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Study on Gennetic Diversity of 7 Rabbit Populations Evidenced by Microsatellite Makers

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Abstract: The genetic diversity and phylogenetic relationship of 7 China rabbit populations were investigated with 15 microsatellite. The results showed that: The value of the average expected heterozygosity (He range from 0.820±0.012-0.675±0.031) Polymorphic Information Content (PIC range from 0.796±0.016-0.625±0.033) and the mean effective number of alleles (Ne = 6.625±0.498) of the seven rabbit populations were high, which indicated that polymorphisms and genetic diversity of genes were abundant. The range of F_{ST} for the whole population was from 0.041 (6L3F8) -0.195 (Sat8). The mean F_{ST} was 0.099±0.010; the average of total inbreeding coeffient (F_{IT}) was -0.004±0.052; the mean inbreeding coeffient among populations (F_{IS}) was -0.114±0.050. The dendrogram by Unweighed Pair-Group Method with Arithmetic averaging (UPGMA) based on Nei's genetic distance and Reynolds' genetic distance was similar. Seven populations were clustered into 4 groups. The Germany Angora Rabbit, American Rex Rabbit and Wan-line Angora Rabbit belonged to the first group; the New Zealand White Rabbit and Zika Rabbit were included in the second group; the Fujian Yellow Rabbit and Fujian Black Rabbit were clustered separately. The results suggested that the 15 microsatellite loci were effective markers for analysis of genetic relationships among rabbit populations.

Key words: Genetic diversity, microsatellite markers, Fujian yellow rabbit, Fujian black rabbit, wan-line angora rabbit, phylogenetic tree

INTRODUCTION

Rabbit raising has been a long history in China. Over the years, systematical rabbit breeding studies has been carried out and some achievements were obtained. Since 1950's, many kinds of rabbit varieties were introduced from overseas. For instance, the Angora rabbit, a kind of wool rabbit was introduced successively from abroad (such as England, Germany, Japan and Hungary). The German and French strains have contributed to Chinese wool rabbit breeding. Many meat rabbit varieties (Japan Big Checkered Rabbit, Chinchilla Rabbit, French Lop Rabbit, German Checkered Rabbit, Flemish, New Zealand Rabbit, Californian Rabbit and Danish White Rabbit) were also introduced. Fur rabbit (Mainly Rex Rabbit) was introduced from US, Germany and France. These foreign strains made tremendous contributions to the development of Chinese Rex Rabbit breeding. China has almost all kinds of world rabbit varieties with outstanding genes (Zi-Lin *et al*., 2008).

Wan-line Angora Rabbit, which belongs to coarse wool type angora rabbit was bred by Germany Angora Rabbit and New Zealand White Rabbit by direct cross, backcross, grading, transversely breed and strict system of selection made. A formal appraisal was passed on October 1991 and was considered as the first coarse wool type Angora rabbit strain in China (Zhao *et al*., 1996).

Documentation of existing genetic resources including the description of the population phenotypic characteristics, performance, cultural importance and genetic uniqueness is one of the main areas of the livestock conservation activities. Description of genetic diversity can also inform on further sustainable intensification of animal production (Traore *et al*., 2009). Among all the types of molecular markers, the microsatellite loci is used most widely on the analysis of genetic diversity and population structure of livestock. Although, few study has been carried out in China rabbit populations, it is known that they present adaptation traits that could be useful in breeding. The objective of this research is to evaluate the actual genetic diversity and the population structure of 7 rabbit populations sampled in China with 15 microsatellite loci to provide a theoretical reference for rabbit breeding in China.

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MATERIALS AND METHODS

Populations and microsatellite primers: Four hundred and forty five induviduals were randomly seleted in China: 31 New Zealand White Rabbit, 30 Zika Rabbit, 77 Germany Angora Rabbit, 79 Wan-line Angora Rabbit, 128 American Rex Rabbit, 40 Fujian Yellow Rabbit from Jinling rabbit farm (Nanjing, China) 60 Fujian Black Rabbit from the rabbit farm of the institute of Animal Science and Veterinary, Fujian Academy of agricultural Science (Fujian, China).

