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Analysis of genetic diversity of four quails by microsatellite markers

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ABSTRACT

Genetic diversity of four quail populations, including Korean quails (maroon quails), Peking white quails, Chinese yellow quails and Chinese black quails, were analyzed by microsatellite markers, aiming to provide scientific basis for new breeds of Chinese black quails for egg production as well as the assessment, protection and utilization of Chinese quail's genetic resources. The results showed that 48 alleles were detected by nine microsatellite markers in the four quail populations, with the mean of 5.33 alleles in each locus. The average effective number of alleles marked by the nine microsatellite markers in Chinese black quails, Peking white quails, Chinese yellow quails and maroon quails were 3.5338, 3.6135, 4.0312 and 3.6508 respectively. The average heterozygosity of the four quail populations were 0.6952, 0.7046, 0.7353 and 0.7096 respectively. The average polymorphic information content of nine microsatellite loci in four quail populations were 0.6204, 0.6587, 0.6942 and 0.6639 , respectively, all of which were greater than 0.5, indicating the four populations' copious genetic diversity. In this study, the average genetic differentiation coefficient among populations was 0.0349, so the genetic variation among populations accounted for 3.49%, which demonstrated that genetic variation among populations was just a small proportion of the total population genetic variation, and there was little differentiation among the four populations. Cluster analysis indicated that Chinese black quails and Peking white quails were firstly clustered, and then Chinese yellow quails and maroon quails were clustered, and finally the two were clustered together.

Key words: Chinese black quails, Genetic distance, Genetic diversity, Microsatellite marker.

INTRODUCTION

As an important part of biodiversity, domestic animals' genetic diversity is a precious resource after a long-term evolution and also the treasure of world animal resources to develop, as well as the basis of studies on genetics and breeding of domestic animals. The genetic diversity of domestic animals (including fowls) refers to the variation of genetics and genotype frequency in domestic animals (fowls) species, including the genetic variation in different species or in different strains of the same species. A great number of alleles mean great possibility of traits in this species controlled by this locus, while the phenotype is diverse resistance and adaptability. Thus, there is still great possibility of species survival when the external environment changes suddenly. During the domestical animal production in recent decades, the pandemic due to strain consistency supports the fact that genetic diversity is decreasing, and species' adaptability to environmental changes is also reducing. Usually described by genetic polymorphism, genetic diversity is studied from the polymorphism of morphology, chromosome, blood protein and DNA. The mutation diversity of quail is also a potential biomedical resource and can be used to explain the pigmentation, morphology, nervous system and metabolic regulatory mechanisms (Pang, 2009).

It is urgent to protect existing varieties and to study the genetic diversity. With high polymorphism and codominant inheritance, microsatellite DNA can be used to analyze genetic variation degree and survival stability of population, evaluate genetic diversity, phylogenetic relationship and genetic differentiation among breeds. Korean quails, Peking white quails and Chinese yellow quails are the major breeds for egg production in China. They can form six autosexing strains applied to production. Peking white quails and Chinese yellow quails are the feather-color mutants of Korean quails, due to the recessive mutation of two chain loci in Z chromosome of Korean quails. The genetic relation of the three colors has been explained (Zhao *et.al*., 2008). Chinese black quails have a feather-color mutation newly discovered by our researchers, which is the hybrid of male Chinese yellow quails and female Korean quails. Hybrid authentication has initially proved that the color mutation is the result of incompletely recessive mutations of autosome (Yu *et.al*., 2009). This study selected nine microsatellite markers to detect the polymorphism of quails of four colors, aiming at investigating the genetic polymorphism of Chinese quails for egg production, and to provide scientific basis for the culture of new black quail breed for egg production as well as the assessment, protection and utilization of Chinese quail's genetic resources.

