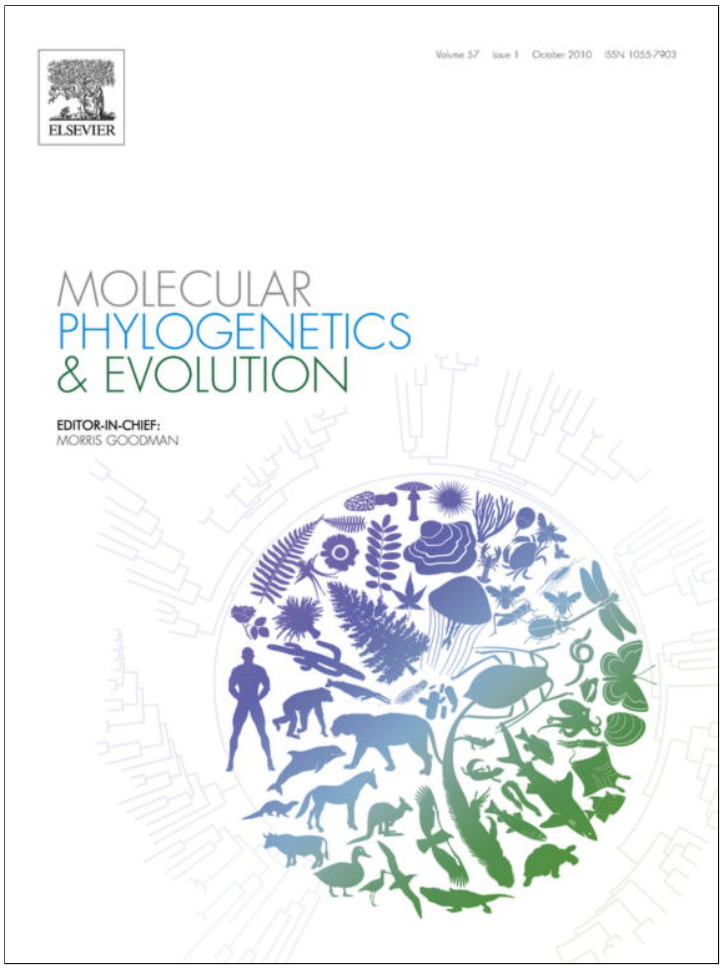

Molecular Phylogenetics and Evolution 57 (2010) 429–433

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Assessment of genetic diversity in Chinese eared pheasant using fluorescent-AFLP markers

Xiujuan Li [a,1], Yaohong Zhu [a,1], Panqi Liu [a], Zengyu Zhuge [a], Guosheng Su [b], Jiufeng Wang [a,*]

[a] College of Veterinary Medicine, China Agricultural University, Beijing 100193, PR China
[b] Department of Genetics and Biotechnology, Faculty of Agricultural Sciences, Aarhus University, DK-8830 Tjele, Denmark

ARTICLE INFO

Article history:
Received 5 May 2010
Accepted 25 May 2010
Available online 1 June 2010

Keywords:
Eared pheasant
AFLP
Genetic diversity
Polymorphic markers

ABSTRACT

The eared pheasant consists of four species: white eared pheasant (*Crossoptilon crossoptilon*), Tibetan eared pheasant (*Crossoptilon harmani*), blue eared pheasant (*Crossoptilon auritum*), and brown eared pheasant (*Crossoptilon mantchuricum*). These species are found only in China, and are also on the list of the world's threatened species. In this paper, 74 individuals from the four eared pheasant species were assessed for population genetic diversity by means of fluorescent-AFLP markers. A total of 429 AFLP peaks were amplified by 11 pairs of fluorescent *Eco*RI/*Taq*I primer combinations. Out of all markers, 329 AFLPs were polymorphic. Each primer combination produced in reactions from 19 to 72 fragments and the polymorphic peaks percentage ranged from 53.33% to 86.11% with an average of 74.36% polymorphic bands. Genetic distance between species and genetic diversity within species were evaluated using Jaccard's similarity coefficients (SC) and the corresponding dendrogram. It was found that there was a moderate genetic distance between the four species (SC = 0.674–0.832). Brown eared pheasant was genetically closely related to blue eared pheasant (SC = 0.832), while white eared pheasant was more closely related to Tibetan eared pheasant (SC = 0.812). Genetic diversity was lower in brown eared pheasant (SC = 0.913) and Tibetan eared pheasant (SC = 0.903) than in white eared pheasant (SC = 0.832) and blue eared pheasant (SC = 0.853).

