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Genetic Diversity of Wild Quail in China Ascertained with Microsatellite DNA Markers

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ABSTRACT : The genetic diversity of domestic quail and two wild quail species, Japanese (*Coturnix coturnix*) and Common quail (*Coturnix japonica*), found in China was studied using microsatellite DNA markers. According to a comparison of the corresponding genetic indices in the three quail populations, such as Polymorphism Information Content (PIC), Mean Heterozygosity ($\overline{H}$) and Fixation Index, wild Common quail possessed rich genetic diversity with 4.67 alleles per site. Its values for PIC and $\overline{H}$ were the highest, 0.5732 and 0.6621, respectively. Domestic quail had the lowest values, 0.5467 and 0.5933, respectively. Wild Japanese quail had little difference in genetic diversity from domestic quail. In addition, from analyses of the fuzzy cluster based on standard genetic distance, the similarity relationship matrix coefficient between wild Japanese quail and domestic quail was 0.937, and that between wild Common quail and domestic quail was 0.783. All of these results showed that the wild Japanese quail were closer to the domestic quail for phylogenetic relationship than wild Common quail. These results at the molecular level provide useful data about quail's genetic background and further supported the hypothesis that the domestic quail originated from the wild Japanese quail. (**Key Words :** Microsatellite, Wild Quail, Genetic Diversity, Phylogenetic Relationship)

INTRODUCTION

Common quail (*Coturnix coturnix*) and Japanese quail (*Coturnix japonica*) belong to Aves, Calliformes, Phasianidae, *Coturnix*. The number of chromosomes is 78, including 6 big pairs, 6 medium pairs, and 27 mini pairs. Common quail includes European quail, African quail, and some kinds of Asian quail. Japanese quail are mainly found in East Asia, including Japan, Korea, China, Mongolia, Siberia, and Kuye Island, ranging from 100 to 150 degrees east longitude, and from 17 to 55 degrees north latitude (Sano et al., 1994). Most areas of China have the two wild species and the number of the Japanese quail is more than that of the Common quail. They are all migratory birds and their ranges greatly overlap. They usually inhabit the plains, coastal regions, and the foothills, especially places sparsely covered with grass. Domestic quail, derived from Japanese quail (*Coturnix joponica*), as laying, meat, and laboratory animals have produced a flourishing industry. At present, there are about 1,050 million quails around the world and 200 million quails in China. Quail ranks second to chicken in the Chinese poultry industry. Moreover, the use of quail is diversified including laying, meat, and laboratory types (Kimura, 1996). However, the existing domestic quails were all bred in Japan, and possess a narrow genetic background. After decades of use, the producing performance obviously does not satisfy the needs for the development of a flourishing industry. On the other hand, the deterioration of the environment in recent years dramatically reduced the number of wild quails; none could be found in some places. This paper analyzed the genetic diversity of the two wild quails with 9 microsatellite DNA markers, and compared them with domestic quails, aiming to determine genetic variance and phylogenetic relationship between wild and domestic quail populations in order to explore new wild quail resources and promote sustainable development of the quail industry.

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MATERIALS AND METHODS

Collection and extraction of genomic DNA

About 400 wild quail samples were captured from two abundant areas where wild quails migrated to and settled down. One was the Weishan Lake region, and the other was the Anyang district, Henan Province. One milliliter blood

Table 1. Characterization of the nine microsatellite sites and PCR conditions

Site	Primer sequences	Mg^{2+} (mmol/L)	Annealing temperature (°C)
*GUJ*0028	5'-TGAACAAAGCAGAAAGGAGC-3'	1.5	54.6
	3'-CCTTACCTACATGAAACGTC-5'		
*GUJ*0029	5'-GAGCATTTCTAGTCTGTCTC-3'	1.2	58
	3'-ATACACAGGTAAGGAAACC-5'		
*UBC*0001	5'-TCTCTAAAATCCAGCCCTAA-3'	1.5	48
	3'-AGCTCCTTGTACCCTATTGC-5'		
*UBC*0002	5'-CAGCCAATAGGGATAAAAGC-3'	1.5	50
	3'-CTGTAGATGCCAAGGAGTGC-5'		
*UBC*0004	5'-TCCTTGGGCAGTAGTTTCAA-3'	1.5	38
	3'-CTCCCATGTTGCTTCTTTAG-5'		
*UBC*0005	5'-GGAACATGTAGACAAAAGC-3'	1.5	57
	3'-AGTAGTCCATTTCCACAGCCA-5'		
*UBC*0006	5'-TTTCTATCCTTCATCTCCAG-3'	1.5	49
	3'-AGACATCCTGCTTTCTCGTG-5'		
*GUQ*0001	5'-TGAACAAAGCAGAAAGGAGC-3'	1.5	56
	3'-CCTTACCTACATGAAACGTC-5'		
*GUQ*0007	5'-GAGCATTTCTAGTCTGTCTC-3'	1.5	58
	3'-ATACACAGGTAAGGAAACC-5'		

