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Analysis on the Polymorphism and the Genetic Effects on Some Economic Traits of Mx Gene S631N Mutation Site in Chicken

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Original Article

Analysis on the Polymorphism and the Genetic Effects on Some Economic Traits of Mx Gene S631N Mutation Site in Chicken

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Abstract

PCR-RFLP technique was performed to detect the distribution differences of the resistant and sensitive alleles of S631N site of Mx gene in 8 chicken populations. The results showed the average frequencies of resistant allele A and sensitive allele G were 0.4995 and 0.5005, respectively, and the average value of observed heterozygosis and Shannon's index were 0.6163 and 0.5232. Three populations were in Hardy-Weinberg equilibrium at this site (p>0.05). The Ewens-Watterson test indicated that this site was neutral in all the other populations except for Rugao. The dendrograms based on allele frequency divided the 8 populations into 3 clusters, which reflected the different charactertics of Mx gene in antiviral property between different populations. The relevance in the three kinds of genotypes and some economic traits of Wenchang and Anka were also analyzed, the result indicated there was almost no significant negative effect between Mx gene S631N and important economic traits.

Keywords: Chicken breed, clustering analysis, economic traits, Mx gene, genetic structure

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บทคัดย่อ

การวิเคราะห์ความหลากหลายทางพันธุกรรม และผลกระทบทางพันธุกรรมของการเกิดการผันแปรทางพันธุกรรมของยีน Mx S631N ต่อลักษณะทางเศรษฐกิจบางลักษณะในไก่

De Qin Luan[1] Guo Bin Chang[1] Zhong Wei Sheng[1] Yan Liu[2] Guo Hong Chen[1]*

เทคนิค PCR-RFLP ถูกใช้ในการตรวจสอบความแตกต่างของการกระจายตัวของ Resistant gene และ Sensitive gene ที่ตำแหน่ง S631N ของ Mx gene ในประชากรไก่ทั้งหมด 8 กลุ่มประชากร ผลการทดลองพบว่า ความถี่เฉลี่ยของ Resistant allele A และ Sensitive allele G คือ 0.4995 และ 0.5005 ตามลำดับ และค่า Observed heterozygosis มีค่าเฉลี่ยเท่ากับ 0.6163 และ Shannon's index มีค่าเฉลี่ยเท่ากับ 0.5232 ประชากรไก่ 3 กลุ่มประชากร อยู่ในสมดุลของ Hardy-Weinberg (p>0.05) การทดสอบด้วย Ewens-Watterson test แสดงให้เห็นว่า ยีนตำแหน่ง S631N นี้ เป็นกลางในทุกประชากร ยกเว้น Rugao จากแผนภูมิ Dendrograms ที่สร้างขึ้นจากความถี่ของยีน สามารถแบ่งกลุ่มประชากรไก่ 8 กลุ่มประชากร ออกเป็น 3 กลุ่ม ซึ่งแต่ละกลุ่มจะแสดงให้เห็นถึงคุณสมบัติการต้านทานไวรัสที่แตกต่างกันของ Mx gene ในกลุ่มประชากรที่ต่างกัน นอกจากนี้ได้ทำการวิเคราะห์ความสัมพันธ์ทางด้านยีนต่อลักษณะที่สำคัญทางเศรษฐกิจของ Wenchang และ Anka ทั้ง 3 กลุ่ม (Cluster) ซึ่งผลการศึกษาพบว่า Mx gene ที่ตำแหน่ง S631N และ ลักษณะที่สำคัญทางเศรษฐกิจนั้นไม่มีผลกระทบในด้านลบต่อกันและกัน

คำสำคัญ: สายพันธุ์ของไก่ การวิเคราะห์การจัดกลุ่มคลัสเตอร์ ลักษณะทางเศรษฐกิจ Mx gene โครงสร้างของยีน

