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Studies on the DNA Barcoding of Two Newly Discovered Chicken Breeds by mtDNA *COI* Gene

Gao Yu-Shi, Tu Yun-Jie, Tang Xiu-Jun, Lu Jun-Xian and Zhang Xiao-Yan
Institute of Poultry Science, Chinese Academy of Agricultural Sciences,
225003 Yangzhou, Jiangsu, P.R. China

Abstract: In order to investigate the genetic diversity of two newly discovered chicken breeds (Jinhu silky and Wahui) and their genetic relationships with other indigenous chicken breed (Langshan), the *COI* gene of these three breeds were analyzed by amplification and direct sequencing. The results showed that the selectived sequence of *COI* had ten SNPs and seven haplotypes, three of SNPs were specific mutation sites, five of haplotypes were specific haplotypes. The Pi of Jinhu silky, Wahui and Langshan were 0.068, 0.051 and 0.369%, respectively and the Hd were 0.4167, 0.3030 and 0.9000, respectively. The Kimura 2-parameter distances between breeds ranged from -0.002 to 0.236. The genetic distances of intrapopulation were less than that of interpopulation. The data suggest that the genetic diversity of Langshan was higher than that of two newly discovered breeds, the specific mutation sites and haplotypes provide the gist for variety identification.

Key words: Indigenous chicken breeds, mtDNA *COI* gene, DNA barcoding, variety identification, genetics, China

INTRODUCTION

DNA barcoding means using a uniform objective DNA list as a tool to assess species which is a new direction of biology taxology in recent years. DNA barcoding has been used in many research fields including assessment and classification of multi-animal species (Hajibabaei *et al*., 2006; Hebert *et al*., 2004). Mt *COI* gene could be used as DNA barcode genes because of its many advantages such as relative conservation, long variation sequence, amplification with universal primer and so on (Xiao *et al*., 2004). The genetic distance of 640 bp sequence of *COI* gene was analyzed to assessed and classified twenty butterfly samples of four kinds which belong to Bhutanitis (Zhu *et al*., 2005). The mt *COI* gene sequence of 270 kinds of fish in Australia was analyzed to found that the DNA barcode could not only form species appraising system but also contained some information of system development (Ward *et al*., 2005).

Wahui chicken in Jiangxi province and Jinhu silky chicken in Fujian province were discovered by investigating local chicken breeds' resources in 2002 which was supported by agricultural important item Genetic Diversity Research of Chinese Indigenous Chicken Breeds. Before 2002, these two chicken breeds were not assessed or included in the protective list of nation livestock and poultry genetic resource. In 2004, these two chicken breeds were collected and conserved in National Gene Pool for Poultry. Langshan is an indigenous chicken breed in China which is distributed in Jiangsu province and has been conserved in gene pool for 20 years. In this study, the variations of selected *COI* gene and population genetic structure of the two newly discovered chicken breeds were investigate and their genetic relationships with Langshan was analyzed, the genetic diversity of these three chicken breeds were also studied by using *COI* gene as DNA barcode. The results may provide bases for breeds identification at the level of mtDNA.

MATERIALS AND METHODS

Experimental population: Nine Jinhu silky hens, twelve Wahui hens and five Langshan hens were randomly collected from conservation populations in National Gene Pool for Poultry.

DNA isolation: DNA was isolated from the whole blood by the method of phenol/chloroform (Sambrook and Russell, 2002).

Genotyping: The primers were as follows: F: 5'GCACA GGATGGACAGTTTAC 3' (371-390), R: 5'ATAGCATAG

Corresponding Author: GAO Yu-Shi, Institute of Poultry Science, Chinese Academy of Agricultural Sciences, 225003 Yangzhou, Jiangsu, P.R. China

GGGGGTCT CAT 3' (1002-1021), designed according to the *COI* gene sequence of Chinese Red Jungle-fowl which was enunciable in GenBank. The length of PCR product was 651 bp. Taq DNA polymerase, dNTPs, $MgCl_2$ and 100+1.5 kb DNA ladder markers used in this experiment were all bought from Shanghai Biology Engineering Corporation.

The 50 μL PCR volume included 100 ng of genomic DNA template, 0.5 μM of each primer, 200 μM of each dNTP, 2.0 mM $MgCl_2$ and 1.0 U Taq DNA polymerase. The reaction condition was initially denatured at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 45 sec, primer annealing at 55°C for 1 min, extension at 72°C for 1 min and a final extension at 72°C for 10 min. The obtained fragments were detected on 2.0% agarose gel. Then PCR products were recovered and sequenced by Shanghai Biology Engineering Corporation.

Statistical analysis: The sequence alignments were managed by CLUSTALW software (Hebert *et al*., 2003). Kimura 2-parameter distances were estimated by MEGA 3.0 software (Kress *et al*., 2005). Haplotypes were counted by DNASP 4.10 software (Rozas *et al*., 2003).