samples were collected from the heart of random samples of birds, including 40 wild Japanese quails (WSH) from the Weishan Lake region, 62 wild Common quails (YCQ) from the Anyang district, Henan Province, and 40 domestic quails (YJQ) from *a* quail breeding farm in Yangzhou City, Jiangsu Province. The genomic DNA was extracted according to the procedures described by Sambrook et al. (1998). DNA was extracted from 100 μl of whole EDTA-blood. Then the mixture solution was made up with 100 μg/ml Proteinase K and 80 μg/ml Dnase-free pancreatic Rnase. After overnight incubation at 37°C, the proteins were removed by phenol and chloroform-isoamyl alcohol extractions and the DNA was precipitated by ethanol.

Source of primers

Nine primer pairs of microsatellite markers were designed according to the literature (Inoue-Murayama and Nomura, 1998; Inoue-Murayama et al., 2001; Kayang et al., 2000, 2002; Read et al., 2000), as shown in Table 1. Though 22 pairs of primers were tested in this work, only 9 pairs of primers were used in the analyses.

Polymerase chain reaction (PCR)

The PCR reaction mixture with a final volume of 25 μl contained 50 ng of template DNA, 2.5 μl of 10×buffer, 1.2 to 2.0 μl of 25 mmol/L $MgCl_2$ (as optimized for each marker), 0.5 μl of 10 mmol dNTP, 1 μl of 5 pmol/μl forward and reverse primers, and 1 u of *Taq* DNA Polymerase; ddH_2O was added to the volume of 25 μl.

The amplification conditions for PCR were: 3 min denaturing at 94°C followed by 30 cycles of denaturation at 94°C for 1 min, annealing for 45 s at 38 to 58°C (as optimized for each marker), and extension at 72°C for 1 min. This was followed by a final cycle at 72°C for 15 min. The PCR products were then separated on 8% denaturing polyacrylamide gels with a molecular weight marker, pBR322 DNA/*Msp*I Markers, on an electrophoresis system, at 100 V for 6 to 7 h, and stained with ethidium bromide. The results were visualized and photographed with the Kodak Digital Science ID Image Analysis System.

Statistical methods

The calculation of Heterozygosity (H) (Nei, 1978), Polymorphism Information Content (PIC) (Bostein et al., 1980), Effective Number of Alleles (N_e) (Kimura and Ohta, 1973), Fixation Index (F) (Wright, 1978), and Coefficient of Gene Differentiation (G_{st}) (Nei, 1973) was done according to procedures described in the literature.

Test of H (Nei and Kimura, 2000) : For all polymorphic sites, the formula, $d_i = \hat{h}_{X_i} - \hat{h}_{Y_i}$, was used to calculate the difference in polymorphism between populations X and Y. $\bar{d}$ and its variance, $V_{(\bar{d})}$ were calculated using the following equations,

$$\bar{d} = \sum_{i=1}^{L'} d_i / L', \qquad V(\bar{d}) = \sum_{i=1}^{L'} (d_i - \bar{d})^2 / [L'(L'-1)]$$

where *L'* is the number of polymorphic sites.

The difference in *H* between two populations was tested by the following formula,

$$t_{L'-1} = \bar{d} / S_{(\bar{d})}$$

Fuzzy cluster analysis (Chang, 1998) : The following

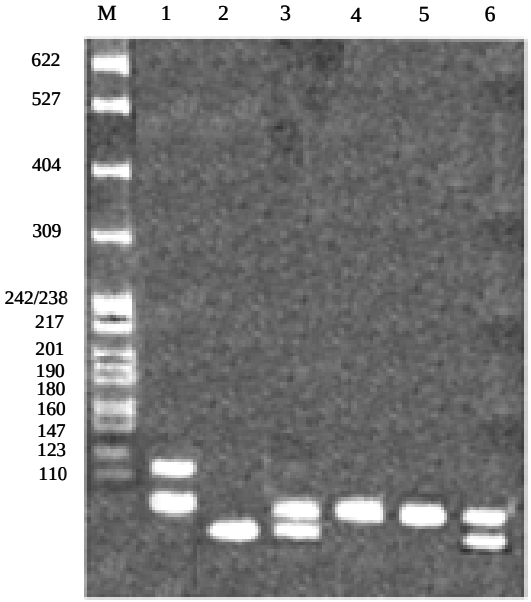

Figure 1. PAGE (Polyacrylamide Gel Electrophoresis) pattern of microsatellite DNA of wild Japanese quail population 1, 2, 3, 4, 5, and 6 represent sample numbers of wild Japanese quail. M stands for pBR322 DNA/*Msp*Ⅰ Marker.