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Introduction

Extensive research about resistance genes in poultry have been undertaken, the major included histocompatibility complex MHC genes, which related to the resistance to diseases such as the Marek's disease and Rous sarcoma (Niikura et al., 2004; Nikolich et al., 2004; Xu et al., 2007). The resistant Salmonella gene, NRAMP1 gene (Liu et al., 2003) and the gene that can produce a variety of broad-spectrum anti-viral protein interferon (IFN) (Wei et al., 2006). So far, the Mx protein (myxo-virus resistance gene) was only found the anti-avian influenza virus genes (Ding et al., 2006). The mouse Mx1 protein, the first protein of the Mx family to be reported, was conferred resistance to influenza virus (Staeheli et al., 1986). It was further confirmed the Mx protein with asparagine (Asn) had the anti-avian influenza virus and rhabdovirus activity contrasted to that with serine (Ser) at position 631 (Ko et al., 2002; Ko et al., 2004). However, such studies were sparingly reported, it was also reported a certain degree of negative correlation exited between resistance traits and economic traits (Balkissoon et al., 2007), therefore, it was quite important to analyze an association between the S631N mutation polymorphism and economic traits of chicken, the possibility and feasibility of genetic improvement of ability of anti-influenza virus in chicken populations were evaluated by Mx gene S631N mutation site. The Republic of China was rich resources of local chicken breeds with distinct characteristic features and strong resistance, which is most valuable disease-resistant breeding material of poultry in the world (Li et al., 2009). Therefore, 7 Chinese indigenous chicken breeds and an alien species were selected as the material to test the distribution of mutation of the 631 amino acid coding site of Mx protein in different chicken breeds, and an indigenous chicken and an alien species were chosen respectively to analysis influence of body weight in various stages, body weight measurements of 12 weeks old, slaughter traits, meat and some other important economic traits effected by the chicken site mutation. In order to provide the basis and methods for the Mx gene resistance detection of different kinds of chicken.

Materials and Methods

Experimental animals: 8 kinds of chicken included 7 Chinese indigenous chicken breeds and an alien species: 44 Zhangzhou gamecocks, 66 Henan gamecocks, 53 Huainan chickens, 38 Wannan chickens, 66 Wenchang chickens, 88 Rugao chickens, 88 Anka chickens, which were obtained from Poultry Institute of Chinese, Academy of Agricultural Science, 32 Red Jungle Flow, which were obtained from the Yunnan Wild Animal Rescue Center of China, the body weight of Wenchang

chicken and Anka chicken were weighed every 2 weeks, body measurements, slaughter traits and meat traits were also determined at 12 weeks old.

Genomic DNA of all kinds of chickens was extracted from chicken venous blood through conventional phenol-chloroform extraction method. DNA was dissolved in TE buffer and stored at -20℃ until use.

Primers design and restriction enzyme selection: The mutations between G and A of Mx cDNA nucleotide sequence at 2032 site resulted in that Ser (AGT) changed into Asn (AAT) of Mx protein at 631 sites (Ko et al., 2002). Primers were designed according to The chicken Mx gene sequence (Z23168) in GenBank F:5'-CCTTCAGCCTGTTTTTCTCCTTTAGGAA-3' (intron 13), and R1: 5'-CAGAGGAATCTGA TTGCTCAGGCGTGTA-3' (exon14), the restriction enzyme cutting site of Rsa I (Figure 1) was introduced, primers were ordered from the Shanghai Public Health and Bio-Engineering Services Limited (Sangon, Shanghai, China), and restriction enzymes were purchased from Po Biological Engineering (Dalian, China) Co., Ltd. (TaKaRa, Japan).