Approximately 5 mL blood was collected from each individual from the central artery vein of the ear into tubes containing 1 mL ACD (Citric acid, Sodium citrate, Dextrose) as anticoagulant and was then preserved in a -20°C freezer.

Fifteen microsatellite primers were designed based on the report of Xin-Sheng *et al.* (2008a) and were synthesized by the Shenggong biological engineering technology company, the information of primers are shown in Table 1.

Genomic DNA extraction and PCR condition: Genomic DNA were extracted according to the Molecular Cloning-A Laboratory Manual (Joseph and David, 2002).

PCR amplification was carried out in 20 μL of a mixture containing 1 μL DNA template (100 ng μL^{-1}) 2 μL 10x PCR Buffer, 1.5 μL dNTP (10 m mol L^{-1}) 1 μL of each primer (10 p mol μL^{-1}) and 0.2 μL Taq DNA polymerase (5 U μL^{-1}). Double-distilled water was added to a final volume of 20 μL. After a denaturing step of 5 min at 94°C, samples were processed through 35 cycles of 40 sec at 94°C, 40 sec at an optimal annealing temperature and 40 sec at 72°C. Then the last elongation step was at 72°C for 10 min.

Agarose gel (0.6%) was used to analyze Genomic DNA and PCR products were visualized by polyacrylamide gel (10%). Analysis of the segmental length of the genes were conducted by ONEDSCAN software.

Statistical method: Allele frequencies were computed by formula as:

$$Pi = (2\,(ii)+(ij_1)+(ij_2)+\ldots+(ij_n))/2N$$

Where:

P_i = The frequency of the ith allele
I = The allele
$j_1, j_2, \ldots j_n$ = The co-dominant alleles to I
n = The total number of the alleles

Polymorphic Information Content (PIC) was calculated by the following formula (Botstein *et al.*, 1980):

Table 1: Microsatellite primer sequences and annealing temperature

Locus	Primer sequence	Allele size (bp)	Annealing Temp. (°C)
5L1E8	CCAGCTGGTAATAGTAGAGA		
	AAGGCATTTGTGGAGTGAA	206-244	53
6L3F8	CTCCTGCCCTGTTCTAT		
	CAGGCTGGTCTTATTAC	114-158	53
7L1B10	TTGGCAGGAAGAAAAGGAAGATT		
	TTTGTCATAAGCATTTGGGAAGTG	175-241	56
12L1E11	AGTGGTAGCGCTTTGGTCTG		
	GCTCCTTGGGGCATTTG	234-294	57
12L4A1	GCTAATTACCCAAAGGAACATACA		
	CAGTGCAAATTTGGAAGGTCT	154-210	56
12L5A6	GGTGTGAACCACTAGATAGAA		
	CAAAATTAGGTCCCTTGTAGT	301-359	53
19L1G5	AGTTGCTCCCACCCGATTTTA		
	TGCTGTTGGGAGTAGATTGACC	142-200	58
D3Utr2	AGGAAGTGAGGGGAGGTGTT		
	ATAATGTGCTGCCAAAATAGAAAT	388-434	55
D6Utr4	CAGAAGGGCATTTGTTTTG		
	GGTGATTCTTTCTTCTGCCTCTTA	190-238	55
D7Utr5	ACACCTGGGGAATAAACAACAAG		
	GAGGGAGGCAGAGGGATAAGA	131-163	58
Sat5	GCTTCTGGCTTCAACCTGAC		
	CTTAGGGTGCAGAATTATAAGAG	231-263	56
Sat8	CAGACCCGCAGTTGCAGAG		
	GGGAGAGAGGGATGGAGGTATG	133-339	60
Sol30	CCCGAGCCCCAGATATTGTTACCA		
	TGCAGCTTCATAGTCTCAGGTC	161-181	60
Sol33	GAAGGCTCTGAGATCTAGAT		
	GGGCCAATAGGTACTGATCCATGT	191-269	55
Sol44	GGCCCTAGTCTGACTCTGATTG		
	GGTGGGGCGGCGGGTCTGAAAC	194-262	58

$$PIC = 1 - \sum_{i=1}^{m} P^2_i - \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} 2P^2_i P^2_j$$

$$= 2\sum_{i=1}^{m-1} \sum_{j=i+1}^{m} P_i P_j (1 - P_i P_j)$$

The formulas of expect Heterozygosity (He) and effective Number of alleles (Ne) are:

$$He = 1 - \sum_{i=1}^{m} P^2_i$$

$$Ne = \sum_{i=1}^{m} P^2_i$$

Where:

m = The number of the allele
P_i and P_j = The frequencies of the ith and the jth allele

