Journal of Genetics and Genomics
(Formerly *Acta Genetica Sinica*)
November 2007, 34(11): 1010−1018

Research Article

Genetic Analysis of Six Endangered Local Duck Populations in China Based on Microsatellite Markers

Ying Su[1], Ruijun Long[2], Guohong Chen [3, ①], Xinsheng Wu[3], Kaizhou Xie[3], Jianghong Wan[4]

1. *Agricultural College*, *Guangdong Ocean University*, *Zhanjiang* 524088, *China*;
2. *College of Pratacultural Science*, *Lanzhou University*, *Lanzhou* 730070, *China*;
3. *College of Animal Science and Technology*, *Yangzhou University*, *Yangzhou* 225009, *China*;
4. *Animal Husband Bureau of Zhanjiang*, *Guangdong Province*, *Zhanjiang* 524088, *China*

Abstract: Seventeen microsatellite loci were used to assess the genetic variation and population structure of six endangered duck populations in China. *PIC*, heterozygosity with two estimators of genetic differentiation (F_{ST} and G_{ST}), and Nei's standard genetic distance were evaluated. The results showed that these six endangered duck populations showed high polymorphism. The proportion of inter-population subdivision among the six duck populations ranged between 17.0 and 14.7%. The average heterozygosity was 0.706, 0.702, 0.691, 0.676, 0.604, and 0.660 in the Gaoyou, Liancheng, Jinding, Beijing, Shaoxing, and Jianchang ducks, respectively. The average *PIC* ranged from 0.561 to 0.663. Finally, the results showed higher genetic diversity and difference above the levels of genetic variation among all the populations.

Keywords: endangered duck breeds; microsatellite DNA; genetic diversity; conservation

Duck breeds (*Anas platyrhynchos*) play important roles in socio-economic and ecological values in both southern and northern parts of China. In addition, their precious values as genetic resources are used for the genetic improvement of duck breeds [1–3]. The Shaoxin ducks, Liancheng ducks, and Jinding ducks are Chinese special egg-type whereas the Beijing ducks, Jianchang ducks, and Gaoyou ducks are special Chinese meat-type [4, 5]. Liancheng ducks are the important medicinal breed in China. Owing to their high yield, Liancheng ducks have brought considerable economic benefits to local farmers. The Beijing ducks are a well-known breed and are used worldwide for meat production. As a consequence, the Beijing ducks have locally adapted to a wide range of environments, showing high levels of phenotypic variability and increased fitness under natural conditions. However, their population numbers were dramatically reduced and they were in danger of extinction in the second half of the twentieth century. Six duck breeds were taken as the Critical and Endangered breeds for conservation [6]. Hence, the Chinese government launched a conservation program for these duck breeds. Thus, determining the appropriate sampling and management procedures is crucial for drawing the conservation strategies of genetic resources. One of the important tasks for effective genetic conservation is to detect the allelic richness of the population or samples that are targeted for conservation. An understanding of the relationships and

Received: 2007−03−02; Accepted: 2007−03−26
This work was supported by the National High-tech Research and Development Program (863 Program) (No. 2001AA243082), and the National Natural Science Foundation of China (No. 30170673).
① Corresponding author. E-mail: ghchen@yzu.edu.cn; Tel: +86-514-797 9230

the culture aspects of these breeds will facilitate the effective conservation of genetic resources in the breed gene pool by strategy decision-making.

Molecular techniques have provided new markers for the study of genetic variation [7–15]. Among these techniques, microsatellite has rapidly become the favorite method for population genetic studies as it shows advantages over other methods, particularly, in conservation projects. Microsatellites are widely distributed in the genome, and thus these exhibit a high degree of polymorphism among breeds and even individuals. The observed genetic diversities arise from the consequence of genetic drift and mutation. Several studies of genetic relationships between duck breeds using the microsatellite method were reported previously [16–19]. In this study, we assessed the genetic structures as well as the phylogenetic relationships among six duck populations in China *via* DNA polymorphisms, to provide useful information for effective strategies of conservation programs for Chinese endangered local duck breeds.

1 Materials and Methods

1.1 Sample collection

Blood samples were obtained from 288 unrelated individuals representing 6 endangered local duck populations in China. These 288 individuals included 50 Jinding (JD) ducks, 50 Liancheng (LC) ducks from Fujian, 50 Shaoxing (SX) ducks from Zhejiang, 38 Gaoyou (GY) ducks from Jiangsu, 50 Beijing (BJ) ducks from Beijing, and 50 Jianchang (JC) ducks from Sichuan. All individuals fit a racial standard and were included in their respective independent selection or conservation programs. All blood samples were stored at –80℃ before analyses.

1.2 DNA extraction and PCR amplification

Genomic DNA was isolated from blood using a modified phenol/chloroform extraction method. Blood was digested in 300 μL lysis buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, 100 mmol/L NaCl, pH 8.0) with 8 μL proteinase K (10 mg/mL) for 12 h at 55℃. The extraction was repeated three times. After precipitation by adding two volumes of ice-cold ethanol, DNA was isolated by centrifugation and then stored at –20℃ for future use. DNA pellets were re-suspended in 30 μL TE buffer, and the total genomic DNA was quantified using agarose gelelectrophoresis. The DNA concentration was calculated according to the standards.

Seventeen microsatellite loci were investigated, including *APL577*, *APL579*, *APL580*, *CMO211*, *CMO212*[17], *AY258*, *AY264*, *AY269*, *AY283*, *AY285*, *AY287*, *AY294*, *AY295*, *AY310*, *AY314*, *CADU086*, and *CADU24* (from GenBank). The PCR primers are listed in Table 1. All primers were synthesized by the Shanghai Bioasia Bio-Tech. Co., Ltd.

Polymerase chain reaction (PCR) amplifications were performed on Biometra T gradient 1702238 thermal cyclers. A total reaction volume of 20 μL with 2 μL of 10×buffer, 2 μL of 25 mmol $MgCl_2$, 0.8 μL of 10 mmol dNTPs, 0.2 μL of 5 U/μL *Taq* DNA polymerase, 1 μL of 10 pmol/μL each primers, and approximately 50 ng of genomic DNA. The reaction was carried out by denaturing at 94℃ for 1 min, annealing at the temperature optimized for each primer pair for 1 min and extending at 72℃ for 1 min for 30 cycles, followed by an extra extension step at 72℃ for 10 min. The optimized annealing temperatures of different primer pairs are also listed in Table 1.

The amplification products were separated by electrophoresis on 8% non-denaturing polyacrylamide gels along with DNA marker (pBR322 DNA/ *Msp* Ⅰ markers) and visualized by silver staining [20]. The images data was analyzed using the Kodak Digital Science ID Image Analysis Software.

1.3 Statistical analyses

The following parameters were calculated for every locus using software FSTAT (Version 2.9.3.2) [21] and Genepop (Version 3.3)[22]: the distribution of allele frequencies, the presence of private alleles, the number of alleles, the observed heterozygosity (H_o),