Figure 1 The RsaI sequence alignment

PCR amplification and detection of restriction enzyme digestion: Polymerase chain reaction (PCR) was carried out in 20 µl volume containing approximately 2 µl of 10×PCR buffer, 10 µmol/l dNTPs, 20 µmol/l each primer, 15 ng/µl genomic DNA, and 1 U Taq DNA polymerase. PCR conditions were as follows: denaturation at 94℃ for 5 min; followed by 35 cycles of denaturation at 94℃ for 60 sec, annealing for 60 sec, extension at 72℃ for 30 sec; with a final extension at 72℃ for 5 min on Mastercycler® 5333 (Eppendorf AG, Hamburg, Germany), and then the products were kept at 4℃. The PCR products were loaded on 10% neutral polyacrylamide gels (acrylamide:bisacrylamide= 29:1). Restriction enzyme reaction was carried out in 10 µl volume containing 5µl of PCR products were separately digested with 2 U Rsa I (New England Biolabs, Beverly, MA, USA) at 37℃ for 3 hrs. The fragments were separated by electrophoresis on 10% neutral polyacrylamide gels in parallel with a 100 bp DNA marker. After electrophoresis, the DNA fragments in the gels were visualized by silver nitrate staining.

Statistical analysis: The Hardy-Weinberg test, Ewens-Watterson test and the Genetic variation analysis for the group of the frequencies of alleles were analyzed by POPGENE in different groups. Cluster analysis by PHYLIP software. The relationship between each trait value and the genotypes was analyzed by the method of least squares estimation for the use of generalized linear models of SPSS16.0 software.

Results

PCR amplified products: The 100 bp (from 1961 bp to 2060 bp) sequence of Mx gene was mismatch PCR amplificated using a pair of primer. The results showed that amplification fragment sizes were consistent with the target ones and had good specificity. PCR products amplified by the primers were shown in Figure 2.

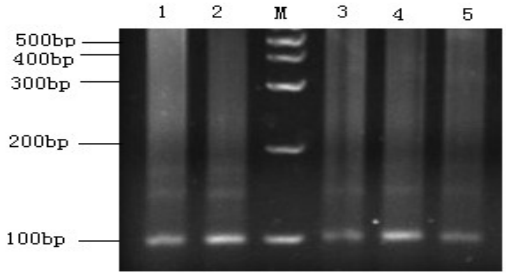

Figure 2 Agarose electrophoresis of MX PCR fragments

1-5: PCR fragment M: DNA Marker

Rsa I analysis of the G2032A of Mx gene: The nucleotide mutation of G2032A leaded to the restriction endonuclease site disappeared of Rsa I , The 100 bp PCR products were completely digested with the restriction endonuclease Mx I and genetic polymorphisms were investigated by PCR-RFLP. According to analyze the results of sequencing and restriction endonuclease map (Figure 3), three genotypes were detected AA (100 bp), AG (100 bp/ 73 bp/27 bp), GG (73 bp/27 bp).

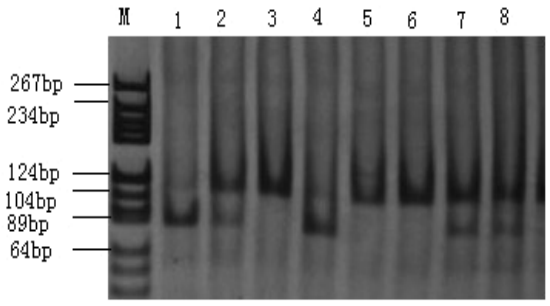

Figure 3 Polyacrylamide gel electrophoresis of digested fragments by Rsa I AA: 3, 5, 6; AG: 2, 7, 8; GG: 1, 4; M; DNA Marker

Allele and genotype frequencies and neutrality test of Mx gene S631N in all populations: In 8 chicken populations, Mx gene of Henan gamecocks and Huainan chickens showed an extreme distributions, which were lack of AA at the S631N resistance site. Gene frequency distribution was tested by Chi-square test and Likelihood ratio test, the result confirmed that except for Zhangzhou gamecock、Wannan chicken, Wenchang chicken, Rugao chicken and Anka chicken, the other three populations were all in Hardy-Weinberg equilibrium at this site (p>0.05) (Table 1).

