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The Genetic Distribution and Polymorphism Analysis of Antiviral Resistant Mx Gene Locus in Fifteen Chinese Indigenous Chicken Breeds

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Abstract: The aim of this study was to examine Single Nucleotide Polymorphism (SNP) of resistant sequence in Mx gene in chicken with a mismatched Polymerase Chain Reaction-restriction Fragment Length Polymorphism (PCR-RFLP) method and thus, the differences of frequency on sequence A2032G in Mx gene in different breeds were detected. The results showed that three genotypes were found on A2032G in 15 breeds of chicken, which were AA, AG, GG and AA genotype was not found in Luyuan, Langshan chicken and Zhongyuan Game chicken, as GG genotype did in Chahua chicken and Red Jungle Fowl. The average frequency of resistance gene (A) and sensitive gene (G) was 0.350 and 0.650, respectively. However, the frequency of A alleles had a wide rangeability from 0.034-0.984 and the results of χ^2-test indicated that Mx gene in all populations was in Hardy-Weinberg equilibrium.

Key words: Chicken breed, Mx gene, genetic polymorphism, allele, China

INTRODUCTION

Domestic and oversea scholars have evolved extensive researches of resistance gene in poultry, mainly including Major Histocompatibility Complex (MHC) genes (Xu *et al*., 2007; Nikolich *et al*., 2004; Niikura *et al*., 2004) related to the resistance of Marek's disease and Lloyd's sarcoma, the gene which can resist Salmonella, Leishmania encoding Natural Resistance Macrophage Protein-1 (NRAMP1) (Liu *et al*., 2003) and Interferon (IFN) (Wei *et al*., 2006) gene, which can produce a variety of broad spectrum antiviral protein and so on. So far, Mx protein gene (myxo virus resistance gene) has been found the only one antiavian influenza virus gene (Ding *et al*., 2006). The research on Mx gene in chicken was started 10 years ago. Bernasconi *et al*. (1995) got the full-length chicken Mx gene from the IFN induced Leghorn fibroblasts by RT-PCR, encoding a total of 705 amino acids. He also found that the chicken Mx protein mainly located in the cytoplasm (Bernasconi *et al*., 1995). Schumacher *et al*. (1994) had studied that the chicken Mx gene contained 14 exons and its translation start codon located in the second exon. Studies reported by Ko *et al*. (2002) showed that in many natural variations of chicken Mx gene, only the 631 (Ser to Asn) mutation was caused by a single nucleotide substitution in 2,032 point, which has antiviral activity. The protein had activity only when G took the place of A in 2,032 point and the 631th amino acid changed from serine to asparagine (Ko *et al*., 2002; Seyama *et al*., 2006). But this kind of research has not been reported in our county.

As China has rich resources of local chicken breeds with distinctive characteristics and strong resistance. They are precious materials of poultry breeding for disease resistance in our country. So it is necessary to detect the distribution of mutation at Mx cDNA 2,032 point. It can provide materials and screening technique for the research of the resistivity of Mx gene in Chinese local chicken breeds.

MATERIALS AND METHODS

Test material: A total of 718 samples in 15 breeds were obtained from Poultry Institute, Chinese Academy of Agricultural Science, including 44 Beijing Fatty chickens, 35 Dagu chickens, 53 Luyuan chickens, 66 Zhongyuan game chickens, 40 Langshan chicken, 48 Xiaoshan chicken, 50 Chongren chicken, 63 Wugu chickens, 46 Gushi chickens, 53 Qingyuan chickens, 78 White ear chickens, 40 Xianju chickens, 44 Tibet chickens, 26 Chahua chickens and 32 Red Jungle fowls. Genomic DNA was extracted from chicken venous blood through classical phenol-chloroform method. DNA was solved by TE and stored at -20.