$$\mu_{\underline{R}}(x,y)=\frac{1}{2}\ln\left(J_{x,y}/\sqrt{J_x\cdot J_y}\right)+1$$

formula was used to analyze phylogenetic relationships between animal populations. Here J_x,J_y and $J_{x,y}$ represent, respectively, population X, population Y, and the probability average among sites of the same alleles acquired at random from populations X and Y; $\mu_{\underline{R}}(x,y)$ stands for the membership function under the fuzzy consistency relationship matrix $\underline{R}$ between populations X and Y. According to the value of the membership function, the fuzzy resemblance matrix was set up, and finally a similarity relationship matrix was obtained through fuzzy operating. Fuzzy cluster analysis of different animal populations was based on the coefficients of the fuzzy similarity relationship matrix.

RESULTS

Calculation of allelic frequency

The electrophoresis results were determined by 8% denaturing polyacrylamide gels using a size marker, pBR322 DNA/*Msp*I (Figure 1). Lanes 1, 3, and 6 represent heterozygotes, and lanes 2, 4, and 5 stand for homozygotes. According to the individual genotypes, the allelic frequencies of 9 microsatellite sites for the two wild quail populations and domestic population were calculated. A total of 37, 37, and 42 alleles in the WSH, YJQ, and YCQ populations were observed, respectively, which indicated

Table 2. Allelic frequencies at nine microsatellite sites in Domestic quail (YJQ), wild Japanese quail (WSH) and Common quail (YCQ) populations

Site	Allele (bp)	YJQ	WSH	YCQ	Site	Allele (bp)	YJQ	WSH	YCQ
GUJ	150	0.4250	0.5600	0.2984	*GUJ*	140	0.2500	0.2600	0.0323
0028	160	0.1375	0.0300	0.2742	0029	142	0.1625	0.1600	0.2903
	170	0.1000	0.1400	0.0645		149	0.1500	0.1000	0.4194
	175	0.2250	0.1400	0.0484		152	0.1250	0.1800	0.0323
	178	0.1125	0.1300	0.1452		159	0.3125	0.3000	0.1452
	185	0.0000	0.0000	0.1694					
UBC	172	0.1250	0.0900	0.0242	*UBC*	204	0.5500	0.6100	0.2742
0001	176	0.3125	0.5800	0.0726	0002	221	0.0250	0.1100	0.4113
	180	0.5625	0.3300	0.2823		231	0.4250	0.2800	0.2339
	196	0.0000	0.0000	0.1694		251	0.0000	0.0000	0.0806
	206	0.0000	0.0000	0.4516					
UBC	227	0.4625	0.3400	0.0484	*UBC*	97	0.5375	0.5400	0.4839
0004	231	0.2250	0.2500	0.0403	0005	107	0.1250	0.1400	0.2339
	236	0.0500	0.0800	0.0161		111	0.1500	0.1600	0.0645
	242	0.0375	0.1100	0.0887		114	0.1875	0.1600	0.1290
	250	0.2250	0.2200	0.3710		125	0.0000	0.0000	0.0887
	275	0.0000	0.0000	0.4355					
GUQ	98	0.0250	0.1000	0.0887	*UBC*	95	0.1625	0.2500	0.2500
0007	108	0.7750	0.6300	0.7016	0006	101	0.2375	0.1600	0.0726
	118	0.2000	0.2700	0.1129		105	0.2250	0.3700	0.1371
	125	0.0000	0.0000	0.0968		108	0.0875	0.1100	0.1694
						112	0.1000	0.0000	0.1290
						118	0.1875	0.1100	0.2419
GUQ	98	0.1625	0.0100	0.0000					
0001	109	0.8125	0.8500	1.0000					
	121	0.0250	0.1400	0.0000					

that YCQ possessed richer genetic diversity than WSH and YJQ (Table 2).