Table 1 The frequency of genotypes and alleles of S631N site in 8 populations

Breeds	No	Genotype frequency			Gene frequency		Chi-square test		Likelihood ratio test	
		AA	AG	GG	A	G	x^2	*p*	G^2	*p*
ZHA	44	0.455	0.545	0.000	0.727	0.273	5.8870	0.0153	8.8480	0.0030
HEN	66	0.000	0.273	0.727	0.136	0.864	1.5440	0.2140	2.6960	0.1006
HUA	53	0.000	0.340	0.660	0.170	0.830	2.0784	0.1494	3.5031	0.0613
WAN	38	0.026	0.579	0.395	0.316	0.684	4.0845	0.0433	4.7489	0.0293
WEN	66	0.258	0.712	0.030	0.614	0.386	16.1464	0.0001	18.4360	0.0001
RUG	88	0.125	0.705	0.170	0.477	0.523	14.5278	0.0001	15.0108	0.0001
RJF	32	0.969	0.031	0.000	0.984	0.016	0.0000	1.0000	0.0000	1.0000
ANK	88	0.232	0.681	0.087	0.572	0.428	10.1946	0.0014	10.6842	0.008
Average	/	0.258	0..483	0.259	0.4995	0.5005				

(Abbreviations: ZHA: Zhangzhou gamecocks, HEN: Henan gamecocks, HUI: Huainan chickens, WAN: Wannan chickens, WEN: Wenchang chickens, RUG: Rugao chickens, ANK: Anka chickens, RJF: Red Jungle Flow. The same below)

The Ewens-Watterson test at the S631N resistance site indicated that except for Rugao chicken, all the other populations were in the 95% confidence interval, belonging to neutral site (Table 2).

Table 2 The Ewens-Watterson test for neutrality of S631N site in 8 Chicken populations

Breed	Observed F*	Mean F*	SE*	L95*	U95*
ZHA	0.6033	0.8105	0.0261	0.5041	0.9775
HEN	0.5346	0.8042	0.0273	0.5031	0.9740
HUA	0.7180	0.8095	0.0284	0.5016	0.9813
WAN	0.5679	0.7976	0.0276	0.5014	0.9740
WEN	0.5258	0.8143	0.0279	0.5029	0.9850
RUG	0.5010	0.8242	0.0294	0.5032	0.9887
RJF	0.9692	0.7804	0.0286	0.5005	0.9692
ANK	0.5105	0.8189	0.0272	0.5017	0.9856
Average	0.6163	0.8075	0.0278		

Analysis of Mx gene population genetic variation: According to the Table 3, An observed heterozygosity of the red jungle fowl (0.9688) was the highest, while Wenchang Chicken (0.2879) was the lowest. Levene's expected heterozygosity and Nei's expected heterozygosity of the forecast the trends were both different from these observations. An effective alleles number of Rugao chicken (1.9959) was the highest, while that of red jungle fowl (1.0317) was the lowest, an average of Shannon's information index was 0.5232 in 8 kinds of Chicken groups.

Clustering analysis based on Mx gene S631N allele frequency: The UPGMA dendrograms based on allele frequency divided the 8 chicken populations into three clusters, The first category included the Red jungle fowl, Zhangzhou gamecock, the second included Wenchang chicken, the third included Wannan chicken, Huainan chicken, Henan gamecock, Anka chicken and Rugao chicken reflected the difference and advantage of Mx antiviral property in 8 chicken populations (Figure 4).

The relationship between Mx gene S631N polymorphism and various stages of body weight and body measurements: The relevance between the genotypes of Anka and body weight of various stages were analyzed as shown in Table 4, three kinds of genotypes at all stages of body weight have no significant difference. In addition to birth weight and the Wenchang Chicken GG and AA, AG genotype significant difference in body weight, the remaining stages of body weight difference was not significant. In addition to Wenchang Chicken GG and AA, AG genotype have significant difference in body weight, the remaining stages of body weight difference was not significant.The relevance between the genotypes both of Wenchang, Anka and body size at 12 weeks of age were analyzed. The results were shown in Table 5, the body size traits of three kinds of genotypes were not significantly different.

