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POLYMORPHISM ANALYSIS OF *CHINESE YELLOW QUAIL* USING MICROSATELLITE MARKERS

J. Y. Bai, Y. Z.Pang, S. J.Wu, M. Q.Yu, X.H. Zhang, S.J. Zhao and H.W. Xu

College of Animal Science and Technology, Henan Science and Technology University, Luoyang 471003, China
Corresponding author email: pyzh2006@126.com

ABSTRACT

This study aims to provide a basis for the evaluation, protection, and utilization of the genetic resources of Chinese Yellow Quails by analyzing the polymorphism of these quail groups with nine microsatellite markers using the polyacrylamide gel electrophoresis. A total of 48 alleles were identified from nine microsatellite markers, with an average of 5.3 alleles per marker. The average heterozygosity was 0.7353, and the average polymorphism information content was 0.6943. The average number of effective alleles was 4.0312. The nine microsatellite markers showed high polymorphism in the Chinese Yellow Quail groups, and could be used as effective genetic markers for group genetic diversity analysis. The chi-square test result showed that the microsatellite marker GUJ0077 remarkably deviated from the Hardy-Weinberg equilibrium. Moreover, extreme deviation of the remaining eight genes with microsatellite markers from the Hardy-Weinberg equilibrium was observed.

Key words: Chinese Yellow Quails, Microsatellite marker, Genetic diversity.

INTRODUCTION

Genetic diversity is the basis of species and ecosystem diversities. It is also the foundation of life evolution and species differentiation, as well as a key element in the evaluation of natural biological resources. The abundance of genetic diversity determines the adaptation and evolutionary potential of species to environmental changes. Research on genetic diversity is conducive to the conservation and utilization of biological resources. Microsatellite markers have been used internationally for the polymorphism analysis of wild quails and domestic quails. Massive effective data have been obtained to evaluate quail genetic resources and to analyze group genetic variability and evolutionary relationship.Wang *et al.*(2004) studied the genetic relationship of domestic quali and wild Japanese quail with 10 microsatellite markers, the result showed that because of domesticated selection, domestic quali differed from wild Japanese quail in some degree. Chang *et al.* (2005) reported that wild common quail possessed the richer genetic diversity, while no clear difference of genetic diversity observed between wild Japanese quail and domestic quali, indicating their closer relative relationship. Olowofeso *et al.* (2006) studied genetic variation of four quail populations in East China with three microsatellite markers, the result showed that average heterozygosity of the four quail populations was 0.4627,0.5146,0.5549 and 0.6345 respectively, indicating the high genetic variation among populations in East China. Amirinia *et al.* (2007) studied the genetic diversity of four Japanese quail strain. Besides the breeding of Beijing White-feather quails, Chinese Yellow Quail is another feather-color mutation species found from North Korean quail groups and cultivated by researchers in China. Chinese Yellow Quails have become a key specialized strain parent in the production of Chinese laying quails（Pang *et al*., 2001）. They can form various autosexing specialized strains with North Korean quails and Beijing white-feather quails, thereby presenting remarkable values in production application and promotion. In this study, through testing the universality, accuracy, and sensitivity of twenty microsatellite markers, nine were selected for the polymorphism test of Chinese Yellow Quails to provide a scientific basis for the evaluation, protection, and utilization of the genetic resources of Chinese Yellow Quails.

MATERIALS AND METHODS

Sample collection: Seventy-five Chinese Yellow Quail individuals obtained from the test ranch of Henan University of Science and Technology were selected. Approximately 2 mL of blood were taken from the heart of each individual, and an Acid citrate dextrose solution （ACD） anticoagulant was applied to the blood with an ACD rate of 6:1. The blood was stored at -20 °C for later use. Genomic DNA was extracted with the blood tissue genomic DNA extraction kit (Tiangen, Beijing, China).

Primer selection and synthesis: Microsatellite loci with high polymorphism were selected from the literature, and the location of the microsatellite loci in the chromosome, characteristics of primer sequences, polymorphism of the microsatellite loci, product size, and other factors were considered. Nine microsatellite markers were identified

as genetic markers in this study. The primer sequences were sent to Shanghai Sangon Biological Engineering Co., Ltd. for synthesis. The microsatellite markers data is shown in Table 1.