Analysis of genetic variance of populations

Based on the allelic frequency of each site, PIC, H, mean H ($\overline{H}$), N_e and Fixation index were calculated (Tables 3 and 4). From Table 3, the value of PIC and $\overline{H}$ in YCQ was the highest being 0.5732 and 0.6621, respectively. Meanwhile, YJQ had the lowest values of 0.5467 and 0.5933, respectively. The difference in $\overline{H}$ presented the same trends, but there was no difference ($p>0.05$) among the three populations. N_e of YCQ was 3.3913, and was higher than those of YJQ and WSH. This value of N_e was close to the observed number per site of YCQ, 4.67. In the three quail populations, most values for the Fixation index were less than zero, except for *GUJ*0028 and *GUQ*0007 in YJQ and WSH, *UBC*0002 in YJQ, and *GUQ*0001 in YCQ. These results indicated that H in the three populations was high, namely there was no obviously deviation from the Hardy-Weinberg equilibrium.

Coefficient of gene differentiation (G_{st})

We can see that the G_{st} value between YJQ and WSH was smaller than that between YJQ and YCQ (Table 6), which indicated that the extent of genetic differentiation between YJQ and WSH was lower, and these two species had a closer phylogenetic relationship compared to the other combinations.

Fuzzy cluster

The fuzzy similarity relationship matrix coefficients of fuzzy cluster of the three quail populations (Table 7) showed that WSH and YJQ firstly clustered together (Figure 2). The coefficient of fuzzy cluster matrix between them was 0.937, whereas the coefficient between YCQ and YJQ was 0.738.

DISCUSSION

Genetic analysis within two wild quail populations and a domestic population

PIC (Polymorphic Information Content), an important index of polymorphism of microsatellite DNA sites, was first used to estimate the polymorphism of gene markers in linkage analysis. The value of PIC indicates the degree of polymorphism. A site is highly polymorphic when PIC>0.5,

Table 3. Polymorphism information content (PIC), observed heterozygosity (H_o), expected heterozygosity (H_e), observed homozygosity ($1-H_O$), and expected homozygosity ($1-H_e$) of nine microsatellite DNA sites in three quail populations

Site	Population	PIC	$1-H_o$	H_o	$1-H_e$	H_e	Mean H
*UBC*0001	YJQ	0.4956	0.3750	0.6250	0.4225	0.5775	0.5996
	WSH	0.4814	0.4200	0.5800	0.4479	0.5521	
	YCQ	0.6294	0.2742	0.7258	0.3126	0.6874	
*UBC*0002	YJQ	0.4024	0.5000	0.5000	0.4772	0.5228	0.5827
	WSH	0.4682	0.4400	0.5600	0.4572	0.5428	
	YCQ	0.6384	0.2419	0.7581	0.2999	0.7001	
*UBC*0004	YJQ	0.6300	0.2750	0.7250	0.3104	0.6896	0.6989
	WSH	0.7148	0.1800	0.8200	0.2374	0.7626	
	YCQ	0.6004	0.3065	0.6935	0.3340	0.6660	
*UBC*0005	YJQ	0.5921	0.3000	0.7000	0.3541	0.6459	0.6526
	WSH	0.5930	0.3600	0.6400	0.3560	0.6440	
	YCQ	0.6398	0.2581	0.7419	0.3120	0.6880	
*GUQ*0007	YJQ	0.3099	0.6500	0.3500	0.6367	0.3633	0.4522
	WSH	0.4529	0.5000	0.5000	0.4745	0.5255	
	YCQ	0.4477	0.4516	0.5484	0.5184	0.4816	
*UBC*0006	YJQ	0.7869	0.1250	0.8750	0.1759	0.8241	0.7914
	WSH	0.7123	0.2200	0.7800	0.2416	0.7584	
	YCQ	0.7821	0.1290	0.8710	0.1838	0.8162	
*GUJ*0028	YJQ	0.6866	0.1935	0.8065	0.2141	0.7859	0.7120
	WSH	0.5915	0.2750	0.7250	0.2636	0.7364	
	YCQ	0.7457	0.5000	0.5000	0.3642	0.6358	
*GUJ*0029	YJQ	0.7397	0.2419	0.7581	0.2893	0.7107	0.7506
	WSH	0.7380	0.1000	0.9000	0.2149	0.7851	
	YCQ	0.6749	0.1600	0.8400	0.2178	0.7822	
*GUQ*0001	YJQ	0.2771	1.0000	0.0000	1.0000	0.0000	0.1902
	WSH	0.2293	0.6750	0.3250	0.6832	0.3168	
	YCQ	0.0000	0.7200	0.2800	0.7396	0.2604	
Mean	YJQ	0.5467	0.4067	0.5933	0.4311	0.5689	0.6034
	WSH	0.5535	0.3522	0.6478	0.3751	0.6249	
	YCQ	0.5732	0.3379	0.6621	0.3647	0. 6353	

Table 4. Effective number of alleles (N_e), observed number of alleles (N_o), and Fixation index (F) of nine microsatellite DNA sites in three quail populations