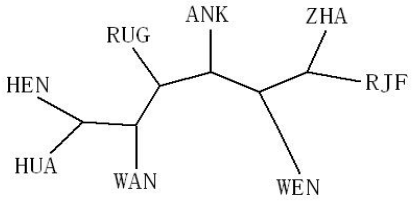

Figure 4 UPGMA dendrogram of 8 chicken breeds

Table 3 The genetic variation analysis of S631N site in 8 chicken breeds

Breed	Observed value		Levene' s Expected value		Nei's Expected heterozygosis (H)	Ne	Shannon' s index
	Hom	Het	Hom	Het			
ZHA	0.4545	0.5455	0.5987	0.4013	0.3967	1.6575	0.5860
HEN	0.7273	0.2727	0.7627	0.2373	0.2355	1.3081	0.3983
HUA	0.6604	0.3396	0.7154	0.2846	0.2820	1.3927	0.4556
WAN	0.4280	0.5789	0.5621	0.4379	0.4321	1.7610	0.6237
WEN	0.2879	0.7121	0.5222	0.4778	0.4742	1.9018	0.6671
RUG	0.2955	0.7045	0.4982	0.5018	0.4990	1.9959	0.6921
RJF	0.9688	0.0312	0.9688	0.0312	0.0308	1.0317	0.0805
ANK	0.3188	0.6812	0.5069	0.4931	0.4895	1.9589	0.6826
Average	0.5177	0.4832	0.6419	0.3581	0.3550	1.6260	0.5232

Table 4 The relationship between Mx gene S631N polymorphism and various stages of body weight

Genotype	WEN			ANK		
	AA	AG	GG	AA	AG	GG
Birth weight(g)	27.63±2.13[a]	27.88±2.38[a]	31.33±3.51[b]	36.00±3.54	38.12±4.23	36.75±2.99
2-week-old weight(g)	79.32±9.46	77.56±9.65	80.33±11.24	165.46±18.13	168.21±25.44	157.75±13.38
4-week-old weight(g)	205.69 ±24.71	200.06 ±28.74	204.33 ±21.39	513.62±62.67	527.29±84.15	504.25±51.56
6-week-old weight(g)	419.13 ±54.55	409.32 ±60.52	427.67 ±53.98	1188.77±143.95	1187.18±192.92	1214.75±130.12
8-week-old weight(g)	705.75 ±102.37	703.12 ±109.98	757.67 ±49.52	1984.46±254.80	1928.06±355.55	2055.50±344.51
10-week-old weight(g)	956.25 ±150.65	914.12 ±159.10	939.33 ±23.86	2431.69±297.82	2338.06±424.68	2459.50±501.24
12-week-old weight(g)	1214.38 ±206.92	1152.42 ±229.09	1256.33 ±68.24	2898.08±399.13	2808.71±558.55	3072.50±653.90

[a] If letters on the same line were the same, the difference was not significant (p>0.05), if not, the difference was significant (p<0.05).

Table 5 The relationship between Mx gene S631N polymorphism and body measurements

Genotype	WEN			ANK		
	AA	AG	GG	AA	AG	GG
Body slope lengh(cm)	19.94±1.15	17.81±1.22	18.00±0.50	24.11±1.48	23.84±1.86	24.34±1.65
Fossil bone lengh(cm)	9.53 ±2.43	8.89 ±2.56	10.30 ±0.82	13.48±1.01	13.41±0.81	13.93±1.30
Coxa width(cm)	7.73 ±2.01	8.17 ±2.40	6.90 ±1.18	9.08±1.68	11.38±11.58	9.64±1.64
Shank lengh(cm)	8.26 ±1.40	7.85 ±0.90	7.77 ±0.75	10.36±1.27	10.11±1.76	9.64±1.64
Shank circumference(cm)	3.85 ±0.34	0.78 ±0.28	3.87 ±0.15	5.09±0.46	5.06±0.45	5.28±0.44