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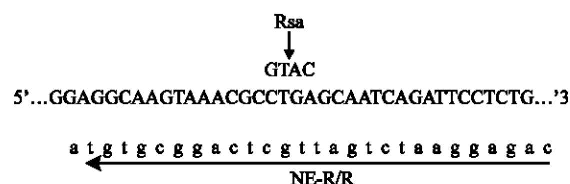
Primers: The primers were designed according to literature (Seyama *et al*., 2006) as following:

NE-F2, 5'CCT TCA GCC TGT TTT TCT CCT TTT AGG AA3'
NE-R/R, 5'CAG AGG AAT CTG ATT GCT CAG GCG TGT A3'

Because of no enzyme, which could recognize single nuclear variation, so we used mismatched primer as the reverse primer. Rsa I could recognize and digest the Mx gene, when the base at 2032 site was G rather than A.



PCR-RFLP: PCR amplify reaction system was 10×PCR buffer 2.0 μL, 10 mmol L^{-1} dNTPs 2 μL, 20 μmol L^{-1} NE-F2 1 μL, 20 μmol L^{-1} NE-R/R 1 μL, Taq polymerase (Takara dalian, China) 1U, 15 ng μL DNA 1 μL, total 20 μL. PCR reaction was conducted under the following cycling conditions: Initial denaturing step at 94 for 5 min; followed by 35 cycles of 94 for 1 min, 60 for 1 min, 72 for 1 min and completed by a final extension at 72°C for 5 min. The PCR products of Mx gene were digested by restriction enzymes Rsa I at 37 for 3 h.

Both PCR products and digested fragments were analyzed on 10% Polyacrylamide Gel Electrophoresis (PAGE) for 3 h at 120 V.

Statistical analysis: Data analysis was conducted using the SPSS12.0 statistical package. Pearson χ^2-test with the suitable degree of freedom was used to evaluate the populations, which were determined to be in Hardy Weinberg equilibrium.

RESULTS AND DISCUSSION

Mismatched PCR: Mismatched PCR was used to amplify the Mx gene from site 1961-2060, which contained 100 bp. Then we used 10% Polyacrylamide Gel Electrophoresis (PAGE) and silver staining (Fig. 1) to distinguish their genotypes. Figure 1 indicated that the gene was very bright and the length of them was 100 bp as a contrast from Marker-B.

RFLP: PCR products could be digested into two fragments 73 and 27 bp, respectively, when the single nuclear of 2032 site was G and produced wild genotype, GG. However, if 2032 mutated to A, the PCR products could not be recognized and digested by Rsa I and

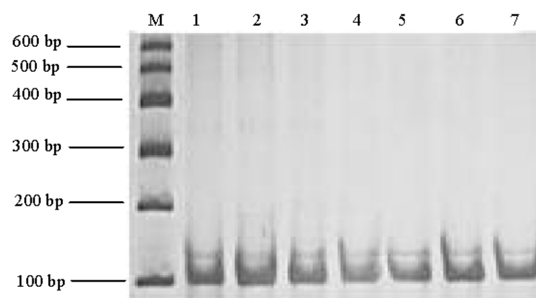


Fig. 1: PCR products of Mx gene, 1-7 PCR products (100 bp), M, DNA marker-B

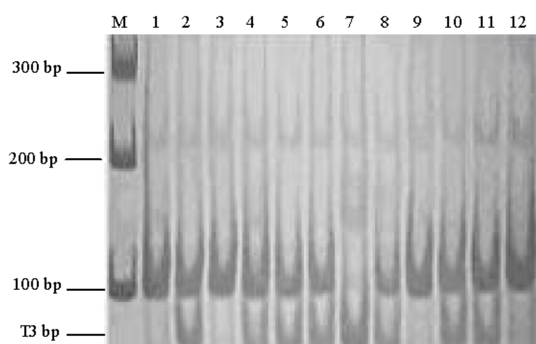


Fig. 2: Image of digested PCR product of Mx gene with Rsa I AA:1, 3, 9, 12; AG; 2, 4, 5, 6, 8, 10, 11; GG:7, M: DNA marker-B

produced the fragment of 100 bp and AA genotype. In another occasion, A and G were simultaneous and produced AG genotype with three fragments of 100, 73 and 27 bp. According to the number of fragments, which from PCR products digested by Rsa I, this research had 3 genotypes, AA, AG, GG (Fig. 2).