Site	Population	N_o	N_e	F	$\overline{N_o}$	$\overline{N_e}$	$\overline{F}$
*UBC*0001	YJQ	3	2.3273	-0.0959	3.6667	2.7289	-0.0738
	WSH	3	2.2056	-0.0611			
	YCQ	5	3.6537	-0.0645			
*UBC*0002	YJQ	3	2.0672	0.0315	3.3333	2.3784	-0.0341
	WSH	3	2.1617	-0.0421			
	YCQ	4	2.9063	-0.0916			
*UBC*0004	YJQ	5	3.1342	-0.0647	5.3333	3.9960	-0.0669
	WSH	5	4.0816	-0.0861			
	YCQ	6	4.7721	-0.0498			
*UBC*0005	YJQ	4	2.7610	-0.0975	4.3333	2.8312	-0.0628
	WSH	4	2.7594	-0.0038			
	YCQ	5	2.9731	-0.0871			
*GUQ*0007	YJQ	3	1.5595	0.0244	3.3333	1.8450	-0.0282
	WSH	3	2.0842	0.0388			
	YCQ	4	1.8912	-0.1478			
*UBC*0006	YJQ	6	5.3691	-0.0753	5.6667	4.9273	-0.0633
	WSH	5	4.0128	-0.0389			
	YCQ	6	5.4000	-0.0758			
*GUJ*0028	YJQ	5	3.6655	0.0030	5.3333	3.6332	0.0580
	WSH	5	2.6983	0.2056			
	YCQ	6	4.5357	-0.0345			
*GUJ*0029	YJQ	5	4.4506	-0.1608	5.0000	4.0910	-0.1069
	WSH	5	4.4326	-0.0847			
	YCQ	5	3.3898	-0.0753			
*GUQ*0001	YJQ	3	1.4552	-0.0390	2.3333	1.2675	0.2916
	WSH	3	1.3473	-0.0861			
	YCQ	1	1.0000	1.0000			

Table 5. p value[1] of heterozygosity test in Domestic quail (YJQ), wild Japanese quail (WSH) and Common quail (YCQ) populations

Population	YJQ	WSH	YCQ
YJQ		0.275	0.281
WSH			0.764
YCQ			

[1] Significant where $p<0.05$ and not significant where $p>0.05$.

normally polymorphic when PIC<0.5, and lowly polymorphic when PIC<0.25. Kong et al. (2006) assessed the genetic variation and established the relationship amongst breeds and strains using 15 chicken specific microsatellite markers, and founded that PIC of UMA1019 was the highest (0.872) and that of ADL0234 was the lowest (0.562). Tu et al. (2006) studied genetic diversity of 14 indigenous grey goose breeds in China based on microsatellite markers, and indicated the highest PIC was in the Xupu (0.6916) and the lowest was in the Yan (0.4985). In contrast to that of chicken and other fowls, quail's microsatellite DNA has not received much attention from researchers. Though few highly polymorphic microsatellite DNA sites of quail were reported, there were 5 highly and 4 normally polymorphic sites in YJQ in this study. WSH had 5 highly polymorphic, 3 normally polymorphic, and 1 lowly polymorphic site, and in the YCQ population, there were 7 highly polymorphic, 1 normally polymorphic, and 1 monomorphic site, which showed obvious differences from the two other populations.

Heterozygosity, a measure of gene diversity, reflects the genetic variance of populations at polymorphic sites. The *GUJ*0028, *GUJ*0029, *UBC*0004, *UBC*0005, and *UBC*0006 sites possessed high polymorphism, rich genetic diversity, and high selection potentials (Table 3). Therefore, they may act as candidate genes to be applied to the study of location of QTL. Liu et al. (2006) studied the correlations between heterozygosity at microsatellite loci, mean d2 and body weight in a Chinese native chicken, and indicated positive correlations were found between microsatellite heterozygosity and body weight in males and females ($p<0.05$). The difference in genetic differentiation level between YJQ and WSH was lower, though the test of H showed no significant differences among the three quail populations ($p>0.05$). Furthermore, N_e showed the same trends as H. It is well known that, the more similar the distribution of alleles in populations, the more close the relation between N_e and the absolute number of alleles tested, which is the reciprocal of homozygosity (Nozawa et al., 1996). Of the 9 microsatellite sites in this paper, the N_e value of *GUQ*0001 and *GUQ*0007 in their own populations was relatively small, indicating that the distribution of

Table 6. Genetic distance coefficients between Domestic quail (YJQ), wild Japanese quail (WSH) and Common quail (YCQ) populations