1. Introduction

The eared pheasants (Galliformes: Phasianidae) consists of four different ecological species: white eared pheasant (*Crossoptilon crossoptilon*), Tibetan eared pheasant (*Crossoptilon harmani*), blue eared pheasant (*Crossoptilon auritum*), brown eared pheasant (*Crossoptilon mantchuricum*). These four species are endemic to China and are distributed from Qinghai, Yunnan, Tibet, Sichuan, Shanxi to Hebei and Beijing. Eared pheasants typically inhabit forested areas where they feed on corn as well as roots, stems, leaves, and buds of plants. They were once abundant, but since the beginning of the 20th century, climatic change, habitat loss, and fragmentation from deforestation has markedly diminished the population size (Bird Life International, 2006). Especially the brown eared pheasant, it is classified as one of the endangered species with higher priority for conservation in China (Zheng and Wang, 1998; CITES, 2006).

Previous studies of brown eared pheasant focused on nest-site selection (Yang et al., 2001), clutch size and its variations (Zhang et al., 1997), breeding-habitat selection (Li et al., 2009), isolation and characterization of microsatellite markers (Fu et al., 2009). There are also studies of the reproductive ecology of Tibetan eared pheasant (Lu and Zhang, 2003), habitat of Tibetan eared pheasant (Lu and Zhang, 2002), *Plasmodium* (*Bennettinia*) *juxtanucleare* infection in captive white eared Pheasant (Murata et al., 2008), and nest-site selection of the white eared pheasant (Wang et al., 2006). To our knowledge we report the first study of blue eared pheasant.

Little is known about genetic diversity and genetic relationship among the four ecological species of eared pheasants. Estimates of genetic diversity will be of potential use in studies of population structure and reproductive strategy of these species. Knowledge of intraspecific genetic variation may help to assess extinction risks and evolutionary potential in a changing world (Hedrick, 2001).

Analysis of amplified fragment length polymorphism (AFLP) is a popular approach to detect genetic diversity. AFLP is an application of the DNA fingerprinting technique proposed by Vos et al. (1995) that uses a combination of restriction fragment length polymorphism (RFLP) (Sreekumar et al., 2001) and random amplified polymorphic DNA (RAPD) (Horng and Huang, 2003; Horng et al., 2004; Yen et al., 2001). In comparison to microsatellite markers, the

* Corresponding author. Address: College of Veterinary Medicine, China Agricultural University, No. 2, Yuanmingyuan West Road, Beijing 100193, PR China. Fax: +86 10 62731274.
E-mail address: jiufeng_wang@hotmail.com (J. Wang).
[1] These authors contributed equally to this work.

1055-7903/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2010.05.024

advantage of AFLP is that a large number of markers can be generated with a smaller number of primer pairs and no prior knowledge of sequence data is required. This is especially true when working in a species for which only a few microsatellite markers are available or when there is no sequence information (Agrama et al., 2002; Guerra et al., 2002; Van Haeringen et al., 2002). Moreover, AFLP is reproducible and has a high throughput and good resolution. In addition, the analysis requires minimal amounts of DNA and can resolve multiple polymorphic markers in each reaction (Lazzaro et al., 2002). Because of these advantages, AFLP has been used to investigate genetic variation in a wide variety of microorganisms, plants, and animals (Schmidt et al., 2004; Jones et al., 2005; Mba and Tohme, 2005; Plastow et al., 2003).

The objective of the current study was to investigate genetic distance between the four ecological species of eared pheasants, and assessed genetic diversity within species using fluorescent-AFLP markers.

2. Materials and methods

2.1. Sample collection

A total of 74 birds (17 brown eared pheasant, 19 white eared pheasant, 20 blue eared pheasant, 18 Tibetan eared pheasant) were sampled from Qinghai, Yunnan, Tibet, Sichuan, Shanxi to Hebei and Beijing. All of them were 2 years old and from Wildlife Refuge and Zoo. The experiment and AFLP analysis were carried out at the Clinical Laboratory College of Veterinary Medicine, China Agricultural University, during the period from January 2007 to December 2008. Venous blood samples were taken from the each individual's wing vein and mixed with anticoagulant (EDTA) and then kept at −20 °C for subsequent DNA extraction.

