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PROCEEDINGS

Genetics – Short papers

GENETIC VARIATION WITHIN AND AMONG FIVE RABBIT POPULATIONS USING MICROSATELLITE MARKERS

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ABSTRACT

Five microsatellite loci (*Sat3, Sat4, Sat7, Sat8 and Sat12*) were used to analyze the genetic variation among 5 breeds or strains of domestic rabbit populations (Vc-I Rex rabbit strain (Vc-I), Vc-II Rex rabbit strain(Vc-II), New Zealand White rabbit breed (NZW), Qingzilan rabbit breed (QZL), Japanese White rabbit breed (JAW)). Five microsatellite loci exhibited a total of 20 alleles from the 5 rabbit populations. One private allele was found at Vc Rex population by *Sat4* and *Sat8*, respectively. PIC and heterozygosity had the same tendencies among 5 rabbit populations, and the Vc-II population had the highest value (0.5526, 0.6202), followed by Vc-I population (0.5240, 0.5906), JAW population (0.4932, 0.5374), QZL population (0.4626, 0.5347), and NZW population (0.4515, 0.5261), which suggested that genetic variation was greater in Vc Rex population than in the other three rabbit populations. The result of cluster displayed that the population whose relationship was nearest to Vc-I Rex rabbit population was Vc-II Rex rabbit population, followed by Japanese white rabbit population , QZL rabbit population, and New Zealand White rabbit population was the furthest one to Vc-I Rex rabbit population in relationship, which conformed to the fact of the Vc Rex rabbit breeding history.

Key words: rabbit, microsatellite, genetic, variation.

INTRODUCTION

Vc Rex rabbit is a new rabbit breed raised by the Quartermaster University of PLA of China since 1990. It has many desirable characters such as big body, large skin, fine fur, being adapted to the climate of the northeast areas of China. Genetic variation should be taken into account to guide genetic conservation programs, while highly polymorphic microsatellite DNA marker provide a good tool to assess the genetic variation (TAUTZ & RENZ, 1984, ESTOUP *et al*.,1993, WEBER & WRONG, 1993). So using microsatellite markers to analyse the genetic variation is critical for the Vc Rex rabbit breeding.

MATERIAL AND METHODS

Sampling and microsatellite typing

Blood or tissue samples were collected randomly from five rabbit breeds (strains) consisting of Vc-I Rex rabbit strain (Vc-I), Vc-II Rex rabbit strain (Vc-II), New Zealand White rabbit breed (NZW), Qingzilan rabbit breed (QZL), Japanese White rabbit breed (JAW). The number of DNA samples were 16 for each rabbit population, respectively. Genomic DNA was extracted by a standard phenol-chloroform protocol with some modification. Five microsatellite loci were chosen (MOUGEL *et al*., 1997) from gene banks (*Sat3* and *Sat4*) or isolated from a genomic library(*Sat7, Sat8*, and *Sat12*). The primers used for the amplification of these microsatellite loci were listed in Table 1. The PCR reactions were carried out in a thermocycler in 25 μl final volume consisting of 200 ng DNA, 10 pM forward primer, 10 pM reverse primer, 100 μM dNTP (HT Biotechnology), 1.0 or 1.5 nM $Mgcl_2$, and 1 U supertaq (HT Biotechnology). After an initial 5 min denaturation at 94°C, 30 cycles were performed as follow: 30 s denaturation at 94°C, 30 s annealing at 55 or 60ºC, and 30 s extension at 72°C. A final elongation was carried out for 10 min at 72ºC. PCR products were separated on denaturing electrophoresis in 8% polyactylamide gels containing 8 M urea (Solano, 1998), and bands were visualized by rapid silver staining (Sanguinetti et al., 1994).