Population	YJQ	WSH	YCQ
YJQ		0.0439	0.0548
WSH			0.0109
YCQ			

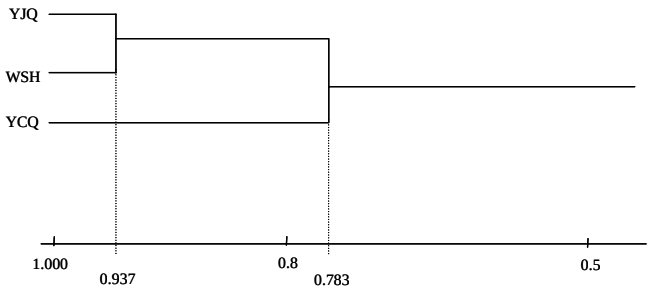

Figure 2. Fuzzy cluster of Domestic quail (YJQ), wild Japanese quail (WSH) and Common quail (YCQ) populations.

alleles was not similar. However, the condition of *UBC*0006 and *GUJ*0029 was the opposite.

At most sites the Fixation Index (F) was negative (Table 4), showing that the heterozygote frequencies were high at these sites. The heterozygote of YCQ was relatively richer than that of YJQ and WSH. However, there existed no significant genetic differentiation and evolutionary divergence force in the three populations.

Through comparison of domestic quail and the two wild quails, we found that the genetic variance level of WSH was close to that of YJQ. The possible reasons for this result include common origin and evolutionary progress, time of species formation, bottleneck effect at the beginning of domestication, gene flow between different species, crosses between different lines, mixing, and human activities.

Genetic analysis of three quail populations

The genetic background could be evaluated through the allele composition of the three quail populations. Thirty-seven alleles were detected in YJQ and WSH at 9 microsatellite sites, with a mean of 4.11 alleles per site. Moreover, every allele was shared by the two populations, and the distribution of alleles in YJQ and WSH was still under equilibrium due to geographical isolation and artificial selection. On the other hand, 42 alleles were detected in YCQ at the same 9 microsatellite sites, with a mean of 4.67 alleles per site. However, there was one homogeneous site in YCQ, which presented a difference from the two other populations, YJQ and WSH.

G_{st} is the right index of calculating relative value of genetic differentiation between sub-populations. Chang et al. (2000) reported that the G_{st} of a goat population in the middle and lower Yellow River in China was 0.0038-0.2118.

Table 7. Fuzzy similarity relationship matrix coefficients of fuzzy cluster of Domestic quail (YJQ), wild Japanese quail (WSH) and Common quail (YCQ) populations

Population	YJQ	WSH	YCQ
YJQ		0.937	0.783
WSH			0.783
YCQ			

Fan et al. (1999) tested G_{st} of 8 native pig breeds in China, and found a value of 0.2030. In this study, G_{st} of 3 quail populations was 0.0109-0.0548; that is, the genetic variance between populations accounted for 1.09%-5.48% of the total genetic variance, which indicated that the variance was mainly produced from within populations.

Recently, there have been many studies of phylogenetic relationship in animal populations with standard genetic distance and classical cluster. Zhang et al. (1998) analyzed the population genetic variance in Guangdong local chicken breeds and clarified the phylogenetic relationship among these breeds. Cao et al. (1999) studied the genetic variance of 5 beef populations with microsatellite markers. Wu et al. (2004) reported the genetic structure of 12 local chicken breeds in China with the microsatellite technique and divided the 12 breeds into 3 clones based on their phylogenetic relationships. Osman et al. (2006) also revealed the genetic variability and relationships of Japanese and foreign chickens assessed by microsatellite DNA profiling and indicated native Japanese chicken breeds and foreign breeds were clearly separated from each other. Olowofeso et al. (2006) also studied genetic distance of the four chicken populations and indicated the Jiangchun and Cshiqishi chickens were closely related breeds. Su et al. (2006) reported the genetic variance of different Chinese duck populations and presented the genetic diversity was improved by crossbreeding. When it came to quail, few reports about phylogenetic relationship were available. Moreover, most reports about the variance were based on allied species with cytogenetic and biochemical genetic techniques, and *the* application of the theory of Nei's standard genetic distance to analyze phylogenetic relationships. It was obvious that this theory had the trend of absolution, overlooking the consistency of difference between species or breeds and did not show the relativity of the results. It belonged to hard clustering (Chang et al., 2001). Fuzzy cluster analysis, combining the characteristics of animal genetics into fuzzy set theory, better fits the objective facts. This paper changed the coefficients of Nei's standard genetic distance into membership functions, and then set up the fuzzy resemblance matrix, after fuzzy operating step by step. Lastly a similarity relationship matrix was obtained. According to the coefficients of the fuzzy similarity relationship matrix, we got the fuzzy clustering of the three quail populations (Figure 2). The figure showed that the difference in genetic diversity

between YJQ and WSH was smaller than that between YJQ and YCQ, and clustered at the level of 0.937. The results further proved that YJQ and WSH were closer in their phylogenetic relationship than YJQ and YCQ. There would be a reasonably deduced common origin and evolutionary progress in fairly recent times between YJQ and WSH.

