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Microsatellite Polymorphism Analysis of Yang Yuan Donkey in China

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Abstract: To analysis the polymorphism of Yang Yuan donkey in China, the polymorphism of 12 microsatellites in 64 Yang Yuan donkeys were studied by PCR, polyacrylamide gel electrophoresis and silver staining. Number of alleles (Na), effective number of alleles (Ne), Polymorphism Information Content (PIC), observed Heterozygosity (Ho) and expected Heterozygosity (He) of each microsatellite were counted. And the Hardy-Weinberg equilibrium was tested as well.

Key words: Yang Yuan donkey, microsatellite, polymorphism, observed heterozygosity, expected heterozygosity

INTRODUCTION

Yangyuan donkeys, a Chinese local breed, presented the characteristics of strong adaptability, disease-resistant, roughage-resistance, easy propagation, short incubation period and other advatages. Mainly distributed in Yangyuan, Weixian, Xuanhua, Zhulu County and Huai'an county, Hebei Province in China with Yangyuan county and Weixian county are the most concentrated. Black, blue, gray, bronze are the most common colors. Adult body size: height, 135.8 cm; length, 136.5 cm; breast circumferences, 149.0 cm; cannon circumferences, 17.4 cm for male; 119.6, 120.6, 136.8 and 14.7 cm for female, respectively. Dressing percentage of 1.5-2.5 years old donkey: 56.05%. Meat percentage: 39.05%. Meat quality: lustrous slight red. Sexual maturity age: 1 years old. Mating age: male, 3 years old; female and 2 years old (Cheng and Xu, 2004). Due to excessive use the number of Yangyuan donkeys is gradually redused at present.

Microsatellite markers is a novel molecular markers which developed in the late 1980s. Because of its widely distributed, highly polymorphic, lower mutation rate, the simple method of the allele and genotype detection, the dominant maker and the versatility of the primers, it is widely used in the study of animal genetic diversity. In order to enrich the microsatellite database of Chinese local breed, researchers evaluated the genetic resources of Yangyuan donkeys at the molecular level by microsatellite molecular markers. The theoretical basis is provided for its protection, research, development and scientific utilization in the study.

MATERIALS AND METHODS

Specimen collection and DAN extraction: The 64 blood samples of Yangyuan donkeys were collected from Yangyuan, Xuanhua county of Hebei Province in China. DNA was isolated from the blood and extracted by phenol/chloroform mixture (Sambrook *et al*., 1989).

Microsatellite primers screening: According to Aranguren-Mendez *et al*. (2001), Jordana *et al*. (2001) and Locke *et al*. (2002), 12 microsatellite primers were screened and synthesized by Shanghai Biology Engineering Technology Ltd. The primer sequence and the condition of reaction is in Table 1.

PCR amplification: The PCR amplification was carried out in a total volume of 20 uL, 10×buffer 2.0 mL, dNTPs 1.2 uL, mix Primer 2 uL, Taq DNA polymerase 0.2 uL, template DNA 2 uL and12.6 uL sterilization distilled water. PCR was performed with the following procedure, 95°C for 5 min followed by 32 cycles of 30 sec at 94°C, 30 sec at annealing temperature 55~66°C, 30 sec at 72°C and a final extension of 10 min at 72°C.

Electrophoresis of the PCR products: The PCR products, first were tested in 1.5% agarose gel electrophoresis and then in 8-12% polyacrylamide gel electrophoresis, take the photos with BIO-CAPT200E Acquisition Software after silver stain andanalysis microsatellite alleles size to determine the genetic type by BIO-CAPT200E System with software.

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Table 1: Information on the 12 pairs microsatellite loci selected in this study

Locus	Primer sequence (5'→3')	Annealing temperature (°C)
HMS3	CCAACTCTTTGTCACATAACAAGA CCATCCTCACTTTTTCACTTTGTT	58
HMS2	CTTGCAGTCGAATGTTTAAATG ACGGTGGCAACTGCCAAGGAAG	58
HMS6	GAAGCTGCCAGTATTCAACCATTG CTCCATCTTGTGAAGTGTAACTCA	61
HMS7	CAGGAAACTCATGTTGATACCATC TGTTGTTGAAACATACCTTGACTGT	61
HTG7	CCTGAAGCAGAACATCCCTCCTTG ATAAAGTGTCTGGGCAGAGCTGCT	55
AHT4	AACCGCCTGAGCAAGGAAGT GCTCCCAGAGAGTTTACCCT	66
HTG6	CCTGCTTGGAGGCTGTGATAAGAT GTTCACTGAATGTCAAATTCTGCT	55
HTG10	CAATTCCCGCCCCACCCCCGGCA TTTTTATTCTGATCTGTCACATTT	55
HMS5	TAGTGTATCCGTCAGAGTTCAAA GCAAGGAAGTCAGACTCCTGGA	60
VHL20	CAAGTCCTCTTACTTGAAGACTAG AACTCAGGGAGAATCTTCCTCAG	63
HTG15	TCTTGATGGCAGAGCCAGGATTTG AATGTCACCATGCGGCACATGACT	61
COR071	CTTGGGCTACAACAGGGAATA CTGCTATTTCAAACACTTGGA	58

Statistical analysis: The population genetic parameters including number of alleles, effective number of alleles, expected heterozygosity and observed heterozygosity were calculated by Statistical Software POPGENE32 (Yeh *et al*., 1999), Hardy-Weinberg equilibrium was tested by the POPGENE32. Polymorphic Information Content (PIC) values were calculated by the PIC_Calc 0.6 (http://www.bbioo.com/Soft/2007/983.htm).