2.2. DNA extraction

Genomic DNA of eared pheasants was extracted using *EasyPure* Blood Genomic DNA Extraction Kit (Beijing TransGen Biotech Co., Ltd., China). The procedure was carried out following the instructions of the manufacturer.

2.3. AFLP analysis

Genomic DNA digestion and adapter ligation procedures were performed according to the method described by Huang et al. (2007). The sequences of TaqI, EcoRI-adapters and primers used in this study were shown in Table 1.

Samples of 300 ng of eared pheasant genomic DNA were digested for 2 h at 65 °C with 10 units of TaqI (New England Biolabs), 1 μL 10 × NEBuffer 3 (1000 mM NaCl, 500 mM Tris–HCl, pH 7.9, 100 mM $MgCl_2$, 10 mM DTT), 0.1 μL 100 × BSA (500 μg/ml BSA, 50% glycerol), and then sterile water to obtain a final volume of 10 μL. Subsequently, the DNA was further digested with 10 units of EcoRI (New England Biolabs), 1.5 μL 10 × NE Buffer (500 mM NaCl, 1000 mM Tris–HCl, pH 7.5, 100 mM $MgCl_2$, 0.25% Triton X-100), and sterile water were added into the tube for a final volume of 15 μL, and then incubated at 37 °C for 3 h.

DNA ligations were performed in a final volume of 10 μL. The samples contained 5 μL of restricted materials, 5 pmol of the EcoRI-adapter, 50 pmol of the TaqI-adapter, 1 μL 10 × DNA ligase buffer (500 mM Tris–HCl, pH 7.6, 100 mM $MgCl_2$, 10 mM ATP, 100 mM DTT, 50% polyethylene glycol-8000), 10 unit of T4 DNA ligase, and sterile water. The ligation reactions were incubated for 12 h at 37 °C. After ligation of adapters, DNA fragments were diluted 10-fold in sterile water.

Table 1
Sequences of the adapters and primers used in the AFLP analysis.

	Name	Sequence
EcoRI-adapter	EcoRIEco top strand	5-CTCGTAGACTGCGTACC
	EcoRIEco bottom strand	5-AATTGGTACGCAGTCTAC
TaqIadapter	TaqITaq top strand	5-GACGATGAGTCCTGAC
	TaqITaq bottom strand	5-CGGTCAGGACTCAT
EcoRIPrimer	EcoRI + A	5-GACTGCGTACCGTACC A
	FAM-EcoRI + AC	5-FAM GACTGCGTACCGTACC AC
	FAM-EcoRI + AAA	5-FAM GACTGCGTACCGTACC AAA
	FAM-EcoRI + AAC	5-FAM GACTGCGTACCGTACC AAC
	FAM-EcoRI + ACA	5-FAM GACTGCGTACCGTACC ACA
	FAM-EcoRI + ATA	5-FAM GACTGCGTACCGTACC ATA
	FAM-EcoRI + ACC	5-FAM GACTGCGTACCGTACC ACC
	FAM-EcoRI + ACT	5-FAM GACTGCGTACCGTACC ACT
TaqIPrimer	TaqI + A	5-GATGAGTCCTGACCGA A
	TaqI + AC	5-GATGAGTCCTGACCGA AC
	TaqI + AAC	5-GATGAGTCCTGACCGA AAC
	TaqI + AAG	5-GATGAGTCCTGACCGA AAG
	TaqI + ATG	5-GATGAGTCCTGACCGA ATG
	TaqI + ACT	5-GATGAGTCCTGACCGA ACT
	TaqI + ACC	5-GATGAGTCCTGACCGA ACC

AFLP analysis was carried out according to the procedures described by Vos et al. (1995) who recommended that the PCR amplifications of DNA fragments be performed in two consecutive reactions. In the preamplification reaction, DNA fragments were amplified with a pair of AFLP primers completely complementary to the adapters and the restriction site sequences except for one selective nucleotide A at their 3′ end (Table 1). Preamplifications were performed in a final volume of 20 μL with 5 μL of the 10-fold dilution of restricted-ligated DNA, 1 μL of TaqI + A primer (50 ng/μL), 1 μL of *Eco*RI + A primer (50 ng/μL), 10 μL Premix Taq® (TaAaKa Taq™ version), and then 3 μL sterile water. Preamplification cycles started with 5 min denaturation at 94 °C, followed by reaction for 30 s at 94 °C, 60 s at 56 °C, and 60 s at 72 °C. Reactions were performed for 30 cycles with a final extension step at 72 °C for 10 min. The pre-amplified products were detected by electrophoresis in 1% agarose gel. The PCR products of preamplification were diluted 20-fold and used as a template for the next reaction, called selective amplification.Eleven pairs of FAM-EcoRI /TaqI primer combinations with two (tAN) and three (tANN) selective nucleotides at the 3′ end were used in this study. The 5′ end of the EcoR tAN or EcoR tANN primer was labelled with FAM fluorescences (Table 1). The reactions of selective PCR were performed with 2 μL of diluted preamplification reaction products, 1 μL of each pair selective amplification primer (50 ng/μL) (Table 2), 10 μL Premix Taq® (TaAaKa Taq™ version). Sterile water was then added to yield a total volume of 20 μL.

