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Microsatellite Analysis of Genetic Diversity in Quail Populations from China

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Chinese black quail, Microsatellite marker,
Polymorphism, Genetic diversity.

ABSTRACT

Polymorphism of three quail communities was analyzed by using 12 microsatellite markers in this paper, aiming to provide scientific references for the evaluation, protection and utilization of quail genetic resources in China. Results demonstrated that the number of observed alleles by 12 microsatellite markers ranges between 4~7. The average polymorphism information contents (PIC) of the Chinese yellow quail, the Chinese black quail and the Korean quail, as detected by 12 microsatellite markers, are 0.6853, 0.6401 and 0.6565, respectively, and average heterozygosity values are 0.7333, 0.6957 and 0.7111, respectively. This indicates that the Chinese yellow quail has the richest genetic polymorphism. According to cluster analysis, the Chinese black quail and the Korean quail have the smallest genetic distance (0.0628), which reflects that they have the closest genetic relationship. The genetic distance between the Chinese yellow quail and the Korean quail is 0.0951. Therefore, the Chinese black quail and the Korean quail are clustered together firstly, and then the Chinese yellow quail.

INTRODUCTION

Microsatellite markers are good genetic markers for studying genetic variation in a same species and among different species. Due to their large quantity, wide and uniform distribution, rich polymorphism, codominant inheritance, as well as simple and convenient analysis method, microsatellite markers are molecular markers that are widely used to evaluate genetic resources of livestock at present.

Microsatellite markers are mainly used in quail breeding for the establishment of the quail genetic map. Kayang *et al.* (2004) developed the first generation of microsatellite linkage maps with 72 microsatellite markers based on 100 microsatellite markers discovered in quail genomes. Quail functional gene mapping and QTL: in 2005, Miwa *et al.* located three blood protein loci Tf, Hb-1 and Pa-1 on QL08, CJA14 and QL13 chromosomes by using three microsatellite primers GUJ0071, GUJ0097 and GUJ0061 (Miwa *et al.*, 2005). Genetic diversity analysis of quails: many polymorphic analyses on wild quail and domesticated quail using microsatellite markers are reported in both China and foreign countries. Studies have accumulated data on the evaluation of quail genetic resources and analyzed population genetic variation and evolutionary relationship (Wang *et al.*, 2004; Olowofeso *et al.*, 2006; Chang *et al.*, 2007; Amirinia, 2007; Kim *et al.*, 2007; Olympe *et al.*, 2010; Wu *et al.*, 2010; Hossein *et al.*, 2011; Farrag *et al.*, 2011; Thakur *et al.*, 2011; Bai *et al.*, 2013).

The quail is an ancient bird and is also called Japanese quail. There are mainly wild quails and domesticated quails. Wild quails can be divided into wild common quail and wild Japanese quail. Although China started quail production late, it achieved rapid development in 1970s, with



unprecedented growth and diversified varieties. Both varieties of wild quails are present in China, but there is a larger number of Japanese quails (Chang *et al.*, 2004). Korean quails, an egg-laying variety of the Japanese quail, can be divided into Longcheng and Huangcheng varieties. After being introduced in China, the Korean Longcheng variety originated the Chinese white-feather quail and the Chinese yellow quail. The Chinese yellow quails a recessive yellow-feather variety, which was discovered and cultivated by Yue Genhua, a young teacher of Nanjing Agricultural University in 1990 (Yue *et al.*, 1994). The Chinese black quail is a feather color mutant recently discovered by our research group. It is the hybrid of male Chinese yellow quail and female Korean quail. The hybridization test confirmed that the Chinese black quail is the consequence of incomplete autosome recessive (Pang *et al.*, 2013).

Due to few available studies on the Chinese black quail, this study employed 12 microsatellite markers for polymorphic analysis of three quail communities (Korean quail, Chinese yellow quail and Chinese black quail) in order to discuss their evolution degree and provide scientific basis for the evaluation, protection, and utilization of Chinese black quail genetic resources.

MATERIALS AND METHODS

The tested quails derived from the test farm of Henan University of Science and Technology. One hundred Chinese black quail mutants (50 males and 50 females), 75 Chinese yellow quails and 75 Korean quails (40 males and 35 females each) were randomly selected, totaling 250 quails.

Two mL of heart blood was collected into tubes with acid-citrate-dextrose (ACD) anticoagulant solution at 6:1 blood to ACD concentration. Blood samples were stored in refrigerator at -20 °C until analyses. Genomic DNA was extracted with the blood tissue genomic DNA extraction kit (Tiangen, Beijing, China).