The relationship between Mx gene S631N polymorphism and slaughter traits, meat traits: The relationship between Mx gene S631N polymorphism and slaughter traits were analyzed (Table 6). There was no significant difference in slaughter weight, eviscerated weight, half-eviscerated weight, breast muscle weight, leg muscle weight, abdominal fat weight, heart weight, liver weight, dressed percentage, percentage of eviscerated yield, percentage of half-eviscerated yield, percentage of breast muscle, percentage of leg muscle between AA and AG, GG in Anka chicken, while in Wenchang chicken there was significant difference between GG and AG, but no significant difference in all other slaughter traits.

The relationship between Mx gene S631N polymorphism and meat traits were showed in table 7, there was a significant difference between AA and GG in tenderness and OD in Anka chicken, while there was no significant difference in all meat traits in Wenchang chicken.

Table 6 The relationship between Mx gene S631N polymorphism and slaughter traits

Genotype	WEN			ANK		
	AA	AG	GG	AA	AG	GG
Slaughter weight(g)	665.31 ±157.06	662.51±150.04	677.33±81.54	1647.41±340.95	1589.42±390.43	1747.38±417.29
Eviscerated weight(g)	593.25±139.84	598.47±141.28	608.67±66.34	1528.18±318.07	1427.44±363.85	1647.63±406.26
Half-eviscerated weight (g)	760.39±172.61	756.08±157.70	732.27±64.27	1904.78±359.07	1840.09±419.25	2006.12±441.75
Breast muscle weight(g)	71.44±8.96	68.27±8.96	96.50±7.21	179.72±33.14	175.18±33.86	200.48±34.19
Leg muscle weight (g)	77.46±16.95	76.77±18.49	78.73±4.98	219.09±52.46	234.74±223.38	244.61±62.25
Abdominal fat weight (g)	9.52±6.76	16.76±13.13	18.90±0.27	90.68±54.70	81.28±44.53	85.88±29.06
Heart weight (g)	6.57±2.13	6.00±1.80	5.67±0.58	14.00±4.34	15.08±4.05	16.37±2.83
Liver weight (g)	23.64±5.38	22.94±4.21	24.20±3.62	53.57±10.62	54.85±12.84	59.39±10.92
Dressed percentage (%)	52.81±3.75	53.33±4.11	52.23±3.79	54.39±3.29	54.05±6.93	2.97±3.59
Percentage of eviscerated yield (%)	47.09±3.37	48.12±4.30	46.94±2.51	50.89±3.40	50.05±6.51	49.87±3. 77
Percentage of half-eviscerated yield (%)	60.44±3.75[ab]	61.07±3.93[a]	56.50±1.57[b]	63.21±3.15	62.70±6.93	61.01±3.18
Percentage of breast muscle (%)	12.53±2.71	11.90±5.14	11.48±1.44	11.91±1.49	12.31±2.80	12.45±1.67
Percentage of leg muscle (%)	13.16±1.17	13.00±2.03	13.61±1.36	14.33±1.45	16.64±1.93	14.85±0.91
Percentage of abdominal fat (%)	1.71±1.35	2.85±2.36	3.03±0.34	5.79±3.31	5.47±3.33	5.30±2.50

[a] If letters on the same line were the same, the difference was not significant (p>0.05), if not, the difference was significant (p<0.05).