Allele frequency, genetypic frequency and χ^2-test: Mx genotypes and alleles frequencies detected by PCR-RFLP in 15 chicken breeds were shown in Table 1. In 15 breeds, most had 2 alleles A and G, 3 genotypes AA, AG and GG, except Langshan chicken, Dagu chicken, Luyuan chicken, Chahua chicken and Red Jungle Fowl, which lost GG or AA genotype. An interesting thing was that A was the highest frequency of allele in Red Jungle fowl the. In addition, the results of χ^2-test indicated that all populations were in Hardy-Weinberg equilibrium (Table 1).

The restriction enzyme used in this experiment can exactly detect the mutation between A and G of Mx gene in every kind of chicken breeds. The average frequency of resistant allele A and sensitive allele G was 0.350 and 0.650, respectively. The allele frequency detected by Seyama *et al*. (2006) in 17 chicken breeds, 36 strains and

Table 1: Allele frequencies, genetypic frequencies and the value of χ^2-test of Mx gene in 15 chicken breeds

		Genotype frequency			Gene frequency		
Breeds	No	AA	AG	GG	A	G	χ^2
Beijing fatty	44	0.023	0.023	0.955	0.034	0.966	0.005
Dagu	35	0.086	0.029	0.886	0.100	0.900	0.003
Luyuan	53	0.000	0.245	0.755	0.123	0.877	0.000
Game chikens	66	0.000	0.273	0.727	0.136	0.864	0.000
Langshan	40	0.000	0.500	0.500	0.250	0.750	0.009
Xiaoshan	48	0.063	0.417	0.521	0.271	0.729	0.000
Chongren	50	0.040	0.480	0.480	0.280	0.720	0.003
Wugu	63	0.095	0.429	0.476	0.310	0.690	0.000
Gushi	46	0.152	0.522	0.326	0.413	0.587	0.001
Qingyuan	53	0.132	0.585	0.283	0.425	0.575	0.008
White ear	78	0.205	0.513	0.282	0.462	0.538	0.000
Xianju	40	0.075	0.775	0.150	0.463	0.537	0.095
Tibet	44	0.237	0.591	0.136	0.586	0.432	0.017
Chahua	26	0.692	0.308	0.000	0.846	0.154	0.000
Red jungle fowl	32	0.969	0.031	0.000	0.984	0.016	0.000

3 primitive species was 0.592 and 0.408, respectively. Balkissoon *et al*. (2007) tested the distribution of allele frequencies at this point in a total of 1,275 individuals. For egg-to-use were 0.779 and 0.221, meat-to-use were 0.021 and 0.979 and either-to-use were 0.281 and 0.719. There are some differences between the results of this study. Li *et al*. (2006) speculated that changes in the environment (avian flu) and selection intensity on production performance might be the reason that different breeds of chicken domestic and abroad had differences on the distribution of resistant allele at this point. But Balkissoon *et al*. (2007), believed that although allele frequencies separated in different types of strains and exsited great differences, the differences had existed at the beginning of the formation of various types, rather than caused by the role of environmental selection and intensity of selection of production trait. In this study, the results of χ^2-test indicated that all populations were in Hardy Weinberg equilibrium.

The allele frequencies at this point have already fixed in all populations and have not been undertaken targeted artificial selection. This is consistent with the result of using sequence alignment to analysis different species Mx gene evolution model by Hou *et al*. (2007). Furthermore, local chicken varieties in our country did not experience the high intensity production performance selection in the history of formation of variety. Therefore, it can be speculated that the differences of allele frequencies in the local chicken breeds have existed at the beginning of the formation of variety.

From the result of genetic polymorphism at Mx gene 2,032 point in 15 typical chicken breeds, the frequency change of resistant allele A and sensitive allele G was effected greater by the physeal environmental and less by the choice of production traits. Thus, it can dispel the concern that the choice of production traits will reduce the resistance. This result has provided instructive significance for the poultry breeding.

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