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MATERIALS AND METHODS

Sample collection: The quails to test came from the experimental farm of Henan University of Science and Technology. 100 Chinese black quail mutants were randomly selected, as well as 80 Peking white quails, 75 Chinese yellow quails and 75 Korean quails, total 330 quails. In Chinese black quails and Peking white quails, half are male and half female. In Chinese yellow quails and Korean quails, there were 40 males and 35 females. 2ml of heart blood was taken and ACD anticoagulant was used. Blood :ACD was 6:1. The blood sample was stored in refrigerator at -20°C. Genomic DNA was extracted with the blood tissue genomic DNA extraction kit (Tiangen, Beijing, China).

The selection and synthesis of primers: According to the local and oversea reports, we selected the microsatellite loci with at least four alleles, giving high polymorphism level. Finally, 9 pairs of microsatellite markers were determined to be the genetic markers of this study (Kayan *et.al*.,2006). Primer sequences were sent to Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. for synthesis, the microsatellite loci information was shown in Table 1.

PCR reaction condition: The total size of the PCR reaction system was 12 μL, including 8.15 μL of ddH_2O, 1.25 μL of 10× buffer, 0.75 μL of Mg^{2+} (25 mmol/L), 0.5 μL of DNA template, 0.5 μL (10 mmol/L) of upstream primers, 0.5 μL (10 mmol/L) of downstream primers, 0.25 μL of dNTPs, and 0.1μL of Taq enzyme. The PCR amplification process was as follows: pre-denaturation for 3 min at 95 °C, then 30 cycles of denaturation for 45 s at 94 °C, annealing for 60 s at 55-60 °C, extension for 60 s at 72 °C were followed, finally, extension for 12 min at 72 °C and preserving at 4 °C.

Polyacrylamide gel electrophoresis: On 8% non-denaturing polyacrylamide gels composition for distilled water for 6 ml, 50% acrylamide as 2 ml,5×TBE as 2 ml, 10% ammonium persulfate as 100ìLÿtetramethylethylenediamine (TEMED) for as 10μL03 μL of the PCR product was subjected to electrophoresis on 8% non-denaturing polyacrylamide gels for 2h under 120 V.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 conten(PIC), effective number of alleles (Ne), and expected heterozygosity (H) of each marker.

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

Where p_i was the frequency of ith allele of a microsatellite DNA

Effective number of allele (Ne) is the reciprocal of genetic homozygosity, which reflects the mutual influence among alleles, so it is used to detect population genetic variation.

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

Where p_i was the frequency of ith and jth allele of a microsatellite DNA

Polymorphic information content (PIC) is employed to assess whether the effective information of some locus is suitable for the linkage analysis probability and reflects the degree of genetic polymorphism of microsatellite locus.

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

Where p_i and p_j were the frequency of ith allele of a microsatellite DNA,and n is allele number.

Genetic differentiation coefficient: Genetic differentiation coefficient is an indicator evaluating the genetic differentiation in many loci among populations and the

TABLE 1: Information of nine microsatellite loci

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

function of whole population's average heterozygosity(H_t) and average sub-population heterozygosity (H_s).

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

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

Genetic polymorphism: As shown by Table 2, nine microsatellite markers produced an average allele number in Chinese black quails, Peking white quails, Chinese yellow quails and maroon quails of 5.1, 4.8, 5.3 and 5.0, respectively. There were a total of 48 alleles in the four quail populations detected by the nine microsatellite markers. GUJ0063 detected the least of three alleles in the four quail populations; GUJ0057 detected the most of seven alleles in the Chinese yellow quails. The average effective number of alleles of the nine microsatellite markers in the four quail populations ranged from 1.8574 to 5.8754, being 3.5338, 3.6135, 4.0312 and 3.6508 separately in Chinese black quails, Peking white quails, Chinese yellow quails and maroon quails.

