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Correlation Analysis of Wool Yield in Wan Line Angora Rabbits Using Microsatellite DNA Markers

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Abstract: Correlation analysis of wool yield at age of one year in Wan line Angora rabbits using microsatellite DNA markers was studied. The results showed that the average number of alleles per locus was 4.5 (from 3 to 6) in Wan line Angora rabbits. The mean heterozygosity and the average polymorphism information content were 0.680 (from 0.630 to 0.721) and 0.642 (from 0.559 to 0.705), respectively. The relationships were not significant between Sat4, Sat13, Sol44 loci and wool yield ($p>0.05$). The relationship was significant between Sol33 locus and wool yield ($p<0.05$) and wool yield of genotype AD and BD were significantly higher than that of the other three genotypes which did not have gene D ($p<0.05$). This may indicated that the gene D could synergize wool yield of Wan line Angora rabbits.

Key words: Wan line Angora rabbit, microsatellite, wool yield

INTRODUCTION

Microsatellite DNA contains that microsatellite nomenclature and both sides of its flanking sequence regions and it is 1~6 bp. Flanking sequences enable microsatellite to be located on a particular position of the genome specificity. The character of microsatellites include highly polymorphic, plenty and well-distributed in the genome. For that, this technology became the second generation molecular markers after the first generation molecular markers (restriction fragment length polymorphism, RFLP) (Nakamura *et al.*, 1987) and had been widely applied in many studies territory of natural populations. Recently microsatellite markers has been used to establish genetic map of chromosome of the human, mouse, rat, silkworm and chicken and many other species and has been widely used in gene location, clone, disease diagnosis, phylogenetic analysis, identification of species, animal breeding, evolutionary trees and other areas. With the development of research, the abundant number of microsatellites polymorphisms has been used to analyze the significant difference among different species in order to search for genetic markers that link main production traits which serve as the scientific bases for molecular breeding and Marker-Assisted Selection (MAS).

Wan line Angora rabbits is a developed Angora breed which was bred from Chinese Angora and German Angora. In this study, Wan line Angora rabbits were

selected randomly which were healthy and one year old. Wool yield at age of one year were measured. Using microsatellite markers, the relationships between 4 polymorphism of microsatellite markers and wool yield at age of one year will be studied to obtain the theory evidence of marker assisted selection for Angora rabbit's wool yield.

MATERIALS AND METHODS

When this study was conducted, 51 Wan line Angora rabbits (11♂-40♀) were from the rabbit farming of the Institute of Animal Science and Veterinary, Anhui Academy of Agricultural Science (Anhui, China). Approximately 3 mL blood was collected from each individual (from the central artery vein of the ear) in tubes containing ACD (Citric acid, Sodium citrate, Dextrose) as anticoagulant and immediately transferred to the ice-box.

Genomic DNA were extracted according to the literature (Xin-Sheng *et al.*, 2005). The 4 pairs of microsatellite primers were synthesized by the Shenggong biological engineering technology company and primer sequences has been shown in Table 1.

The total 25 μ L volume of PCR reaction mixture comprised as follows: 100 ng of template DNA, 10 \times PCR buffer 2.5 μ L, 25 mM MgCl₂, 2.0 μ L of dNTP (200 μ M), 1.0 μ L of each primer (10 pmol L⁻¹), 1 U of TakaRa Taq enzyme.

Table 1: Primer information of four microsatellites

Locus	Serial No.	Primer sequence	No. of Allele	Allele scope
Sat4	M33582	F: 5' GGCCAGTGTCCCTTACATTTGG3' R: 5' TGTTCAGCGAATGGGG3'	3	214~241
Sat13	X99892	F: 5' CAGTTTTGAAGGACACCTGC3' R: 5' GCCTCTACCTTTGTGGGG3'	6	124~158
Sol33	X94683	F: 5' GAAGGCTCTGAGATCTAGAT3' R: 5' GGGCCAATAGGTACTGATCCATGT3'	5	214~248
Sol44	X94684	F: 5' GGCCCTAGTCTGACTCTGATTG3' R: 5' GGTGGGGCGCGGGTCTGAAAC3'	4	210~234

PCR conditions was as follows: initial denaturation at 94°C for 5 min; followed by denaturation at 94°C for 1 min, annealing at 55 to 60°C for 1 min, extension at 72°C for 1 min for 34 cycles and final extension at 72°C for 10 min.

PCR products were electrophoresed on denaturing polyacrylamide (arcylamide: bisacrylamide = 29: 1) gel to separate PCR products with different sizes at 200V for 6~8 h. The gels were silver stained to visualize gene fragments. According to the different rate of migration of different gene fragments, the length of fragments were analyzed by Kodak imaging software KDSZD2.0.