PCR reaction condition: The total size of the PCR reaction system was 12.5 μL, including 8.65 μL of ddH_2O, 1.25 μL of 10 X buffer, 0.75 μL of Mg^{2+} (25 mmol/L), 0.5 μL of DNA template (50ng/μL) , 0.5 μL (10 mmol/L) of upstream and downstream primers, 0.25 μL of dNTPs, and 0.1μL of Taq DNA polymerase (Tiangen, Beijing, China). The PCR amplification process was as follows: an initial denaturation for 3 min at 95 °C, followed by 30 cycles of 45 s at 94 °C, 60 s at variable temperatures given in table 1 and 60 s at 72 °C. Finally, an extension of 72 °C for 12 min was followed. The annealing temperature is shown in Table 1.

Table 1. Primer information of nine microsatellite markers.

Locus name	Type of repeat	Primer sequence (5'→ 3')	T_A(℃)	Genbank accession number	Chromosome NO.
GUJ0023	(CA)7TA(CA)11	GAGAGGTACAGCAACACTTT CGTTTCTTTCTGGAGTGTCT	55	AB035833	CJA14
GUJ0028	(CA)9	TGAACAAAGCAGAAAGGAGC CCTTACCTACATGAAACGTC	55	AB035838	QL08
GUJ0029	(CA)11CT(CA)2	GAGCATTTCTAGTCTGTCTC ATACACAGGCTAAGGAAACC	55	AB035839	CJA 06
GUJ0057	(CA)12	GGAATGGAAAATATGAGAGC CAGGTGTTAAAGTCCAATGT	60	AB063125	QL03
GUJ0059	(CA)10	GACAAAGTTACAGCTAGGAG TAGGTGCGAAAATCTCTGAC	50	AB063127	CJA05
GUJ0063	(CA)7CT(CA)2CT(CA)7	GCTCAGGTTCTCAGCTGATG GGGAGAGATCAAGGGAACAG	55	AB063131	CJA02
GUJ0077	(CA)8	TATAAGATGGGGAGTGGCAG ATTTTGCTGACCCCCTTCTG	56	AB063145	CJA01
GUJ0083	(CA)11	CCATCTCTGTGCCTTTCCAA GCTGAAAACATTGGGCGTAG	58	AB063151	QL10
GUJ0097	(CA)14	GGATGCTCAGTGTGGAAAAG GAGCAAGAGGTGAGTGTTTC	58	AB063165	CJA14

Polyacrylamide gel electrophoresis: A 1% agarose gel was prepared using a 1.0 X TBE buffer to test the existence of PCR amplification products. Approximately 3 μL of the PCR amplification products and the isometric loading buffer were mixed for sample application and electrophoresis. DNA marker I was used as the control sample to observe the availability of the needed bands on the UV transmittance analyzer. If the specific bands are bright and if the hybrids are insignificant, they can be tested using 8% non-denaturing acrylamide gel electrophoresis at 120 V for about 2 h. The bands were then stained by silver nitrate and photographed using optical coherence tomography for reservation and analyzed. The allele size was detected to identify the individual genotype of each microsatellite marker based on the standard of the pBR322DNA/Msp I marker.

Statistical analysis: The molecular biology software POPGENE (Version1.32) was used to analyze polymorphism information content (PIC), effective number of alleles (Ne), heterozygosity (H) and other parameters for each marker.

RESUITS AND DISCUSSION

Polyacrylamide gel electrophoresis of microsatellite markers: The nine microsatellite markers presented significant polymorphism in the Chinese Yellow Quail groups. The electrophoresis diagram of the microsatellite marker GUJ0077 shown in Figure 1 indicated that the microsatellite marker GUJ0077 contained rich polymorphism.