The average heterozygosity of the nine microsatellite markers in Chinese black quails, Peking white quails, Chinese yellow quails and maroon quails was separately 0.6952, 0.7046, 0.7353 and 0.7096, indicating that these populations have high polymorphism, and Chinese yellow quails has the most copious genetic polymorphism, while Chinese black quails have the lowest one. The average PIC of the nine microsatellite markers in Chinese black

TABLE 2: Number of alleles locus, PIC and Heterozygosity over 9 microsatelliate

Populations	Locus name	Number of alleles locus(Na)	Effective number of alleles (Ne)	Polymorphism information content(PIC)	Heterozygosity(H)
Chinese black quails	GUJ0023	6	3.8760	0.701	0.7420
	GUJ0028	5	4.6555	0.5057	0.7852
	GUJ0029	6	3.7258	0.6992	0.7316
	GUJ0057	6	3.0544	0.6329	0.6726
	GUJ0059	5	3.2755	0.643	0.6947
	GUJ0063	3	1.8574	0.372	0.4616
	GUJ0077	6	4.4504	0.7392	0.7753
	GUJ0083	4	2.7255	0.5703	0.6331
	GUJ0097	5	4.1841	0.72	0.7610
	Mean	5.1	3.5338	0.6204	0.6952
Peking white quails	GUJ0023	6	4.6598	0.7519	0.7854
	GUJ0028	5	4.4603	0.7391	0.7758
	GUJ0029	6	4.2808	0.7331	0.7664
	GUJ0057	5	3.7161	0.6861	0.7309
	GUJ0059	4	2.8810	0.5978	0.6529
	GUJ0063	3	2.1030	0.4639	0.5245
	GUJ0077	5	4.3937	0.7364	0.7724
	GUJ0083	4	2.8137	0.573	0.6446
	GUJ0097	5	3.2134	0.6472	0.6888
	Mean	4. 8	3.6135	0.6587	0.7046
Chinese yellow Quails	GUJ0023	6	4.7348	0.7575	0.7888
	GUJ0028	5	3.9386	0.6999	0.7461
	GUJ0029	6	3.4710	0.6705	0.7119
	GUJ0057	7	5.2219	0.7814	0.8085
	GUJ0059	6	3.5613	0.6819	0.7192
	GUJ0063	3	2.3518	0.5098	0.5748
	GUJ0077	6	5.8754	0.806	0.8298
	GUJ0083	4	3.6430	0.6774	0.7255
	GUJ0097	5	3.4831	0.6633	0.7129
	Mean	5.3	4.0312	0.6942	0.7353
Korean quails(maroon quails)	GUJ0023	6	3.8760	0.7051	0.742
	GUJ0028	5	4.2123	0.723	0.7626
	GUJ0029	6	3.9968	0.7132	0.7498
	GUJ0057	4	2.7005	0.5678	0.6297
	GUJ0059	6	3.5224	0.6649	0.7161
	GUJ0063	3	2.0657	0.454	0.5159
	GUJ0077	6	4.1102	0.7193	0.7567
	GUJ0083	4	3.6140	0.6715	0.7233
	GUJ0097	5	4.7596	0.7561	0.7899
	Mean	5.0	3.6508	0.6639	0.7096

TABLE 3: The chisquare test for Hardy-weinberg in four quail population

Populations	Locus name	Chi-square value	df	$X^2_{0.01}$	Locus name	Chi-square value	df	$X^2_{0.01}$
Chinese black quails	GUJ0023	56.31	20	37.57	GUJ0077	270.47	20	37.57
Chinese yellow Quails		57.62	20	37.57		35.24	20	37.57
Peking white quails		33.81	20	37.57		67.59	14	29.14
Korean quails(maroon quails)		64.09	20	37.57		41.30	20	37.57
Chinese black quails	GUJ0028	89.99	14	29.14	GUJ0083	50.29	9	21.69
Chinese yellow Quails		80.08	14	29.14		139.67	9	21.69
Peking white quails		93.44	14	29.14		142.74	9	21.69
Korean quails(maroon quails)		108.66	14	29.14		139.53	9	21.69
Chinese black quails	GUJ0029	74.04	20	37.57	GUJ0097	96.13	14	29.14
Chinese yellow Quails		38.29	20	37.57		84.91	14	29.14
Peking white quails		28.34	20	37.57		73.84	14	29.14
Korean quails(maroon quails)		48.95	20	37.57		194.68	14	29.14
Chinese black quails	GUJ0059	47.03	14	29.14	GUJ0057	150.80	20	37.57
Chinese yellow Quails		67.66	20	37.57		67.58	27	46.96
Peking white quails		72.57	9	21.69		38.37	14	29.14
Korean quails(maroon quails)		95.10	20	37.57		74.60	9	21.69
Chinese black quails	GUJ0063	200.00	5	15.09				
Chinese yellow Quails		111.89	5	15.09				
Peking white quails		124.86	5	15.09				
Korean quails(maroon quails)		82.53	5	15.09				