Table 7 The relationship between Mx gene S631N polymorphism and meat traits

Genotype	WEN			ANK		
	AA	AG	GG	AA	AG	GG
Rate of water loss (%)	26.15±7.12	28.73±8.36	26.07±6.71	29.56 ±8.43	31.73 ±9.45	28.95 ±5.34
Tenderness	2.38±0.48	2.05±0.69	2.24±0.42	1.75 ±0.53[a]	2.01 ±0.71[ab]	2.42 ±0.93[b]
PH	5.82±0.16	5.85±0.15	5.88±0.32	5.80±0.23	5.74±0.13	5.77±0.16
OD	0.36±0.12	0.42±0.34	0.27±0.78	0.28±0.12[a]	0.43±0.22[ab]	0.60±0.64[b]

[a] If letters on the same line were the same, the difference was not significant (p>0.05), if not, the difference was significant (p<0.05).

Discussion

Allele frequency distribution difference of S631N site of Mx gene: One kind of restriction endonucleases (Rsa I) was used to detect the mutation between the resistant allele A and sensitive allele G of Mx gene in 8 different kinds of chicken accurately, frequency distribution of resistant allele A and sensitive allele G were significantly correlated to the growth geographical environment, the range of resistant allele A frequency distribution was 0.136-0.984, simultaneously, the range of sensitive allele G frequency distribution was 0.016-0.864, which was the same as the conclusion of Seyama et al. (2006), yet different from Balkissoon's (2007) conclusion, which was the allele frequency distribution in a total of 1275 individuals of 28 egg typed, meat typed and both eggs and meat typed chickens: respectively, egg typed ,A: 0.779, G: 0.221, meat typed A: 0.021, G: 0.979, both eggs and meat typed A: 0.281, G: 0.719. According to Li et al. (2006), the reason caused differences of the resistant and sensitive alleles distribution of S631N site of Mx gene of chicken at home and abroad was the changes in the environment (such as avian flu, etc.) and the selection intensity of production performance possibly. The frequency distribution of resistant allele A and sensitive allele G were significantly correlated to the growth geographical environment, that the resistant allele A frequency of chickens was lower that grown in better living environment contrasted to that in poor growing environment. The study of Sironi et al. (2010) shows the need to carefully choose the Mx gene template region for primer design and to carry out repeated genotyping, preferably using different primer sets. The author choose the 100 bp (from 1961 bp to 2060 bp) sequence of Mx gene to mismatch PCR amplificated for the original work. In order to detect the differences of the resistant and sensitive alleles distribution of S631N site of Mx gene in different chicken populations.

The frequency of resistant allele A of Zhangzhou gamecock was 0.727, in the two gamecocks, higher than that of all the other indigenous populations (except for the red jungle fowl), the reason for this may be the different distribution of the unique geographical location of the one. Under the long-term natural selection, the resistance increased, while the feeding of Zhangzhou gamecock was relatively extensive, along with the level of artificial selection was low, resulting in that the status of the red jungle fowl were retained , and the allele A frequency of Mx gene was relatively high. The frequency of resistant allele A of Henan gamecock was 0.136, belonging to a relatively low level and closed to that of the other indigenous populations, the reason should be that geographical location of Henan gamecock was different from the geographical location of Zhangzhou gamecock, in additional, the growth environment of Henan gamecock was more favorable and selection pressure was more less, causing the resistance to disease relatively low. Because of genetic stability, geographical distribution, ecological environment, and population isolation and so on, the red jungle fowl still retained the unique genetic characteristics (Bao et al., 2008), the frequency of homozygote AA reached 96.9%, higher than that of other kinds of chicken.

In 8 chicken populations, Mx gene of Henan gamecocks and Huainan chickens showed the extreme distributions, which were lack of AA at the S631N resistance site, it may be due to the high selection intensity and the environmental impact in actual production, resulting in resistance to disease of two kinds breeds significantly reduced. The gene frequencies of alleles A, G of the alien species, Anka chicken, were respectively 0.572, 0.428, which were not different from Chinese indigenous chicken breeds, the resistance to disease of alien species, especially broiler chickens, were not reduced because of different geographical, the main result may be the chickens all grown in same growth environment and artificial selection.