The F-statistic was calculated by the followed:

$$1-F_{ST} = (1-F_{IS}) \times (1-F_{IT})$$

Where:

F_{IT} = The fixation indices of individuals relatived to its subpopulations
F_{IS} = The fixation indices of individuals relatived to the total population
F_{ST} = The fixation indices of subpopulation relatived to the total population

Reynolds' genetic distance (Dr) was calculated by the followed formula:

$$Dr = -\ln(1-F_{ST})$$

The value of gene flow (Nm) was calculated by the followed formula:

$$Nm = (1- F_{ST})/(4\times F_{ST})$$

The dendrogram between different populations based on the Nei *et al*. (1983)'s genetic distance and Reynolds' genetic distance were estimated by Unweighed Pair-Group Method with Arithmetic Averaging (UPGMA) by PHYLIP 3.0 software.

RESULTS AND DISCUSSION

Figure 1 shows the agarose gel (0.6%) of Genomic DNA, Fig. 2 shows one of the Polyacrlamide Gels (PAGE) of PCR products. The total number of alleles in 15 microsatellite loci of seven populations was 151.

The effective Number of alleles (Ne) and the Number of alleles (Na): The effective number of alleles is also an index used to reveal the genetic diversity of the populations. The results for the effective number of alleles were showed in Table 2. The value of 15 microsatellite loci varied from 2.860 (Sat8)-9.920 (sol44) and the mean was 6.625±0.498, the average value of Na was 10.067±0.720.

The expected Heterozygosty (He) and Polymorphism Information Content (PIC): As shown in Table 2, the microsatellite D6Utr4 in the Rex Rabbit population showed the highest expected heterozygosty (He = 0.889) while the locus Sat5 in Fujian Yellow Rabbit population had the lowest He (He = 0.161). The average He of all loci and populations was range from 0.675±0.031 in Fujian Black Rabbit population to 0.820±0.012 in American Rex Rabbit population.

The Polymorphism Information Content (PIC) was summarized in Table 3. PIC was the highest in Rex Rabbit population (0.796±0.016) and the lowest in Fujian Black Rabbit population (0.625±0.033).

F-statistic analysis: The F-statistic was used to test the genetic differentiation among subpopulations. The F-statistics were calculated for 15 microsatellite locus in this study, the results are shown in Table 4. The range of F_{ST} was from 0.041 (6L3F8)- 0.195 (Sat8). The means of F_{ST}, F_{IT} and F_{IS} were 0.099±0.010, -0.004±0.052 and -0.114±0.050, respectively.

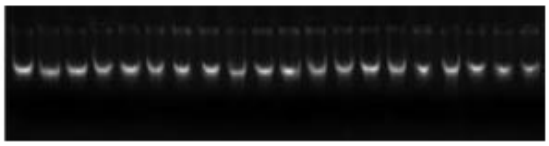

Fig. 1: Genomic DNA visualized by agarose gel (0.6%)

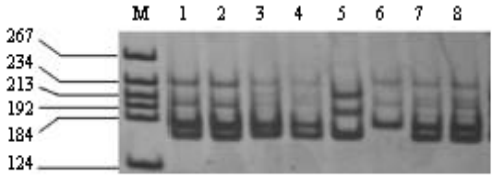

Fig. 2: The result of microsatellite Sol30 visualized by un-denatured PAGE, 1, 2, 3, 4, 7and 8 (177/161) 5(161/161) 6 (177/177) M (markerpBR322 DNA/ BsuRI)

Dr genetic distances and Nm among the 7 rabbit populations: The Reynolds' genetic distance (Dr) and Nm was calculated by F_{ST} and the results are summarized in Table 5. Genetic distance between Germany Angora Rabbit and American Rex Rabbit was the nearest (0.041) while the genetic distance between Fujian Yellow Rabbit and Fujian Black Rabbit was the farthest (0.267). The highest value of gene flow (Nm) was 6.031 (between Germany Angora Rabbit and American Rex Rabbit) and the lowest was 0.818 (between Fujian Yellow Rabbit and Fujian Black Rabbit).