quails, Peking white quails, Chinese yellow quails and maroon quails was separately 0.6204, 0.6587, 0.6942 and 0.6639, all of which were greater than 0.5, indicating the copious genetic diversity of four quail populations. The average polymorphism content of total quail population is 0.6593, showing that the population has copious genetic diversity.

Hardy-weinberg equilibrium detection: When a population is genetically studied, it should be tested by Hardy-weinberg equilibrium state to determine whether the population has the condition of genetic balance. This study employed Chi-square fit test, and the result was shown in Table 3. As shown by Table 3, only GUJ0077 in yellow population, and GUJ0023, GUJ0029 in white population meet Hardy-Weinberg law ($P>0.05$), other markers significantly deviate from the law($P<0.01$).

Genetic differentiation coefficient: Table 4 gave the four quail populations' genetic differentiation coefficient (G_{st}), total heterozygosity (H_t) and sub heterozygosity (H_s) by the nine microsatellite markers. GUJ0077 had the highest average heterozygosity in sub-population (0.7836) and GUJ0063 had the lowest (0.5192). In total population, GUJ0077 had the highest H_t (0.8090) and GUJ0063 had the lowest (0.5244). GUJ0059 had the largest genetic differentiation (0.0853) and GUJ0063 had the smallest (0.0099). The four sub populations' average heterozygosity was 0.7112, while the average total heterozygosity was 0.7376, and the population genetic differentiation coefficient was 0.0349, which manifested that the genetic variation among populations accounted for 3.49% of total genetic variation. It indicated that the genetic variation among population covered just a small proportion of total genetic variation and there was slight differentiation between the four quail populations.

TABLE 4: Total population average heterozygosity (H_t), Averrage heterozygosity within earth population (H_s) and confficient of gene differentiation (G_{st}) of 9 microsatellite loci

Locus name	Coefficient of gene differentiation	Total population average heterozygosity	Averrage heterozygosity within earth population
GUJ0023	0.0191	0.7795	0.7646
GUJ0028	0.0155	0.7795	0.7674
GUJ0029	0.0263	0.7599	0.7399
GUJ0057	0.0738	0.7670	0.7104
GUJ0059	0.0853	0.7606	0.6957
GUJ0063	0.0099	0.5244	0.5192
GUJ0077	0.0314	0.8090	0.7836
GUJ0083	0.0260	0.6998	0.6816
GUJ0097	0.0266	0.7584	0.7382
Mean	0.0349	0.7376	0.7112

Cluster analysis: As indicated by Table 5, the genetic distance between Chinese black quails and Peking white quails was the smallest (0.0717), indicating their close genetic relationship. The genetic distance between Chinese yellow quails and maroon quails was 0.0946, also showing a close genetic relationship. Chinese yellow quails and Peking white quails had the greatest distance of 0.1726. Figure 1 also demonstrated that Chinese black quails and