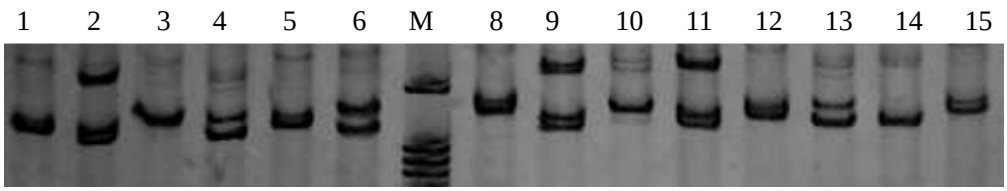

Figure.1 Banding patterns of PCR product of GUJ0077 microsatelite

Note :244/265：2；265/265：1；265/285：4、6、13；270/280：9、11；275/275：3、14；275/285：5、12、15；285/285：8、10

Allele frequencies of microsatellite markers: Table 2 showed that 48 alleles were identified from nine microsatellite markers with an average of 5.3 alleles detected per marker. Among these markers, GUJ0057 detected the most alleles (7) in the Chinese Yellow Quail groups, following it were four markers including GUJ0023, GUJ0029, GUJ0059, and GUJ0077, which detected 6 alleles per locus. The remain four markers , GUJ0028 and GUJ0097 detected 5 alleles per locus, whereas GUJ0083 and GUJ0063 detected 3 alleles per locus. The expected segment sizes of GUJ0023, GUJ0028 GUJ0029, GUJ0057 GUJ0059, GUJ0063 GUJ0077, GUJ0083, and GUJ0097 were 219 bp to 244 bp, 160 bp to 185 bp, 147 bp to 175 bp, 145 bp to 180 bp, 230 bp to 246 bp, 242 bp to 246 bp, 260 bp to 285 bp, 130 bp to 155 bp, and 130 bp to 170 bp, respectively. The nine microsatellite markers exhibited 15, 11, 15, 19, 12, 7, 19, 10, and 15 genotypes in the Chinese Yellow Quail groups, respectively. Aside from GUJ0028, which has two dominant genes (160 and 180 bp), other microsatellite markers had one dominant gene. The dominant genes of GUJ0023, GUJ0029, GUJ0057, GUJ0059, GUJ0063, GUJ0077, GUJ0083, and GUJ0097 marker were 238bp, 160 bp, 155 bp, 238 bp, 244 bp, 285 bp, 147 bp, and 147 bp, respectively.

The Hardy-Weinberg equilibrium test for the nine microsatellite markers was conducted, and the χ^2 of GUJ0077 was between 0.05 and 0.01 of the chi-square critical value, that was, $\chi^2_{0.05}<\chi^2<\chi^2_{0.01}$. The gene distribution of GUJ0077 presented significant deviations from the genetic equilibrium ($P<0.05$) of the Chinese Yellow Quail groups. The remaining eight microsatellite markers showed a value of $\chi^2>\chi^2_{0.01}$, which indicated that the gene distribution of these markers presented significant deviations from the genetic equilibrium ($P<0.05$). Genetic equilibrium was related with the test sample content; several gene loci might fail to detect all the alleles when the sample content was not sufficient enough. Baker *et al.* (1994) pointed out that 25 samples should be analyzed to test each species, and that the sample number should reach 50 to avoid errors. For the current study, the sample content (75) reached the sampling requirement, which indicated that the genetic disequilibrium should not be attributed to the samples but to the excessive artificial selection or inbreeding and other factors of the Chinese Yellow Quail groups. This condition was related with a small group preservation of Chinese Yellow Quails.

Table 2. Allele size(bp)and frequencies of microsatellite loci.

Locus name	Observ-ation alleles number	Allele size(bp) (Allele frequencies)							Chi-square value	df	$\chi^2_{0.01}$
GUJ0023	6	219 (0.0597)	230 (0.0746)	238 (0.3060)	242 (0.2313)	240 (0.1866)	244 (0.1418)		57.62	20	37.57
GUJ0028	5	160 (0.2887)	170 (0.2887)	175 (0.0141)	180 (0.2465)	185 (0.1620)			80.08	14	29.14
GUJ0029	6	147 (0.0357)	150 (0.0786)	160 (0.4357)	165 (0.2714)	170 (0.0643)	175 (0.1143)		38.29	20	37.57
GUJ0057	7	145 (0.1066)	147 (0.1148)	155 (0.2623)	160 (0.2049)	162 (0.2295)	170 (0.0328)	180 (0.0491)	67.58	27	46.96
GUJ0059	6	230 (0.0984)	236 (0.0328)	238 (0.4426)	240 (0.2295)	242 (0.1311)	246 (0.0656)		67.66	20	37.57
GUJ0063	3	242 (0.2465)	244 (0.5775)	246 (0.1760)					111.89	5	15.09
GUJ0077	6	260 (0.1301)	265 (0.1849)	270 (0.1712)	275 (0.1507)	280 (0.1575)	285 (0.2056)		35.24	20	37.57
GUJ0083	4	130 (0.1959)	140 (0.2162)	147 (0.3851)	155 (0.2028)				139.67	9	21.69
GUJ0097	5	130 (0.2266)	147 (0.3984)	155 (0.2656)	160 (0.0703)	170 (0.0391)			84.91	14	29.14
Average	5.3										