Phylogenetic tree: The dendrogram between different populations based on the Nei *et al*. (1983)'s genetic distance similar to which based on Reynolds' genetic distance were estimated by Unweighed Pair-Group Method with Arithmetic Averaging (UPGMA) with PHYLIP 3.0 software, respectively. As shown in Fig. 3 and 4 the 7 populations were clustered into 4 groups. The Germany Angora Rabbit, American Rex Rabbit and Wan-line Angora Rabbit were belong to the first group; the New Zealand White Rabbit and Zika Rabbit were included in the second group; the Fujian Yellow Rabbit and Fujian Black Rabbit were clustered separately.

Amongst all the mammalian species used for animal production in China, though the rabbit populations are probably the smallest populations at present. Few study on the analysis of genetic diversity and population structure of has been carried out in China rabbit populations (Xin-Sheng *et al*., 2008b) by microsatellite markers, while they were used most widely in other livestock (Ting-Long *et al*., 2009; Mukesh *et al*., 2009; Lacis *et al*., 2009; Sollero *et al*., 2009; Wu *et al*., 2009). In this study, the genetic diversity and

Table 2: The expected Heterozygosty (He), effective Number of alleles (Ne) and Number of alleles (Na) of 15 microsatellite loci in 7 rabbit populations

Locus	N	Z	FY	FB	A	W	R	Ne	Na
5L1E8	0.839	0.674	0.741	0.719	0.705	0.787	0.813	5.749	9
6L3F8	0.825	0.741	0.831	0.723	0.779	0.791	0.814	5.574	9
7L1B10	0.836	0.672	0.697	0.741	0.792	0.754	0.778	5.181	15
12L1E11	0.796	0.669	0.757	0.572	0.821	0.819	0.858	9.077	13
12L4A1	0.794	0.823	0.752	0.737	0.825	0.833	0.841	7.995	10
12L5A6	0.765	0.766	0.800	0.636	0.811	0.777	0.803	5.756	9
19L1G5	0.837	0.858	0.813	0.757	0.844	0.803	0.875	9.223	13
D3Utr2	0.752	0.774	0.691	0.743	0.786	0.805	0.804	5.963	7
D6Utr4	0.812	0.825	0.789	0.805	0.866	0.773	0.889	8.599	11
D7Utr5	0.754	0.789	0.840	0.446	0.764	0.574	0.779	5.444	8
Sat5	0.789	0.788	0.161	0.789	0.837	0.751	0.837	6.696	8
Sat8	0.367	0.465	0.495	0.406	0.457	0.580	0.694	2.860	8
Sol30	0.736	0.771	0.492	0.603	0.791	0.730	0.820	5.415	6
Sol33	0.767	0.811	0.755	0.714	0.832	0.688	0.819	5.922	10
Sol44	0.790	0.836	0.650	0.741	0.847	0.776	0.879	9.920	15
Mean	0.764	0.751	0.684	0.675	0.784	0.749	0.820	6.625	10.067
(SE)	(0.029)	(0.026)	(0.046)	(0.031)	(0.026)	(0.020)	(0.012)	(0.498)	(0.720)

N: New Zealand white rabbit; Z: Zika rabbit; FY: Fujian Yellow rabbit; FB: Fujian Black rabbit; A: Germany Angora rabbit; W: Wan-line Angora rabbit; R: American Rex rabbit

Table 3: The Polymorphism Information Content (PIC) of 15 microsatellite loci in 7 rabbit populations