RESULTS AND DISCUSSION

The selected *COI* gene sequence of 26 chickens from two newly discovered breeds and Langshan were assayed, ten variation sites were discovered. Among those variation sites, six were single polymorphism sites while four were simple information sites. Additionally, there were seven kinds of haplotypes in the variation sites (Table 1). H1 was share haplotype of three indigenous chicken breeds and H2 was share haplotype of Jinhu silky and Wahui which indicated that the indigenous chicken breeds had a higher hereditary similarity. The reason may be that when these breeds formed and evolved, the cross-breeding and reciprocal influence happened among the different chicken breeds. It was also found that H3 was particular type in Wahui, H4 was particular type in Jinhu silky, H5-H7 were particular types in Langshan which could provide a foundation for variety identification.

Each breed had its own mutation sites. Jinhu silky were mutated at the 93rd site, Wahui were mutated at the 113th site and Langshan were mutated at the 403rd site, mutation rates were 11.1, 8.33 and 20%, respectively (Table 2). It is possible that natural selection and artificial selection under different periods and surroundings result in different mutation types. The haplotype diversities and nucleotide diversities are primary indicators for evaluating the mtDNA's variation degree because the value is bigger, the genetic diversity is richer (Smith *et al*., 2006; Pan *et al*., 2006). In this study, the average nucleotide difference, haplotype diversities and nucleotide diversities of Langshan were the largest which were 2.4, 0.9000 and 0.00369, respectively. Wahui and Jinhu silky were 0.3333, 0.3030, 0.00051 and 0.4444, 0.4167, 0.00068, respectively (Table 3). It is possible that Wahui and Jinhu silky had smaller population size were closed in the thickly forested mountains for a long time, formed natural isolation barrier and had specific phenotypic characteristics, impossible to exchange genes with other chicken breeds so, the degree of variation are less, the genetic diversity are poorer and the breed purities are higher.

Langshan are raised in Nantong of Jiangsu province where has convenient traffic and a developed poultry industry. Before being collected to the gene pool, Langshan may have already had some gene exchanges with other chickens. After being conserved in the gene pool, they are selected mating by family in equality so, they have kept a higher genetic diversity until now.

Kimura 2-parameter distance and net distance (Da) among three indigenous chicken populations showed that the intrapopulation genetic distances ranged from 0.000-0.077 and the interpopulation genetic distances ranged from 0.068-0.370. The interpopulation genetic distance of Langshan was 0.370 which was the largest of all. The interpopulation genetic distances of Wahui and

Table 1:The no. of *COI* gene haplotype in three indigenous chicken breeds

Haplotype	Jinhu silky	Wahui	Langshan
H1	7	8	2
H2	1	3	-
H3	-	1	-
H4	1	-	-
H5	-	-	1
H6	-	-	1
H7	-	-	1

Table 2: The mutation sites and frequency of *COI* gene in three indigenous chicken breeds

Sites	Mutation types	Mutation frequency in Jinhu silky (%)	Mutation frequency in Wahui (%)	Mutation frequency in Langshan (%)
93	TTAGCA*A*G G→A	11.11	0.00	0
113	CTAGG*G*GCC A→G	0.00	8.33	0
403	CCCA*C*GCAG T→C	0.00	0.00	20

Table 3: The K, Pi and Hd of *COI* gene in three indigenous chicken breeds

Breeds	No.	No. of variable sites	K	Pi	Hd
Jinhu silky	9	2	0.4444	0.00068	0.4167±0.191
Wahui	12	2	0.3333	0.00051	0.3030±0.136
Langshan	5	6	2.4000	0.00369	0.9000±0.161

K means average nucleotide difference, Pi means nucleotide diversity, Hd means Haplotype diversity

Table 4: Kimura 2-parameter distance and net distance (Da) among 3 indigenous chicken populations

Breeds	Interpopulation	Jinhu silky	Wahui	Langshan
Jinhu silky	0.068	-	0.077	0.000
Wahui	0.089	- 0.002	-	0.007
Langshan	0.370	0.218	0.236	-

Above dialogue was net distance (Da), down diagonal was Kimura 2-parameter distance of *COI* gene among seven chicken breeds; all of the value was enlarged 100 times

Jinhu silky were 0.089 and 0.068, respectively (Table 4). The intrapopulation genetic distances were less than the interpopulation genetic distances. This result indicates that the degree of differentiation is low in Chinese indigenous chicken breeds and the variation of interpopulation is bigger than intrapopulation.

Before identifying the chicken breeds, the difference of morphology, physiology, biochemistry, cytogenics and performance in breeds must be found in addition, all these information must be collected and synthesized then we could scientifically and exactly identify breeds.

Compared with other indigenous chicken breeds protected in nation poultry directory, these two indigenous chicken breeds have specificity traits. Jinhu silky have ten full traits which are yellow-black feather, mulberry comb, gray head, green ear, beard, dry foot, skirt feather, black skin, black meat and black bone. Wahui have five grey traits, namely, gray feather, gray skin, gray-dry foot, gray beak and gray comb.

AFLP fingerprint technology was used to analyze the genetic diversity of twelve indigenous chicken breeds, four and two special Gershgorim band were found in Wahui and Jinhu silky, respectively (Gao *et al*., 2007).

CONCLUSION

In this study, the 651 bp segment in mt *COI* gene of 26 chickens were detected. The results showed that both of these two indigenous chicken breeds have specific mutation sites and haplotype. Those specific genetic markers provide objective foundation for variety identification and could be used for establishing rapid, convenient, scientific method for variety identification.

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