Twelve microsatellite primers were synthesized by Shanghai Sangon Biological Engineering Technology Co. Primer sequences are shown in Table 1. The total size of the PCR reaction system was 12.5 µL, including 8.65 µL of ddH₂O, 1.25 µL of 10× buffer, 0.75 µL of Mg²⁺ (25 mmol/L), 0.5 µL of DNA template, 0.5 µL (10 mmol/L) of upstream and downstream primers, 0.25 µL of dNTPs, and 0.1 µL of Taq enzyme. The PCR amplification process was as follows: denaturation for 3 min at 95°C; denaturation for 45 s at 94°C, annealing for 60s at X°C, extension for 60s at 72°C and 30 cycles, extension for 12 min at 72°C, and preserving at 4°C. The annealing temperature is shown in Table 1. The PCR products

were processed with 10% native polyacrylamide gel electrophoresis for 6~8h under stable voltage 150~180V, and then fixed for silver nitrate staining, development, and other processes. Finally, in the gel imaging system under the photo shoot.

Table 1 – Relational information for microsatellite locus

Locus name	Primer sequence (5'→ 3')	T _A (°C)
UBC0004	F:TCCTTGGGCAGTAGTTTCAA R:CTCCCATGTTGCTTCTTAG	49
UBC0005	F:GGAACATGTAGACAAAAGC R:AGTAGTCCATTCCACAGCCA	55
UBC0006	F:TTTCTATCCTTCATCTCCAG R:AGACATCCTGCTTCTCGTG	46
GUJ0001	F:GAAGCGAAAGCCGAGCCA R:CAGCACTTCGGAGCACAGGA	55
GUJ0013	F:ACCAAACCCGAGATCCGACA R:AGCGTTCGCGTTCCTCTTTC	54
GUJ0034	F:CGTAACGGTCCAATATGGAT R:TCCACGATGCAGAGGATTT	55
GUJ0049	F:GAAGCAGTGACAGCAGAATG R:CGGTAGCATTTCTGACTCCA	55
GUJ0054	F:GTGTTCTCTCACTCCCAAT R:ATGTGAGCAATTGGGACTG	56
GUJ0055	F:GCATACTGCAATATACCTGA; R:TTGACATACTTGGATTAGAGA	56
GUJ0070	F:AAACCCCAAAGAAGCTGTCC R:ACGTTGTCAACATCAGCTTG	54
GUJ0071	F:AGATCCTGCTCCTGGAATTG R:CAGCTGCACCTAATACAGGC	58
GUJ0086	F:AGCTGCCATATCTACTGCTC R:TGGCTTAGTGCTTTCAGAGG	55

The allele frequency and size range of alleles were calculated using the Excel Microsatellite Toolkit. The molecular biology software POPGENE (Version 1.32) was used to analyze polymorphism information content (PIC), effective number of alleles (Ne), and miscellaneous heterozygosity (H) of each marker.

Heterozygosity:

$$H = 1 - \sum_{i=1}^n p_i^2$$

Where p_i was the frequency of i^{th} allele of a microsatellite DNA

Effective number of alleles:

$$N_e = 1 / \sum_{i=1}^n p_i^2$$

Where p_i was the frequency of i^{th} and j^{th} allele of a microsatellite DNA

Polymorphic information content:

$$PIC = 1 - \left(\sum_{i=1}^n p_i^2 \right) - \left(\sum_{i=1}^{n-1} \sum_{j=i+1}^n 2p_i p_j^2 \right)$$

Where p_i and p_j were the frequency of i^{th} allele of a microsatellite DNA, and n is allele number.



Genetic differentiation coefficient:

$$G_{st} = 1 - H_s / H_t$$

Where H_t is total population average heterozygosity, H_s is average heterozygosity of different quail populations, and G_{st} is coefficient of gene differentiation.

RESULTS AND DISCUSSION

The test results of polymorphism of microsatellite marker UBC0006 in Chinese black quails are represented in Figure 1, which shows that the microsatellite marker

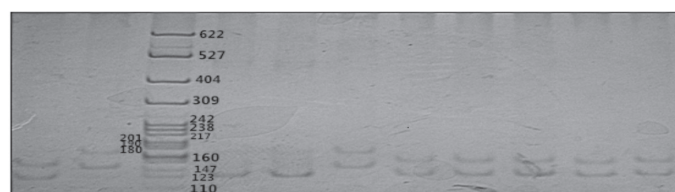


Figure 1- Detection results of microsatellite marker UBC0006

Note: M: markers, 1:AD, 2, 5:CE, 3, 4: BB, 6, 7, 8, 9, 10: BD

UBC0006 in Chinese black quails has a relatively rich polymorphism, with five alleles detected.

The allele frequencies of 12 microsatellite markers in the three quail communities are listed in Table 2.