In the selective amplification reaction, a touch-down thermal cycling was used. The first cycle was 30 s at 94 °C, 30 s at 65 °C, and 80 s at 72 °C, followed by 13 cycles of 0.7 °C lower annealing temperature each cycle, and then 30 cycles of 30 s at 94 °C, 30 s at 56 °C, and 60 s at 72 °C.

2.4. Statistical analyses

Fluorescent peak signals for each primer combination were collected with the ABI 3730XL automatic DNA sequencer data collection 3.0 (Applied Biosystems, USA). The electropherograms were scanned and analyzed by the Genemaper 3.0 software package (Applied Biosystems, USA), which displayed the AFLP fingerprints and quantified the polymorphic peaks.

Peaks representing AFLP fragments were scored as binary format with "1" for the presence of a band and "0" for its absence. The percentage of polymorphism (PP) was calculated by using formula PP = total number of polymorphic bands/total number of

Table 2
Primer combinations and number of detected polymorphisms.

Primer combinations	No. of fragments	No. of polymorphic fragments	Proportion of polymorphic fragments (%)	Size of fragment (bp)
FAM-EcoRI + AAA/TaqI + AAC	41	28	68.29	46–448
FAM-EcoRI + AAA/TaqI + AAG	39	30	76.92	40–464
FAM-EcoRI + AAC/TaqI + AAC	34	29	85.29	40–483
FAM-EcoRI + AC/TaqI + AC	72	62	86.11	46–448
FAM-EcoRI + AC/TaqI + ATG	49	40	81.63	40–488
FAM-EcoRI + ATA/TaqI + AAC	36	25	69.44	58–420
FAM-EcoRI + ACT/TaqI + AAC	32	20	62.50	40–380
FAM-EcoRI + ACA/TaqI + AAC	30	16	53.33	38–415
FAM-EcoRI + ACC/TaqI + ACT	44	30	68.18	51–450
FAM-EcoRI + ACA/TaqI + ATG	19	11	57.89	35–360
FAM-EcoRI + ACT/TaqI + ACC	33	28	84.85	40–420
Mean	39	29	74.36	
Total	429	329	76.69	

bands multiplied with 100. Data analysis was performed using NTSYSpc-2.10 to obtain the Jaccard's similarity coefficients (Jaccard, 1908), applying the formula $S_{ab} = N_{ab}/(N_{aa} + N_{ab} + N_{bb})$, where S_{ab} is the similarity index between ath and bth genotype, N_{ab} the number of bands present in both genotypes, N_{aa} the number of bands present in ath genotype but absent in bth genotype, and N_{bb} is the number of bands present in bth genotype but absent in ath genotype. Cluster analysis based on the similarity coefficient matrix was performed applying unweighted pair-group method of arithmetic analysis (UPGMA), using the NTSYSpc program (version 2.10). The goodness of fit in the UPGMA cluster analysis was evaluated by the Mantel's correlation test (Mantel, 1967).

3. Results

3.1. AFLP profile

The PCR products amplified by 11 pairs of FAM-*Eco*RI/*Taq*I primer combinations yielded a total of 429 AFLP peaks, corresponding to 429 DNA fragments (Table 2). Among these fragments, 329 were polymorphic. It was observed that various primer pairs produced clearly different numbers of bands with an average of 39 per primer pair. A maximum of 72 fragments was amplified with the E-AC/T-AC primer pair, and a minimum of 19 fragments with the E-AC/T-ATG primer pair. The percentages of polymorphic fragments ranged from 53.33% to 86.11% with an average of 76.69% per primer pair. The sizes of the amplified fragments were between 35 and 488 bp with the narrowest range for the E-ACA/T-ATG fragments (35–360) and the widest range for the E-AC/T-ATG fragments (40–488).