The mysteries concerning the origin and domestication of quail

Presently, academic circles tend to believe that the domestic quail originated from wild quail in East Asia, but whether the origin of domestic quail is from the Japanese Islands, China, or Korea is a controversial issue. Recent studies showed that the wild quail populations of the Japanese Islands cluster alone in the phylogenetic clustering with domestic populations of different parts of the world. This indicates that the phylogenetic relationship of domestic quail is distant from that of wild quail in the Japanese Islands (Chang et al., 2001). According to the literature, it has been 1,000 years since China began to domesticate quail, 400 years earlier than Japan. China and Japan are separated only by a strip of water. Cultural exchange between the two countries has a long history. During the period that the Sui and Tang Dynasties (581 A.D.-907 A.D.) ruled China, with the return of Japanese students studying abroad, envoys to the Sui and Tang Dynasties, and learned monks, the custom of quail-fighting spread from China to Japan; so, Chinese quail culture had some effect on that of Japan. This historical background shows the possibility that domestic quail originated from WSH in China. In addition, recent reports proposed that WSH and domestic quail populations have a closer phylogenetic relationship than with the Japanese wild quail based on the analysis of enzyme polymorphisms (Chang et al., 2005). This paper further supported on the DNA level that domestic quail may originate from WSH. All of the alleles at 9 microsatellite sites were shared by WSH and YJQ, which provided an objective foundation for determining the origin of quail and the location of early domestication.

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REFERENCES

Bostein, D., R. L. White, M. Sckolnick and R. W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32:317-331.

Cao, H. H., Y. C. Wang, Y. C. Chen, Y. Zhang and L. Ferretti. 1999. Study on five microsatellite markers in beef cattle population. Scientia Agricultura Sinica. 32(1):69-73.

Chang, G. B., H. Chang, X. P. Liu, W. M. Zhao and O. Olowofeso. 2005. Developmental research on origin and phylogeny of quail. World's Poult. Sci. J. 61(1):105-112.

Chang, G. B., H. Chang, H. L. Zhen, X. P. Liu, W. Sun, R. Q. Geng, Y. M. Yu, S. C. Wang, S. M. Geng, X. L. Liu, G. Q. Qin and W. Shen. 2001.Study on phylogenetic relationship between wild Japanese quails in the Weishan Lake area and domestic quails. Asian-Aust. J. Anim. Sci. 14(5):603-607.

Chang, H. 1998. In: Study on Animal Genetic Resources of China. Xian: Shaanxi People's Education Publishing House, China. pp. 243-249.

Chang, H., K. Nozawa, X. L. Liu, S. M. Geng, Z. J. Ren, G. Q. Qin, X. G. Li, J. M. Sun, H. L. Zheng, J. Z. Song, Y. Kurosawa, A. Sano, Q. Jia and G. H. Chen. 2000. Study on phylogenetic relationships among native goat populations along the middle and lower yellow river valley. Asian-Aust. J. Anim. Sci. 13(2):137-148.

Chang, H., K. Nozawa, X. L. Liu, S. M. Geng, Z. J. Ren and G. Q. Qin. 2000. Study on phylogenetic relationship of native goat populations along the middle and lower yellow river valley. Scientia Agricultura Sinica 33(3):79- 87.

Fan, B., K. Li, Z. Z. Peng, Y. Chen, C. Moran, Y. C. Gong and S. H. Zhao. 1999. Genetic variation of 27 microsatellite sites in three Hubei indigenous pig breeds. Chinese Biodiversity 7(2): 91-96.

Inoue-Murayama, M., B. B. Kayang, K. Kimura, H. Ide, A. Nomura, H. Takahashi, Y. Nagamine, T. Takeda, H. Hanada, K. Tatsuda, M. Tsudzuki, Y. Matsuda, M. Mizutani, Y. Murayama and S. Ito. 2001.Chicken microsatellite primers are not efficient markers for Japanese quail. Anim. Genet. 32:7-11.

Inoue-Murayama, M. and A. Nomura. 1998. Application of chicken microsatellite markers to Japanese quail and Chinese painted quail. Anim. Genet. 29 (Suppl.1):46.