Table 2 – Polymorphism information content(PIC) of microsatellite loci

Locus name	Populations	Number of alleles locus(Na)	Effective number of alleles (Ne)	Fixation index(F)	Polymorphism information content(PIC)	I	Heterozygosity(H)	Chi-square
UBC0004	Chinese yellow quails	4	2.2321	0.3245	0.4698	0.9402	0.5567	126.0020**
	Chinese black quails	4	2.4388	0.8941	0.5271	1.0346	0.5946	235.5987**
	Korean quails	6	2.4208	0.6068	0.5005	1.0469	0.5926	138.3258**
UBC0005	Chinese yellow quails	5	2.9500	-0.5128	0.6006	1.2081	0.6667	78.8815**
	Chinese black quails	4	2.6992	-0.5637	0.5583	1.0715	0.6345	62.3844**
	Korean quails	4	2.9391	-0.5157	0.5975	1.1738	0.6662	51.0510**
UBC0006	Chinese yellow quails	5	3.7531	-0.0629	0.6927	1.4508	0.7398	65.0606**
	Chinese black quails	5	3.4348	0.4269	0.6582	1.3681	0.7144	125.2977**
	Korean quails	5	3.4490	-0.2729	0.6588	1.3589	0.7170	92.7116**
GUJ0001	Chinese yellow quails	6	4.5682	-0.2803	0.7526	1.6573	0.7878	51.0140**
	Chinese black quails	4	3.6136	-0.1234	0.6703	1.3230	0.7290	18.1220**
	Korean quails	6	3.8138	-0.3554	0.7031	1.5075	0.7450	39.1210**
GUJ0013	Chinese yellow quails	6	5.4819	0.0257	0.7914	1.7411	0.8246	27.3916*
	Chinese black quails	6	3.8460	-0.2880	0.7013	1.5204	0.7458	38.0336**
	Korean quails	6	4.2921	-0.0280	0.7293	1.5639	0.7745	47.8137**
GUJ0034	Chinese yellow quails	6	4.6600	-0.2516	0.7518	1.6270	0.7921	165.9295**
	Chinese black quails	5	2.8714	-0.5344	0.5872	1.2288	0.6569	126.9049**
	Korean quails	6	3.7608	-0.3622	0.6903	1.4736	0.7412	130.6089**
GUJ0049	Chinese yellow quails	6	3.4620	-0.0487	0.6691	1.4263	0.7172	169.8493**
	Chinese black quails	5	2.8356	-0.1586	0.6050	1.2728	0.6524	53.0964**
	Korean quails	4	3.5696	-0.2289	0.6711	1.3300	0.7268	51.4800**
GUJ0054	Chinese yellow quails	6	3.4448	0.0208	0.6776	1.4932	0.7158	49.7565**
	Chinese black quails	6	4.0878	-0.0756	0.7149	1.5080	0.7613	200.4585**
	Korean quails	6	3.3137	-0.1017	0.6625	1.4465	0.7050	21.6544ns
GUJ0055	Chinese yellow quails	7	3.8507	-0.3050	0.7019	1.5619	0.7466	171.8327**
	Chinese black quails	7	4.5360	-0.2828	0.7478	1.6782	0.7857	192.3515**
	Korean quails	6	3.8879	-0.2686	0.7046	1.5408	0.7500	212.6933**
GUJ0070	Chinese yellow quails	5	3.3042	-0.4340	0.6457	1.3503	0.7033	175.0000**
	Chinese black quails	4	3.0330	-0.4686	0.6112	1.2071	0.6756	73.5027**
	Korean quails	4	3.2248	-0.3380	0.6354	1.2658	0.6966	36.1802**
GUJ0071	Chinese yellow quails	7	5.9862	-0.2006	0.8105	1.8435	0.8401	205.4907**
	Chinese black quails	7	4.6152	-0.2367	0.7494	1.6254	0.7895	83.1230**
	Korean quails	5	3.0314	-0.2340	0.6239	1.3135	0.6766	77.7222**
GUJ0086	Chinese yellow quails	5	3.3649	-0.3746	0.6594	1.3828	0.7088	75.2757**
	Chinese black quails	7	2.5222	-0.3981	0.5510	1.1872	0.6083	32.8646*
	Korean quails	7	3.8058	-0.1738	0.7012	1.5522	0.7444	170.6024**
Mean	Chinese yellow quails	5.6667	3.9215	-0.1750	0.6853	1.4735	0.7333	
	Chinese black quails	5.3333	3.3778	-0.1507	0.6401	1.3354	0.6957	
	Korean quails	5.4167	3.4591	-0.1894	0.6565	1.3811	0.7111	



The number of observed alleles ranged between 4~7. A total of 197 observed alleles were detected. The maximum number of GUJ0055 and GUJ0071 alleles observed in Chinese yellow quail and Chinese black quail was 7, and the maximum number of GUJ0086 alleles observed in Korean quail was 7.