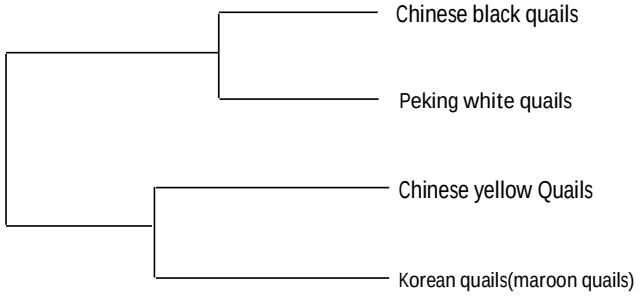

FIG 1: Dendrogram of quail populations

TABLE 5: Genetic distance of four quail populations

Populations	Chinese black quails	Chinese yellow Quails	Peking white quails	Korean quails(maroon quails)
Chinese black quails	—	0.8797	0.9308	0.8510
Chinese yellow Quails	0.1282	—	0.8415	0.9097
Peking white quails	0.0717	0.1726	—	0.8657
Korean quails(maroon quails)	0.1614	0.0946	0.1443	—

Note :Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Peking white quails were clustered into a group, while Chinese yellow quails and maroon quails were clustered into another group, and then the two clusters joined together.

Genetic heterozygosity: Genetic heterozygosity is also named as genetic diversity, indicating the proportion of heterozygous individuals in microsatellite loci, reflecting the genetic variation degree of microsatellite loci in domestic animal populations, so it is generally considered to be the optimal parameter to measure population genetic variation. The average heterozygosity can approximately reflect the degree of genetic variation. Higher heterozygosity means greater genetic diversity in populations and greater genetic variation. On the contrary, the genetic variation among populations is less. The population hyterozygosity calculated by microsatellite is usually within the range of 0.3 and 0.8. In this study, the nine microsatellite markers' average heterozygosity in the four quail populations ranges from 0.5192 to 0.7899, showing these markers are of high polymorphism. Their average hyterozygosity is separately 0.6952 (0.7046) 0.7353 and 0.7096, while the hyterozygosity of maroon population is close to the result of Meng *et.al.* (2007), (0.7111). Our study showed that the average hyterozygosity of four quail populations was higher than Olowofeso *et. Al.* (2006). (0.4627-0.6345), Amirinia *et.al.* (2007), (Japanese quails, 0.113–0.654), Farrag *et.al.* (Japanese quails, 0.636), Wang *et.al.* (2004) (Japanese quails, 0.5967) and Chang *et.al.* (2005) (Japanese quails, 0.662). The mean heterozygosity of the four quail populations was higher than that of Olowofeso *et.al.* (2006) (0.4627-0.6345), Amirinia *et.al.* (Japanese quails, 0.113 - 0.654), Farrag *et.al.* (2011) (Japanese quails, 0.636) , Wang *et.al.* (2004) (Japanese quail, 0.5967) and Chang *et.al.*(Japanese quail, 0.662). This study demonstrated that the four quail populations were of high polymorphism, and the Chinese yellow quails have the most copious genetic diversity.

Effective number of allele: Effective number of allele is the reciprocal of genetic homozygosity, reflecting the mutual influence among alleles in microsatellite loci. The effective number of allele is close to the detected allele's absolute number, showing the even distribution of alleles in populations(Hines,1981). Generally, due to the high frequency of some alleles and low frequency of some rare alleles in domestic animals, the effective number of allele is samller than the detected number in experiments. The detected allele number cannot but only the markers with the same frequency of alleles can completely reflect the populations' genetic variation. The effective number of alleles of the nine microsatellite markers in the whole quail population ranges from 4.2992 to 2.0799. The effective number of allele in this study was higher than that reported by Wang *et.al.* (2004) (Japanese quails, 2.8058).