Table 1. The sequence of 5 microsatellite primers and the condition of PCR system

locus	Primer sequence	fragment (bp)	Mg^{2+} (mM)	anealling (ºC)
Sat3	Forward 5' GGAGAGTGAATCAGTGGGTG 3' Reverse 5'GAGGGAAAGAGAGAGACAGG 3'	146-162	1.5	60
Sar4	Forward 5'GGCCAGTGTCCTTACATTTGG 3' Reverse 5'TGTTGCAGCGAATTGGGG 3'	195-240	1.0	60
Sat7	Forward 5'GTAACCACCCATGCACACTC 3' Reverse 5'GCACAATACCTGGGATGTAG 3'	183-195	1.5	60
Sat8	Forward 5'CAGACCCGGCAGTTGCAGAG 3' Reverse 5'GGGAGAGAGGGATGGAGGTATG 3'	136-158	1.0	60
Sat12	Forward 5'CTTGAGTTTTAAATTCGGGC 3' Reverse 5'GTTTGGATGCTATCTCAGTCC 3'	122-138	1.0	55

Statistical analysis

Comparative measures of genetic variation for each rabbit population were calculated in the form of allelic diversity (total number of alleles, mean number of alleles per locus, frequency of every allele in each rabbit population), observed polymorphism information content (PIC) and heterozygosity of each rabbit population by the software of PPAP (Z.GUO, X.LI, 1994) based on allelic frequency. Genetic distance(D_A) was also calculated by the PPAP software, and the Phylogenetic trees were constructed using UPGMA based on the genetic distance(D_A).

RESULTS AND DISCUSSION

Genetic variation within populations

Five microsatellite loci exhibited a total 20 alleles from the 5 rabbit populations and individuals analyzed in this study. The number of alleles were 4, 3, 2, 5 and 6 for *Sat3 , Sat4, Sat7, Sat8 and Sat12,* respectively. This result diverged from the documents (VAN HAERINGES, 1996, OLYMPIA, 2001). VAN HAERINGES reported 7, 5, 6, 4 and 4 alleles for *Sat3 , Sat4, Sat7, Sat8 and Sat12*. Olympia also reported 5, 9 and 18 alleles for *Sat7, Sat8* and *Sat12*. It was probably due to the reason that the allelic number of these microsatellite loci in the documents came from the Eroupean wild rabbit populations living in open. While the domestic rabbit populations in this study were fed in enclosed environment which may have lead to the loss of some alleles. In additional, one private allele (defined here as allele found in single population throughout the studied populations) was found at Vc Rex population by *Sat4* and *Sat8*, respectively. Another private allele was found at NZW population by *Sat12*. This indicated that Vc Rex breed makes an unique part of rabbit genetic resources.

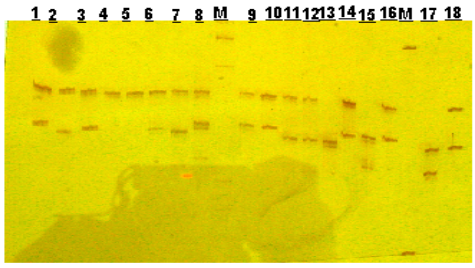

Figure 1. The amplification map of primer *Sat12*

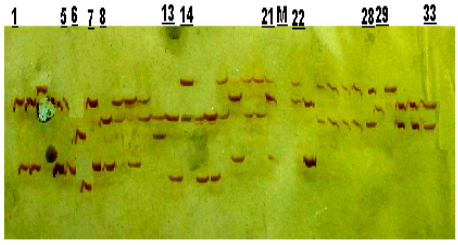

Figure 2. The amplification map of primer *Sat8*

PIC and heterozygosity (Table 2) had the same tendencies among 5 rabbit populations. The Vc-II population has the highest value (0.5526, 0.6202) followed by Vc-I population (0.5240, 0.5906), JAW population (0.4932, 0.5374), QZL population (0.4626, 0.5347), and NZW population (0.4515, 0.5261). The results suggested that genetic variation was greater in Vc Rex population than in the other three rabbit populations. The possible reason was that the NZW, QZL, and JAW rabbit breeds have a long-time breeding history and stationary genetic property, while the Vc Rex rabbit breed has only ten years of breeding history. Though the productive ability of it was already approached to the level of famous rabbit breeds, the genetic properties still have a small distance to the level of famous rabbit breeds unless it was continuously selected.