Locus	N	Z	FY	FB	A	W	R
5L1E8	0.819	0.619	0.705	0.681	0.652	0.752	0.790
6L3F8	0.801	0.707	0.809	0.677	0.749	0.761	0.788
7L1B10	0.816	0.626	0.649	0.697	0.771	0.719	0.746
12L1E11	0.767	0.610	0.732	0.484	0.798	0.793	0.842
12L4A1	0.772	0.802	0.717	0.694	0.802	0.811	0.821
12L5A6	0.725	0.729	0.772	0.565	0.784	0.743	0.774
19L1G5	0.817	0.842	0.790	0.716	0.826	0.775	0.862
D3Utr2	0.709	0.739	0.643	0.697	0.755	0.777	0.779
D6Utr4	0.788	0.802	0.760	0.776	0.852	0.743	0.878
D7Utr5	0.721	0.761	0.819	0.393	0.731	0.494	0.746
Sat5	0.755	0.754	0.148	0.757	0.815	0.706	0.817
Sat8	0.300	0.357	0.373	0.358	0.406	0.514	0.634
Sol30	0.698	0.737	0.371	0.525	0.761	0.681	0.795
Sol33	0.730	0.785	0.719	0.662	0.810	0.630	0.794
Sol44	0.760	0.814	0.585	0.698	0.831	0.741	0.867
Mean	0.732	0.712	0.639	0.625	0.756	0.709	0.796
(SE)	(0.033)	(0.031)	(0.050)	(0.033)	(0.028)	(0.024)	(0.016)

Table 4: F-value for all locus

Locus	F_{IT}	F_{ST}	F_{IS}
5L1E8	0.125	0.084	0.045
6L3F8	-0.179	0.041	-0.229
7L1B10	0.021	0.062	-0.044
12L1E11	0.048	0.141	-0.107
12L4A1	-0.122	0.083	-0.224
12L5A6	-0.191	0.074	-0.286
19L1G5	-0.106	0.075	-0.195
D3Utr2	-0.179	0.074	-0.274
D6Utr4	-0.118	0.062	-0.192
D7Utr5	0.369	0.161	0.247
Sat5	-0.060	0.139	-0.231
Sat8	0.277	0.195	0.103
Sol30	0.427	0.114	0.354
Sol33	-0.186	0.078	-0.286
Sol44	-0.088	0.117	-0.232
Mean	-0.004	0.099	-0.114
(SE)	(0.052)	(0.010)	(0.050)

phylogenetic relationships of 7 rabbit populations in China were investigated with microsatellite markers. We hope this research could provide a theoretical reference for rabbit breeding in China.

Table 5: Dr genetic distances and Nm among the 7 rabbit populations. The upper-triangular data matrix was the Nm and the lower-triangular data matrix was the Dr genetic distance

Population	N	Z	FY	FB	A	W	R
N		3.578	1.320	1.755	3.421	2.528	3.917
Z	0.068		1.447	1.375	4.287	1.976	4.485
FY	0.173	0.159		0.818	1.235	1.127	1.666
FB	0.133	0.167	0.267		1.708	1.856	1.849
A	0.071	0.057	0.184	0.137		3.910	6.031
W	0.094	0.119	0.200	0.126	0.062		4.053
R	0.062	0.054	0.140	0.127	0.041	0.060	

Within population diversity: Frankham *et al.* (2002) pointed out that the average number of alleles per locus also known as the allele diversity, is an important parameter of genetic diversity. The effective number of alleles can also be used as an indicator of genetic variation. In this study, the mean number of alleles was 10.067±0.072; the average effective number of alleles was 6.625±0.498, indicating that the gene polymorphisms and genetic diversity were abundant. There were Significant difference between the effective number of alleles and

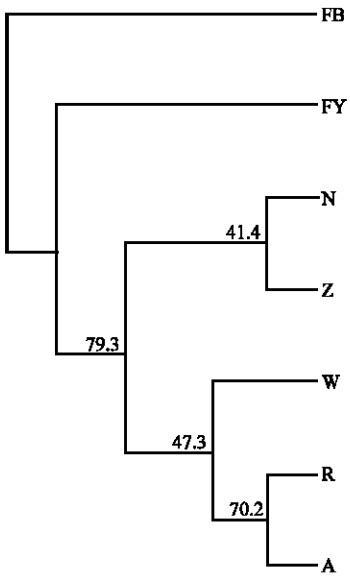

Fig. 3: The dendrogram based on the Nei *et al.* (1983)'s genetic distance were estimated by UPGMA, numbers at the nodes are percentage bootstrap values from 1000 replications with resampled loci