Kayang, B. B., M. Inoue-Murayama, T. Hoshi, K. Matsuo, H. Takahashi, M. Minezawa, M. Mizutani and S. Ito. 2002. Microsatellite sites in Japanese quail and cross-species amplification in chicken and guinea fowl. Genet. Sel. Evol. 34:233-253.

Kayang, B. B., M. Inoue-Murayama, A. Nomura, K. Kimura, H. Takahashi, M. Mizutani and S. Ito. 2000. Fifty microastellite markers for Japanese quail. J. Heted. 91(6):502-505.

Kimura, M. 1996. Wild quails. Study on Animal Products. 50:197-202.

Kimura, M. and T. Ohta. 1973. Mutation and evolution at the molecular level. Genet. 73 (Suppl):19-35.

Kong, H. S., J. D. Oh, J. H. Lee, K. J. Jo, B. D. Sang, C. H. Choi, S. D. Kim, S. J. Lee, S. H. Yeon, G. J. Jeon and H. K. Lee. 2006. Genetic variation and relationships of Korean native chickens and foreign breeds using 15 microsatellite markers. Asian-Aust. J. Anim. Sci. 19(11):1546-1550.

Liu, G. Q., X. P. Jiang, J. Y. Wang and Z. Y. Wang. 2006. Correlations between heterozygosity at microsatellite loci, mean d2 and body weight in a Chinese native chicken. Asian-Aust. J. Anim. Sci. 19(12):1671-1678.

Nei, M. 1973. The theory and estimation of genetic distance in Genetic structure of populations. Honolulu: University of Hawaii Press. United States. pp. 45-54.

Nei, M. 1978. Estimation of average hetorozygosity and genetic distance from a small number of individuals. Genet. 89:583-590.

Nei, M. and S. Kimura. 2000. in Molecular Evolution and Phylogenetics. Oxford: Oxford Univesity Press. England. pp. 235-236.

Nozawa, K., H. Chang and Z. J. Ren. 1996. Effects of domestication to animal genetic variation. J. Husb. Vet. 3:55-56.

Olowofeso, O., J. Y. Wang, P. Zhang, G. J. Dai, H. W. Sheng, R. Wu and X. Wu. 2006. Genetic analysis of Haimen chicken populations using decamer random markers. Asian-Aust. J. Anim. Sci. 19(11):1519-1523.

Osman, S. A. M., M. Sekino, A. Nishihata, Y. Kobayashi, W. Takenaka, K. Kinoshita, T. Kuwayama, M. Nishibori, Y. Yamamoto and M. Tsudzuki. 2006. The genetic variability and relationships of Japanese and foreign chickens assessed by microsatellite DNA profiling. Asian-Aust. J. Anim. Sci. 19(10):1369-1378.

Read, K. M., K. M. Mendoza and C. W. Beattie. 2000. Comparative analysis of microsatellite sites in chicken and turkey. Genome. 43(5):796-802.

Sambrook, J., E. F. Fritch and T. Maniartis. 1998. In Molecular Clone: A laboratory manual (2nd ED). Beijing: Science Press. pp. 483-486.

Sano, A., N. Goto and M. Kimura. 1994. Genetic differentiation within commercial quail populations. Jpn. Poult. Sci. 31:276-286.

Su, Y., C. W. Liu, L. Liu, C. H. Ye, W. Q. Cao, Y. Q. Huang, J. Zheng, D. Y. Cai and O. Olowofeso. 2006. Studies on genetic variance of different Chinese duck populations with random amplified polymorphic DNA analysis. Asian-Aust. J. Anim. Sci. 19(4):475-481.

Tu, Y. J., K. W. Chen, S. J. Zhang, Q. P. Tang, Y. S. Gao and N. Yang. 2006. Genetic diversity of 14 indigenous grey goose breeds in China based on microsatellite markers. Asian-Aust. J. Anim. Sci. 19(1):1-6.

Wright, S. 1978. In: Variability within and among natural populations. Chicago: The Univ. of Chicago Press. United States. pp. 35-49.

Wu, X. S., G. H. Chen, D. Q. Wang, X. Y. Zhang, K. H. Wang, R. Cheng, B. Liu, Q. Xu and Q. L. Zhou. 2004. Analysis of genetic relationship among Chinese native chicken breeds using microsatellites marker. Acta Genetica Sinica. 31(1):43-50.

Zhang, X. Q., X. M. Lu, Y. H. Yang, J. S. Liu, G. F. Yang and X. H. Wu. 1998. Population genetic variability of microsatellite polymorphisms and RAPDs in Chinese chicken breeds in Guangdong. Acta Genetica Sinica. 25(2):112-119.