In this study, the Hardy-Weinberg test indicated that Zhangzhou gamecock, Wannan chicken, Wenchang chicken, Rugao chicken and Anka chicken were deviated from the Hardy-Weinberg equilibrium. This perhaps had a relationship with the choice of experimental groups, as well as the pressure of selection, the stability of gene frequency was rather poor. While gene frequency were stable in the other 3 populations, and artificial selection was not targeted; the Ewens-Watterson test indicated that this site was neutral in all the other populations, except for Rugao chicken. These sequences were similar to the report from Hou et al (2007) through the analysis of Mx genes in different species. The frequency of change in resistant gene A and sensitive gene G were considerably influenced by the growth of the environmental, and not by production traits, and the results had a guiding significance for the poultry breeding, while it dispelled the fears that reduction in resistance while selecting the production traits. Taking the extensive management based on increasing the production traits may be helpful to improve disease resistance. With the commercialization of market-oriented development and in-depth study of Mx genes applications, it will have an important influence on the entire poultry industry or the public health community.

Genetic variation analysis and cluster analysis of S631N site of Mx gene: Heterozygosity and Shannon's index (Zhu et al., 2006) was a measure of genetic polymorphism between groups and level of variation. In this study the average value of observed heterozygosis and Shannon's index at this site were 0.6163 and 0.5232. The result indicated that the genetic diversity in groups was very rich and had a higher level of genetic variation at this site (Li et al., 2009). The UPGMA dendrograms based on allele frequency divided the 8 chicken populations into three clusters, which reflected the difference and advantage of Mx antiviral property, The first category was High-resistance category, the average frequencies of A was 0.727-0.984, including the Red jungle fowl, Zhangzhou gamecock; the second category was

medium-resistance category, the average frequencies of A was 0.614, including Wenchang chicken;the third category was low-resistance category, the average frequencies of A was 0.170-0.572, including Wannan chicken, Huainan chicken, Henan gamecock, Anka chicken, Rugao chicken. More experiment was needed to validate that weather the results conform to the actual, and the results will further provide a theoretical basis for the choice of breeding material.

The association analysis between Mx gene S631N polymorphism and economic traits: It was reported a certain degree of negative correlation exited between resistance traits and important economic traits (Muller et al., 1992), how were the important economic traits of AA, which was considered as the anti-influenza virus resistance genotype, this question was the concern in the study on disease resistance in chicken. The results showed that: although there was difference between AA and GG for tenderness and OD in meat traits, there was no significant difference in body weight of various stages, body measurements, and the slaughter traits among AA, AG and GG for Anka chicken, and in Wenchang chicken except for birth weight and percentage of half-eviscerated yield, there was no difference in all other traits, the result showed that there was almost no significant negative effect between Mx gene S631N mutation site and important economic traits in Wenchang chicken and Anka chicken, which was why groups retained some AA genotypes, on the other hand, it provided valuable reference for the future research for resistance, resistant individuals which have high performance and efficient production will be produced through marker-assisted selection and the development of scientific breeding programs in the chickens like Wenchang and Anka with high or a certain frequency of resistant genes.

Conclusions

PCR-RFLP technique was used to detect the differences of the resistant and sensitive alleles distribution of S631N site of Mx gene in different chicken populations. Except for Zhangzhou gamecock, Wannan chicken, Wenchang chicken, Rugao chicken and Anka chicken, the other three populations were all in Hardy-Weinberg equilibrium at this site. The Ewens-Watterson test indicated that this site was neutral in all the other populations except for Rugao. The relevance among three kinds of genotypes and some economic traits of Wenchang chicken and Anka chicken were analyzed, there was almost no significant negative effect between Mx gene S631N mutation site and important economic traits in Wenchang chicken and Anka chicken.

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