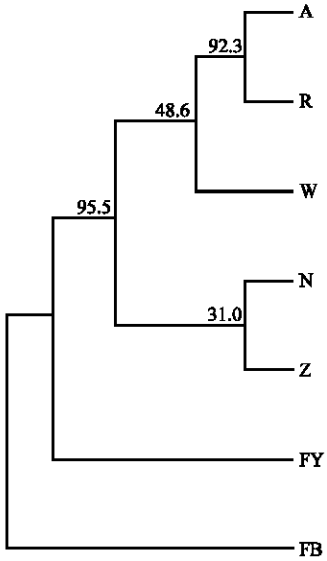

Fig. 4: The dendrogram based on the Reynolds' genetic distance were estimated by UPGMA, numbers at the nodes are percentage bootstrap values from 1000 replications with resampled loci

the number of alleles at some locus (7L1B10, Sat8) because some alleles were not present in all populations.

Expect heterozygosity is one of the indices used to assay the genetic variation of each population. The values of He indicate the diversity level of the molecular marker. When the value is high, the molecular marker's diversity is high too. Among all loci studied, the numbers of He at the locus Sat8 were lower in all populations universally, the highest value was 0.889 at the D6Utr4 locus in American Rex Rabbit population, while the locus Sat5 had the lowest He value (0.161) in Fujian Yellow Rabbit population. In all populations, the He of American Rex Rabbit population was the highest (0.820±0.012) followed by Germany Angora Rabbit population (0.784±0.026) and Fujian Black Rabbit population was the lowest (0. 675±0.031).

The PIC was a good index for genetic diversity evaluation. Botstein *et al.* (1980) first reported that PIC index can be used to evaluate the level of gene variation, when PIC>0.5, the locus was of high diversity; when PIC<0.25, the locus was of low diversity and the locus was of intermediate diversity, when PIC between 0.25 and 0.5. In this study, the mean PIC of each population all showed high diversity (range from 0.625±0.033-0.796±0.016). The data indicated that genetic diversity of all rabbit populations in the study was high. This may be related to the breeding history and environment of each population.

Between population divergences: The F-statistic was used in testing the genetic differentiation among subpopulations. The F_{IS} and F_{IT} may range from -1 to 1, however, the F_{ST} values were always positive. The mean of F_{IT} and F_{IS} were -0.004±0.052 and -0.114±0.050, respectively, which indicated that heterozygotes were abundant in 7 rabbits populations, gene exchange among populations frequently (Wright, 1978) pointed out that that: if the F_{ST} value of the groups between 0 and 0.05, suggested that differentiation did not exist in subgroup; moderate differentiation if the value of F_{ST} between 0.05 and 0.15; a high degree of differentiation if F_{ST} value was range from 0.15-0.25. In this study, the average F_{ST} value was 0.099±0.010 revealling that moderately differentiated in all populations, 9.9% genetic variation between the populations and 90.1% within the populations.

The dendrogram based on the Nei *et al.* (1983)'s genetic distance is similar to that based on Reynolds' genetic distance. The seven populations were clustered into four groups. The Germany Angora Rabbit, American Rex Rabbit and Wan-line Angora Rabbit belonged to the first group, the Germany Angora Rabbit and Wan-line Angora Rabbit were wool type rabbit, Wan-line Angora Rabbit was bred by Germany angora rabbit and New Zealand white rabbit, but American Rex Rabbit belongs to pur type rabbit was included in the same group, the reason could be that the microsatellite loci in the study were not linked with the gene that controlled the

economic character; the New Zealand White Rabbit and Zika Rabbit were included in the second group, Zika Rabbit was bred by Germany Rabbit Breeding Center and Munich University, introduced the bloodvine of New Zealand White Rabbit into its origin was well-known as one of meat rabbit strains in the world, thus, it is reasonable that they were included in the same group the Fujian Yellow Rabbit and Fujian Black Rabbit were clustered separately, they are both local varieties in Fujian province of China, the genetic distance between them and other populations were farther.

CONCLUSION

All populations in the study showed affluent genetic diversity, the result of Phylogenetic Tree is both consistent with its cultivation process and the genetic distance, which suggested that microsatellite still can be used as a tool to understand the genetic variability and phylogenetic relationship among rabbit breeds, but more number of samples and various sets of primers are required for further study on the genetic relationship in rabbit breeds.

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