RESULTS AND DISCUSSION

Assessment of genetic diversity on alleles at different microsatellite loci: The microsatellite loci with four alleles at least can be applied to the assessment of genetic diversity according to microsatellite selection criteria supported by the draft of genetic distances among global livestock breeds. A total of 82 alleles were detected and the genetic diversity on the 12 loci can be done in the study as is shown in Table 2. From the evolutionary perspective, the compositions of alleles and the genetic diversity within populations were derived from a long-term evolution (Meyer *et al*., 1997). The interaction between alleles is indicated by the fact that the effective number of alleles within populations is less than the actual. And the effective number of alleles is closer to the absolute value of detected alleles if the distribution of alleles within populations is more evenly. The observed alleles number varied from 4-13 with an average of 6.8333. While effective alleles number varied from 1.9263-6.5852 with an average of 3.6959.

Table 2: Assessment of genetic diversity on 12 loci of Yangyuan donkey

						Hardy-Weinberg equilibrium	
Locus	Na	Ne	PIC	Ho	He	χ^2	p-value
HMS-2	13.0000	6.5852	0.8306	0.4688	0.8616	170.47	0.00
HMS-3	6.0000	1.9733	0.4490	0.1290	0.5013	112.08	0.00
HMS-6	4.0000	3.4609	0.6576	0.0000	0.7235	96.15	0.00
HMS-7	4.0000	2.0750	0.4582	0.0312	0.5263	71.67	0.00
HTG-7	8.0000	4.4620	0.7492	0.0385	0.7911	224.92	0.00
AHT-4	8.0000	4.4169	0.7410	0.2857	0.7877	118.55	0.00
HTG-6	6.0000	3.6046	0.6725	0.4643	0.7357	82.52	0.00
HTG10	8.0000	3.7245	0.6961	0.5000	0.7448	40.39	0.06
COR-71	7.0000	3.8151	0.7052	0.3571	0.7513	80.39	0.00
HMS-5	5.0000	1.9263	0.4086	0.1786	0.4896	71.64	0.00
VHL-20	7.0000	5.2441	0.7855	0.5357	0.8240	98.77	0.00
HTG-15	6.0000	3.0625	0.6177	0.5000	0.6857	51.80	0.00
Mean	6.8333	3.6959	0.6476	0.2907	0.7019	-	-

χ^2-test for Hardy-Weinberg equilibrium

Assessment of genetic diversity in Yangyuan donkey: PIC is a index for measuring the extent of gene polymorphisms proposed by Botstein *et al*. (1980) showing highly, medium or lowly polymorphic with a threshold of PIC>0.5, 0.25<PIC<0.5 or PIC<0.25, respectively. The 12 loci of Yangyuan donkey were all high genetic diversity, for the average PIC values is higher than 0.5 as is shown in Table 2. The observed Heterozygosity (Ho) is the measurement units of heterozygosity within populations and the detected genetic marker diversity. And it represents the frequency of heterozygous in the detected loci within populations. Generally speaking, the populations had not been highly selected and were all abundant polymorphic if their heterozygosity were higher than 0.5. The expected Heterozygosity (He) is the probability of heterozygous gene with two copies in the loci which drawn from a gene bank. The average Ho was 0.2907 in the study. Among the 12 loci, the difference between the HMS6 and VHL20, HTG10, HTG15 were significant. The observed heterozygosity of HMS6 was 0 while VHL20, HTG10 and HTG15 were not lower than 0.5 andthe average. He was 0.7019, this shows that the HMS6 was homozygous in samples. The heterozygosity of Yangyuan donkey was low and its genetic variation is affected by some factors based on the study.

The polymorphic information content and heterozygosity are both the target of genetic variation within populations. If PIC and H values were all higher, the genetic consistency was worse. Similarly, the genetic variation and the selective potential were greater. The average polymorphic information of 12 loci in Yangyuan donkey were highly polymorphic while the average observed heterozygosity was low just 0.2907. The results showed that the gene polymorphisms information of Yangyuan donkey were rich but the homozygosity of gene were greater. Researchers speculate that the

Yangyuan donkey were selected by unnatural factors based on this phenomenon. Among the 12 loci, only HTG10 was in Hardy-Weinberg equilibrium. The reason for disequilibrium of Hardy-Weinberg may be the gradual decrease in the population size especially the male livestock. It was related with the high levels inbreeding which was unfavourable for protecting the Yangyuan donkey.

Yangyuan donkey, a Chinese native breed with rich gene polymorphisms has not been selected systematically. In order to protect this valuable species, the highly homozygous gene should be avoided at present. Because it may cause the loss of beneficial genes resulting in inbreeding depression.

CONCLUSION

The results showed that the average Na, Ne, PIC, Ho and He of 12 microsatellites in Yang Yuan donkey were 6.8333, 3.6958, 0.6476, 0.2907 and 0.7019, respectively which indicated that the genetic diversity of Yang Yuan donkey were highly polymorphic while the observed heterozygosity was low. All microsatellite loci except for the HTG10 were in unbalanced based on the Hardy-Weinberg equilibrium test. So, should protect this valuable species.

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