Polymorphic information content: Polymorphic information content (PIC) refers to the possibility that a progeny acquires some allelic markers from its father or mother, which is used to describe the variation degree of microsatellite loci. PIC is ideal to measure allele polymorphism. When PIC is greater than 0.5, this locus is high polymorphic; 0.25<PIC<0.5 means the locus is medium polymorphic; PIC<0.25 means this locus is low polymorphic (Botstein *et al.*, 1980). The average heterozygosity of the nine microsatellite markers in the whole quail population is from 0.4499 to 0.7502; except that GUJ0063 is moderate polymorphic locus, the other eight microsatellite loci are all high polymorphic and can taken as effective genetic markers of quail population to analyze genetic diversity of population. In genetics, the microsatellite locus with PIC>0.7 is considered as the ideal selectable marker, because parent generation is usually heterozygous in this locus, and the separation of allele in its posterity can be distinctly observed. The selected nine microsatellite loci, GUJ0023, GUJ0028, GUJ0029 and GUJ0077 contain greater PIC than 0.7, so they can be the candidate genes for the further genetic analysis of quails. The average PIC of the nine microsatellite loci in the four quail populations are greater than 0.5(0.6204, 0.6587, 0.6942 and 0.6639), and the average PIC of maroon quail is close to the study result of Meng *et.al.*,(2007), In this study, the average polymorphism of four quail populations was higher than that of Olowofeso *et.al.* (2006) (0.3767-0.5713), Wang *et.al.* (2004) (Japanese quails,0.5445), Chang *et.al.* (2005) (Japanese quail, 0.5732), but lower than that of Amirinia *et.al.* (2007) (Japanese quails, 0.764).

The average PIC of quail populations and the study results of average population heterozygosity are almost consistent, manifesting the copious genetic diversity of the four quail populations. The average PIC of nine microsatellite markers in the whole quail population is 0.6593, showing the copious genetic diversity of the whole quail populations.

Genetic analysis among populations: G_{st}, H_t and H_s give an account of the genetic variation among populations, while H_s is the average heterozygosity of all populations, and G_{st} refers to the degree of genetic differentiation. The value of genetic differentiation coefficient of variation among populations varies from 0 to 1. When all alleles of the populations are almost the same, G_{st} is approximately 0, and there is almost no differentiation among populations; when the proportions of genetic diversity of the populations in H_t decreases, and the genetic differentiation among population increases, the genetic diversity exists between nearly all populations, and G_{st} is close to 1.

Our study achieves the differentiation coefficient of 0.0349, which is 0.016 lower than that obtained by Chang *et al*., (2005) (0.0365). Chang's study covers the relation between wild and domestic quails, so the differentiation degree must be higher than that of our four quail populations. Compared with Chang's populations studied, we conclude that the differentiation degrees of populations based on differentiation coefficient, and the results are consistent with actual situations, at least there is no conflict. Peking white quails and Chinese yellow quails come from Korean quails, being the consequences of its recessive mutation of B/b and Y/y loci, respectively. The two breeds were successively cultured in 1991 and 2002 separately, taken as male parent, with Korean quails, to be applied in production. Chinese black quails is newly discovered mutant, originating the hybrid of Korean quails and Chinese yellow quails which are separated and purified (Pang,2009). Studies prove that black quail is the recessive mutation of autosome. Regarding to the differentiation degree, white, yellow and Chinese black quails originate from Korean quails. Apart from the different genes, they must have the same background genotype. By means of analyzing genetic differentiation coefficient, this study demonstrates that the four quail populations are highly homologous. Further studies into the relation between differentiation coefficient and population differentiation are required.

CONCLUSIONS

In this four quail populations we studied, the total population genetic differentiation coefficient is 0.0349, meaning that the genetic variation between populations accounts for 3.49% of the total population genetic variation. It is a small proportion, which indicates that the variation largely comes from internal populations. Differentiation coefficient reflects the differentiation degree between groups, but it is just a relative index and only used for quantitative description. As differentiation coefficient is related to the selected locus number, the polymorphism of locus and the number of samples, there is no global quantitative criteria to measure differentiation degree by differentiation coefficient. As a result of the different numbers of selected microsatellite, loci and samples, it is hard to compare various study results.

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