3.2. Genetic diversity of eared pheasants

Each of the 429 AFLP fragments were used to calculate Jaccard's similarity coefficients. The genetic similarities are shown in Table 3. The individuals in the brown eared pheasant population had highest similarity (0.913 in average), followed by Tibetan eared pheasant (0.903), then blue eared pheasant population (0.853). The lowest similarity was observed in the white eared pheasant (0.832). Across species, similarity was highest between brown eared pheasant and blue eared pheasant (0.832), and between white eared pheasant and Tibetan eared pheasant (0.812). The lowest similarity was observed between brown eared pheasant and Tibetan eared pheasant (0.674), and the similarity between other pairs ranged from 0.713 to 0.723.

3.3. Cluster analysis

A dendrogram constructed based on the similarity matrix is shown in Fig. 1. The dendrogram was strongly supported by high value of correlation coefficient in the Mantel's test of goodness of fit ($r = 0.931$). The dendrogram of 74 individuals was divided into four groups. All individuals in the same species belonged to the same cluster group. The brown eared pheasants and the blue eared pheasants were clustered together, and the white eared pheasant and the Tibetan eared pheasant were clustered together.

4. Discussion

In this study, we used AFLP analysis to detect genetic differences among 74 individuals from among four eared pheasant species. The AFLP technique combines the reliability of restriction enzyme digestion with the ability of PCR to successfully amplify small amounts of genomic DNA without prior knowledge of the target DNA sequence (Masiga and Turner, 2004). Similar to random amplified polymorphic DNA (RAPD) (Welsh and Mc Clelland, 1990; Williams et al., 1990), the AFLP technique generates individual specific profiles from template DNA by amplifying anonymous fragments from sites scattered through the genome (Borowsky, 2001). However, AFLP differs from the RAPD method in technical details and has several advantages, such as higher temperature stringency, resulting in more reliable, reproducible data (Majer et al., 1996; Maugham et al., 1996; Sanchez et al., 1999; Reineke et al., 1999). In our experiments, we fixed on 300 ng eared pheasant genomic DNA as the amount of starting template for the AFLP reaction. In addition, fluorescent labelling and automated sequence

Table 3
Average and range (within brackets) of Jaccard's similarity coefficients for interspecific and intraspecific comparisons, calculated from AFLP data.

	Brown eared pheasant	Blue eared pheasant	White eared pheasant	Tibetan eared pheasant
Brown eared pheasant	0.913 (0.872–0.938)			
Blue eared pheasant	0.832 (0.797–0.886)	0.853 (0.825–0.880)		
White eared pheasant	0.713 (0.681–0.743)	0.723 (0.688–0.763)	0.832 (0.792–0.885)	
Tibetan eared pheasant	0.674 (0.646–0.708)	0.714 (0.664–0.754)	0.812 (0.740–0.852)	0.903 (0.835–0.929)