The number of observed alleles, number of effective alleles, PIC, and fixation indexes of 12 microsatellite markers in the three quail communities are shown in Table 2. The average number of observed alleles in Chinese yellow quails, Chinese black quails and Korean quails were 5.6667, 5.3333 and 5.4167, respectively. Out of the 12 microsatellite markers, UBC0004 presented the least effective alleles (2.2321 in Chinese yellow quails) and GUJ0071 showed the most effective alleles (5.9862 in Chinese yellow quails). The average numbers of effective alleles in Chinese yellow quails, Chinese black quails and Korean quails were 3.9215, 3.3778, and 3.4591, respectively. Their mean PICs were 0.6853, 0.6401 and 0.6565, respectively. Chinese yellow quails had slightly higher PIC than the other two varieties.

Expected heterozygosity and average heterozygosity in the three quail communities are shown in Table 2. The average heterozygosity of 12 microsatellite markers in the quail communities were 0.7333, 0.6957 and 0.7111, indicating that all three quail communities present high polymorphism. The Chinese yellow quail showed the richest genetic polymorphism, while the Chinese black quail had lower genetic polymorphism. During the genetic research of a community, the Hardy-Weinberg equilibrium state test is necessary to determine whether there is population genetic equilibrium. Table 2 shows that, although GUJ0054 complied with the Hardy-Weinberg law in Korean quails, the other markers deviated significantly or

extremely significantly from the Hardy-Weinberg law. In other words, genes of these markers were in Hardy-Weinberg imbalance state.

Polymorphism information content (PIC) expresses possibility of an offspring to obtain the same allelic marker from its father (or mother). It is a statistical magnitude used to describe the heterogeneity degree of microsatellite locus, and it is an ideal evaluation index of polymorphism of an allele segment. When $PIC > 0.5$, the locus is a highly polymorphic locus. When $0.25 < PIC < 0.5$, the locus is moderately polymorphic. When $PIC < 0.25$, the locus is a low polymorphic locus. In this study, all microsatellite markers belonged to highly polymorphic loci, except for UBC0004, which showed the smallest PIC (0.4698) and belonged to a moderately polymorphic locus. The average PICs of the 12 microsatellite markers in Chinese yellow quails, Chinese black quails, and Korean quails were 0.6853, 0.6401 and 0.6565, respectively, and all were higher than 0.5. The average PIC of Korean quails was similar to the findings (0.6945) of Meng *et al.* (2007).

Heterozygosity reflects an approximated degree of genetic variation. Higher heterozygosity is accompanied of higher genetic diversity in the community and of higher degree of genetic variation. Heterozygosity of the evaluated communities and calculated using microsatellite markers was generally between 0.3 and 0.8. In this studies, 11 microsatellite markers reported high heterozygosity in the three evaluated quail communities, except for UBC0004. This indicates that all three quail communities present high polymorphism and that the Chinese yellow quail has the richest genetic polymorphism.

The coefficient of genetic differentiation (GST) reflects the degree of genetic variation among communities. It varies from 0~1. When communities

Table 3 – Genetic differentiation coefficient (G_{st}) of microsatellite loci

Loci	Total population average heterozygosity (H_t)	Average heterozygosity of different quail populations (H_s)	Genetic differentiation coefficient (G_{st})
UBC0004	0.6094	0.5813	0.0461
UBC0005	0.6626	0.6558	0.0103
UBC0006	0.7449	0.7237	0.0284
GUJ0001	0.7683	0.7539	0.0187
GUJ0013	0.7964	0.7816	0.0185
GUJ0034	0.738	0.7301	0.0107
GUJ0049	0.7153	0.6988	0.0231
GUJ0054	0.7386	0.7274	0.0152
GUJ0055	0.7699	0.7608	0.0119
GUJ0070	0.7498	0.6918	0.0773
GUJ0071	0.7918	0.7687	0.0291
GUJ0086	0.6992	0.6872	0.0172
Mean	0.7320	0.7134	0.0255



have almost same effective alleles, no differentiation can be observed when G_{ST} approaches 0, but evident differentiation exists when G_{ST} approaches 1. Table 3 shows that the G_{ST} of the three studied quail communities varied between 0.0103~0.0773, indicating low differentiation, but high homology among them. Table 3 shows that the genetic distance between the Chinese black quail and the Korean quail was the shortest, of 0.0628. This implies that the Chinese black quail and the Korean quail have the closest genetic relationship. The second shortest genetic distance was between the Chinese yellow quail and the Korean quail, of 0.0951. Figure 2 further confirms that the Chinese black quail and the Korean quail cluster first, and then the Chinese yellow quail.

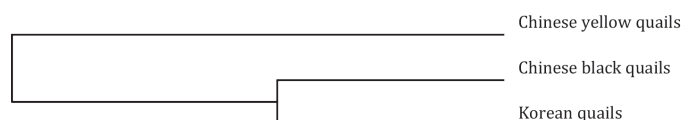


Figure 2 – Dendrogram of quail populations

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