Heterozygosity and polymorphic information content of microsatellite markers: The polymorphic information content (PIC) was an ideal indicator for the measurement of allele polymorphism. The loci was taken as highly polymorphic when PIC>0.5; it was taken as moderately polymorphic when PIC ranged from 0.25 to <0.5; and was taken as lowly polymorphic when PIC<0.25 (Botstein *et al.*, 1980). Table 3 showed that the highest PIC of GUJ0077 was 0.8060 and the lowest PIC of GUJ0063 was 0.5098. The average PIC of the nine

markers was 0.6942, which indicated that the nine microsatellite markers contained highly polymorphic loci in the Chinese Yellow Quail groups. In the genetic diversity analysis, microsatellite markers with PIC>0.7 were taken as the most ideal selected markers. In this case, the parental generation was practically heterozygous for the loci. Allele separation could be clearly observed in its offspring (Heame *et al.*, 1992). From the selected microsatellite markers in this study, the PIC of GUJ0023, GUJ0057, and GUJ0077 exceeded 0.7, which indicated that these loci could be used as genetic markers for the Chinese Yellow Quails genetic diversity analysis.

Table 3. Number of effective alleles, PIC and Heterozygosity of microsatelliate loci.

Locus name	Polymorphism information content (PIC)	Number of effective alleles (Ne)	Heterozygosity (H)
GUJ0023	0.7575	4.7348	0.7888
GUJ0028	0.6999	3.9386	0.7461
GUJ0029	0.6705	3.4710	0.7119
GUJ0057	0.7814	5.2219	0.8085
GUJ0059	0.6819	3.5613	0.7192
GUJ0063	0.5098	2.3518	0.5748
GUJ0077	0.8060	5.8754	0.8298
GUJ0083	0.6774	3.6430	0.7255
GUJ0097	0.6633	3.4831	0.7129
Average	0.6942	4.0312	0.7353

Heterozygosity reflected the degree of genetic variation of the microsatellite loci in livestock. A high heterozygosity indicated a high genetic diversity as well as a high degree of genetic variation. The heterozygosity values calculated with microsatellite markers were generally between 0.3 and 0.8. Meng *et al.* (2007) studied the average heterozygosity of 12 microsatellite markers in North Korean quails, and obtained a value of 0.7111. Wu *et al.* (2010) studied the average heterozygosity of nine microsatellite markers in North Korean quails, and obtained a value of 0.7096. Farrag *et al.* (2011) studied the average heterozygosity of 13 microsatellite markers in 3 Japanese quail species, and obtained a value of 0.6090. Hossein (2011) studied the average heterozygosity of 12 microsatellite markers in 4 Japanese quail species, and obtained a value of 0.4343 to 0.7902. The Chinese Yellow Quails originated from the North Korean quails. In the current study, the average heterozygosity of the nine microsatellite markers was 0.7352, which indicated that the degree of genetic variation of the Chinese Yellow Quail groups was slightly higher than that of the North Korean quails, and was significantly higher than those of Japanese quails and wild Japanese quails (Wang *et al.*, 2004), as well as of wild normal quails (Chang *et al.*, 2005). The degree of heterozygosity was related to the number and types of microsatellite markers in this study, as well as sample content and other factors. In this study, the selected microsatellite markers showed high polymorphism, which indicated the relatively rich genetic variation of Chinese Yellow Quails. The results of this study could provide a scientific basis for the evaluation and utilization of genetic resources of the Chinese Yellow Quails.

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