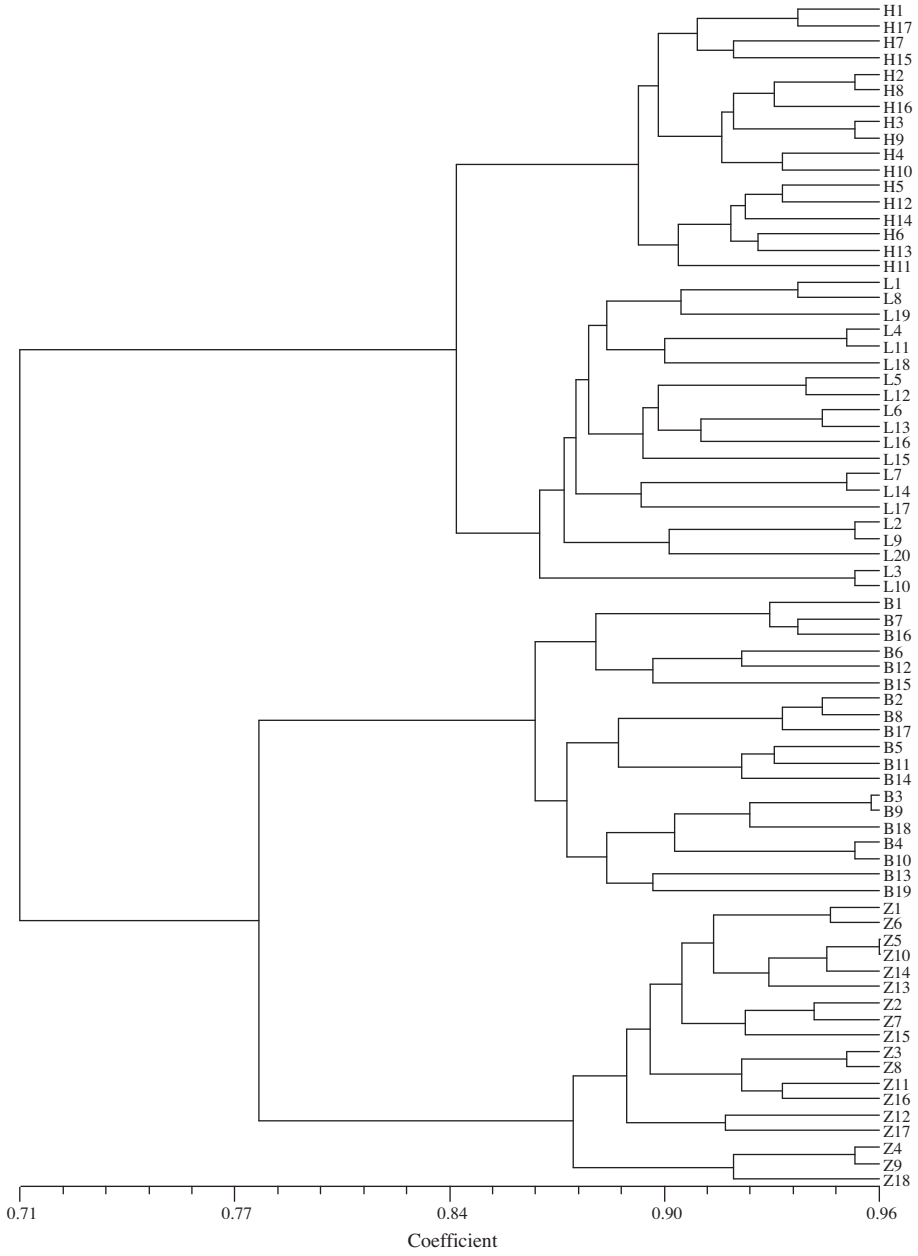

Fig. 1. Dendrogram of 74 individuals resulting from UPGMA analysis based on Jaccard's similarity coefficient: (H) brown eared pheasant; (L) blue eared pheasant; (B) white eared pheasant and (Z) Tibetan eared pheasant.

analysis, instead of the previous techniques of radioactive tagging, was used to monitor the outcome of the AFLP analysis (Knorr et al., 1999; Wong et al., 2004; Papa et al., 2005). A total of 429 AFLP peaks were amplified using 11 pairs FAM-fluorescent *Eco*RI/*Taq*I primer combinations. The polymorphic peaks percentage ranged from 53.33% to 86.11%, and on average 76.69%, thus 329 fragments were polymorphic. The results indicate that it is feasible to use AFLP for the study on genetic diversity of eared pheasants.

Genetic similarity coefficients between the four species ranged from 0.674 to 0.832, indicating a moderate genetic distance among the four species of eared pheasants. The blue eared pheasants, the white eared pheasants and the Tibetan eared pheasants are mainly distributed in north-western China, while the brown eared pheasants only exist in Shanxi, Hebei, and Beijing. According to the Jaccard similarity coefficients and the dendrogram in the current study, Tibetan eared pheasant had a higher genetic similarity with white eared pheasants than others, which was consistent with the geographical distance between the two species. However, blue eared pheasant had a higher genetic similarity with brown eared pheasants than with white eared pheasants, suggesting the genetic similarity is not necessary to be associated with geographical distance.

It was found that genetic similarity between individuals within species was higher in brown bared pheasant (0.913) and Tibetan eared pheasant (0.903) than those in blue eared pheasant (0.853) and white eared pheasant (0.832). The estimated genetic similarities appear to be a strong association with the population sizes of the four ecological species, and in line with the rank of the IUCN Red List of Threatened Species. It is known that the number of brown eared pheasants was smallest among four eared pheasant species, and also classified as one of the endangered species with high priority for conservation in China (Zheng and Wang, 1998; CITES, 2006). Small population size of brown eared pheasants could lead to an increase of consanguineous mating and conse-

quently a reduction of genetic diversity. Tibetan eared pheasant is classified "near threatened" in the IUCN Red List. This is the only species to inhabit relatively poor shrub vegetation as well as primary forest in eastern Tibet. Therefore this species might encounter more dangers than the others. The low genetic diversity of this species is an indicator that the population size might have been reduced during recent years and that the species is at risk of becoming extinct.