Table 2. The PIC and Heterozygosity within 5 rabbit populations

			allelic frequency					
locus	allelic number	allele	Vc-I	Vc-II	QZL	NZW	JAW	H
Sat3	4	a	0.3750	0.1250	0.2500	0.2143	0.2000	0.6666
		b	0.4375	0.4375	0.2500	0.5000	0.4000	
		c	0.1250	0.1250	0.3125	0.2857	0.4000	
		d	0.0625	0.3125	0.1875	0.0000	0.0000	
		PIC	0.5815	0.6273	0.6943	0.5512	0.5632	
Sat4	3	a	0.6875	0.5625	0.6875	0.7500	0.6000	0.4696
		b	0.1875	0.2500	0.3125	0.2500	0.4000	
		c	0.1250	0.1875	0.000	0.000	0.000	
		PIC	0.4275	0.5197	0.3374	0.3047	0.3648	
Sat7	2	a	0.3750	0.3750	0.1875	0.7500	0.2422	0.3969
		b	0.6250	0.6250	0.8125	0.2500	0.7578	
		PIC	0.3589	0.3589	0.2583	0.3047	0.4995	
Sat8	5	a	0.5000	0.4375	0.2222	0.0000	0.5000	0.6116
		b	0.0625	0.0625	0.0000	0.0000	0.0000	
		c	0.3125	0.2500	0.0000	0.0000	0.1000	
		d	0.1250	0.1875	0.4444	0.5000	0.0000	
		e	0.0000	0.0625	0.3333	0.5000	0.4000	
		PIC	0.5703	0.6568	0.5676	0.3750	0.4198	
Sat12	6	a	0.3125	0.4286	0.0625	0.0000	0.2000	0.6645
		b	0.1250	0.1429	0.5000	0.1875	0.5000	
		c	0.3750	0.3571	0.0000	0.3750	.03000	
		d	0.0000	0.0000	0.0000	0.1875	0.0000	
		e	0.0625	0.0714	0.4375	0.1250	0.0000	
		f	0.1250	0.0000	0.0000	0.1250	0.0000	
		PIC	0.6816	0.6003	0.4555	0.7219	0.5478	
PIC within each population			0.52396	0.5526	0.46262	0.4515	0.4932	
Heterozygosity within each population			0.5906	0.6202	0.5347	0.5261	0.5374	

Genetic relationship among 5 rabbit populations

The genetic distance among 5 rabbit populations was listed in Table 3 from which the phylogenetic trees were conducted as Figure 3. The results showed that the population which genetic distance was nearest to Vc-I population was Vc-II population (0.0497), followed by JAW population (0.1558), QZL population (0.2365), and NZW population (0.3262). This was conformed to the fact of Vc Rex rabbit breeding history. Because both Vc-I strain and Vc-II strain were the filial generation of JAW and Californian rabbit breed, and JAW was the maternal relation to Vc Rex rabbit breed. QZL breed was the descendent of Himalayan rabbit breed which has the similar characters of " eight black blots" with Vc Rex rabbit breed. This indicated a little connection between the two rabbit breeds. NZW population was furthest to Vc Rex rabbit population in relationship was due to the long geographical distance and nature barriers between New Zealand and China.

Table 3. Genetic distance index between 5 rabbit populations

population	Vc-I	Vc-II	JWR	QZL	NZR	
Vc-I	0.0000	0.0497	0.1558	0.2365	0.3262	
Vc-II	0.0497	0.0000	0.1606	0.2130	0.3251	
JWR	0.1558	0.1606	0.0000	0.2081	0.3290	
QZL	0.2365	0.2130	0.2081	0.0000	0.3135	
NZR	0.3262	0.3251	0.3290	0.3135	0.0000	

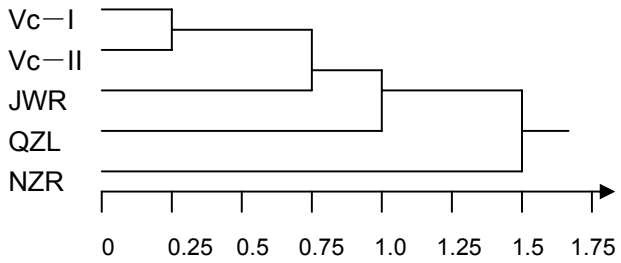

Figure 3. The UPGMA genetic cluster among 5 rabbit populations

CONCLUSIONS

This research showed high variation within and between the rabbit populations in this study and also proved that microsatellite genotyping is an useful tool to evaluate the variation and evolutional relationships among animal populations such as rabbit and to guide the breeding of the Vc-Rex rabbit breed.

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