The present study is the first investigation on genetic distance between four species of eared pheasants and genetic diversity within the species. The results indicate moderate genetic distance between the four eared pheasant species, and moderate genetic diversity within blue eared pheasant and white eared pheasant but low genetic diversity within brown eared pheasant and Tibetan eared pheasant. Such information provides a foundation for the conservation of these endangered birds and the understanding of the evolution and genetic improvement of eared pheasants.

Acknowledgments

This study was supported by grants from the National Natural Science Foundation of China (Project No. 30972232), the Programme for New Century Excellent Talents in University of China (Project No. NCET-08-0816).

References

Agrama, H.A., Houssin, S.F., Tarek, M.A., 2002. Cloning of AFLP markers linked to resistance to *Peronosclerospora sorghi* in maize. Molecular Genetics and Geonmics 267, 814–819.

Bird Life International, 2006. Species factsheet: *Crossoptilon mantchuricum*. Available from: <http://www.birdlife.org>.

Borowsky, R.L., 2001. Estimating nucleotide diversity from random amplified polymorphic DNA and amplified fragment length polymorphism data. Molecular Phylogenetics and Evolution 18, 143–148.

CITES, 2006. Convention on international trade in endangered species of wild fauna and flora (CITES) appendices I, II and III. Available from: <http://www.cites.org/eng/app/appendices.shtml>.

Fu, Y.M., Li, S.P., Li, D.M., Wu, M.L., Wu, Y.F., 2009. Isolation and characterization of microsatellite markers from brown eared-pheasant (*Crossoptilon mantchuricum*). Conservation Genetics 10, 1993–1995.

Guerra, M.M., Bernardo, F., McLauchlin, J., 2002. Amplified fragment length polymorphism (AFLP) analysis of *Listeria monocytogenes*. Systematic and Applied Microbiology 25, 456–461.

Hedrick, P.W., 2001. Conservation genetics: where are we now? Trends in Ecology & Evolution 16, 629–636.

Horng, Y.M., Chen, Y.T., Wu, C.P., Jea, Y.S., Huang, M.C., 2004. Cloning of Taiwan water buffalo male-specific DNA sequence for sexing. Theriogenology 62, 1536–1543.

Horng, Y.M., Huang, M.C., 2003. Male-specific DNA sequences in pigs. Theriogenology 59, 841–848.

Huang, C.W., Cheng, Y.S., Rouvier, R., Yang, K.T., Wu, C.P., Huang, M.C., 2007. AFLP fingerprinting for paternity testing in ducks. British Poultry Science 48, 323–330.

Jaccard, P., 1908. Nouvelles recherches sur la distribution florale. Bulletin de la Société Vaudoise des Sciences Naturelles 44, 223–270.

Jones, S.W., Dobson, M.E., Francesconi, S.C., Schoske, R., Crawford, R., 2005. DNA assays for detection, identification, and individualization of select agent microorganisms. Croatian Medical Journal 46 (4), 522–529.

Knorr, C., Cheng, H.H., Dodgson, J.B., 1999. Application of AFLP markers to genome mapping in poultry. Animal Genetics 30, 28–35.

Lazzaro, B.P., Sceurman, B.K., Carney, S.L., Clark, A.G., 2002. fRFLP and fAFLP: medium-throughput genotyping by fluorescently post-labeling restriction digestion. BioTechniques 33 (3), 539–546.

Li, D.M., Gao, S.P., Wu, Y.F., Sun, L.H., Guo, S.B., Li, J.L., 2009. Habitat selection of breeding brown eared-pheasants (*Crossoptilon mantchuricum*) in Xiaowutaishan national nature reserve, Hebei Province, China. Frontiers of Biology in China 4 (1), 102–110.

Lu, X., Zhang, G.M., 2002. Habitat use of Tibetan eared pheasant (*Crossoptilon harmani*) flocks in the non-breeding season. Ibis 144, 17–22.

Lu, X., Zhang, G.M., 2003. Reproductive ecology of Tibetan eared pheasant *Crossoptilon harmani* in scrub environment, with special reference to the effect of food. Ibis 145, 657–666.

Majer, D., Mithen, R., Lewis, B.G., Vos, P., Oliver, R.P., 1996. The use of AFLP fingerprinting for the detection of genetic variation in fungi. Mycological Research 100, 1107–1111.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27, 209–220.

Masiga, D.K., Turner, C.M.R., 2004. Amplified (restriction) fragment length polymorphism (AFLP) analysis. Methods in Molecular Biology 270, 173–185.

Maugham, P.J., Saghai Maroof, M.A., Buss, G.R., Huestis, G.M., 1996. Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance and near-isogenic line analysis. Theoretical and Applied Genetics 93, 392–401.

Mba, C., Tohme, J., 2005. Use of AFLP markers in surveys of plant diversity. Methods in Enzymology 395, 177–201.

Murata, K., Nii, R., Sasaki, E., Ishikawa, S., Sato, Y., Sawabe, K., Tsuda, Y., Matsumoto, R., Suda, A., Ueda, M., 2008. *Plasmodium* (*Bennettinia*) *juxtanucleare* infection in a captive white eared-pheasant (*Crossoptilon crossoptilon*) at a Japanese zoo. The Journal of Veterinary Medical Science 70 (2), 203–205.

Papa, R., Troggio, M., Ajmone Marsan, P., Nonnis Marzano, F., 2005. An improved protocol for the production of AFLP™ markers in complex genomes by means of capillary electrophoresis. Journal of Animal Breeding and Genetics 122, 62–68.

Plastow, G., Siggens, M., Bagga, M., Brugmans, B., Heuven, H., Peleman, J., 2003. Utilization of AFLP for genetic distance analysis in pigs. Archives de Zootecniche 52, 157–164.

Reineke, A., Karlovsky, P., Zebitz, C.P.W., 1999. Amplified fragment length polymorphism analysis of different geographic populations of the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae). Bulletin of Entomological Research 89, 79–88.

Sanchez, G., Restrepo, S., Duque, M.C., Fregene, M., Bonierbale, M., Verdier, V., 1999. AFLP assessment of genetic variability in cassava accessions (Manihot esculenta) resistant and susceptible to the cassava bacterial blight (CBB). Genome 42, 163–172.

Schmidt, H., Taniwaki, M.H., Vogel, R.F., Niessen, L., 2004. Utilization of AFLP markers for PCR-based identification of *Aspergillus carbonarius* and indication of its presence in green coffee samples. Journal of Applied Microbiology 97, 899–909.

Sreekumar, G.P., Smyth Jr., J.R., Ponce de Leon, F.A., 2001. Molecular characterization of the Smyth chicken sublines and their parental controls by RFLP and DNA fingerprint analysis. Poultry Science 80, 1–5.

Van Haeringen, W.A., Den Bieman, M.G., Lankhorst, A.E., Van Lith, H.A., Van Zutphen, L.F.M., 2002. Application of AFLP markers for QTL mapping in the rabbit. Genome 45, 914–921.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T.V.D., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23, 4407–4414.

Wang, N., Jia, F., Zheng, G.M., 2006. Nest-site selection of the white eared pheasant in southwestern Sichuan Province, China. Frontiers of Biology in China 1, 66–70.

Welsh, J., Mc Clelland, M., 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18, 7213–7218.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18, 6531–6535.

Wong, K.Y., Chuan, Y.C., Aggarwal, A., Tham, L., Kong, W.M., Tan, P., 2004. Identifying patterns of DNA for tumor diagnosis using capillary electrophoresis amplified fragment length polymorphism (CE-AFLP) screening. Journal of Bioinformatics and Computational Biology 2 (3), 569–587.

Yang, F.Y., Wang, R.Q., Zhang, J., Li, S.G., Qiu, F.C., 2001. Study on the nest site choosing of crossoptilon mantcuricum in Shanxi. Journal of Shanxi University (Natural Science Edition) 24 (2), 151–154.

Yen, N.T., Tai, C., Cheng, Y.S., Huang, M.C., 2001. Relative genetic effects of Duroc and Taoyun breeds on the economic traits of their hybrids. Asian–Australasian Journal of Animal Science 14, 447–454.

Zhang, Z.W., Zheng, G.M., Yang, X.M., Wu, J.Y., Li, X.P., 1997. Clutch size and its variations of brown eared pheasant. Acta Zoologica Sinica 43, 83–86.

Zheng, G.M., Wang, Q.S., 1998. China Red Data Book of Endangered Animals (Aves). Science Press, Beijing, China.