Statistical methods: Allele frequencies were calculated using the following formula.:

$$P_i = [2(ii)+(ij_1)+(ij_2)+ \dots (ij_n)]/2n$$

In the formula, P_i represent frequency of the i th allele; i represent the i th allele at a certain seat of microsatellite; J_1, J_2, \dots, J_n represent alleles from the first to the n^{th} which show the codominance with i ; n represent the seat number of microsatellite.

Microsatellite is codominant inheritance, therefore, allele frequency can be attained by simple statistic measurement of the detected genotype.

Heterozygosity (H) was calculated using the following formula:

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

In the formula, n represent the seat number of microsatellite; P_i represent frequency of the i th allele.

Polymorphism Information Content (PIC) is a indicatrix which reflected variation of microsatellite DNA mutation, reflected polymorphic of microsatellite DNA level, the formula as follows:

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

In the formula, n represent the seat number of microsatellite; P_i represent frequency of the i^{th} allele and P_j represent frequency of the j^{th} allele.

Data analysis: Using SPSS11.5 Generalized Linear Model (GLM) to analyze the relationships between polymorphism of microsatellite markers and wool yield at age of one year in Wan line Angora rabbits and analyzed with least square equation.

RESULTS

The PCR product and polymorphism of microsatellite:

The amplified results of 4 pairs of microsatellite primers were as shown in Fig. 1-4, respectively.

In Wan line Angora rabbits, the total number of alleles was 18 at 4 microsatellite loci. The number of alleles per locus ranged from 3 to 6 (Table 2). The average number of alleles of 4 microsatellite loci was 4.5 ± 1.29 .

Heterozygosity and polymorphism information content of Wan line Angora rabbits:

According to the allele frequencies at 4 microsatellite loci, heterozygosity and genetic polymorphism information content of Wan line Angora rabbits were calculated in Table 3. Table 3 indicated that the average heterozygosity of Wan line Angora rabbits was 0.680. The highest heterozygosity level was 0.721 at Sol33 locus; the lowest heterozygosity level was 0.630 at Sat4 locus. The average polymorphism information content of Wan line Angora rabbits was 0.642, the highest polymorphic information content level was 0.705 at Sol33 locus; the lowest polymorphic information content level was 0.559 at Sat4 locus.

The relationships between microsatellite locus and wool yield:

The relationships between polymorphism of microsatellite markers and wool yield at age of one year in Wan line Angora rabbits were analyzed by least square equation (Table 4). The relationship between Sol33 locus and wool yield was significant ($p < 0.05$). The relationships were no significant between Sat4, Sat13, Sol44 loci and wool yield ($p > 0.05$). Five genotypes were discovered at Sol33 locus and wool yield of genotype AD and BD were

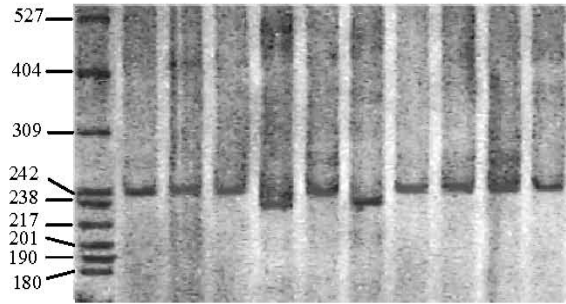


Fig. 1: PAGE of PCR product of Sat4 locus

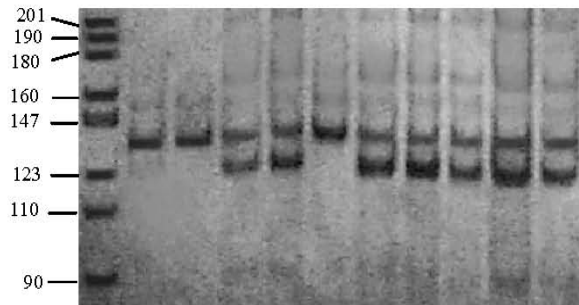


Fig. 2: PAGE of PCR product of Sat13 locus

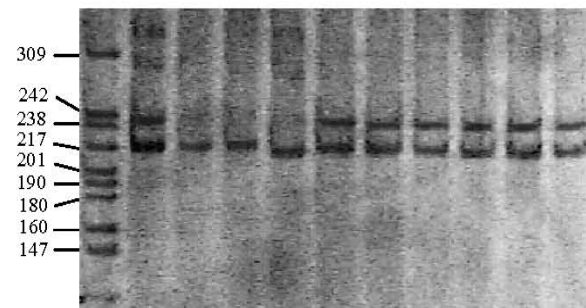


Fig. 3: PAGE of PCR product of Sol33 locus

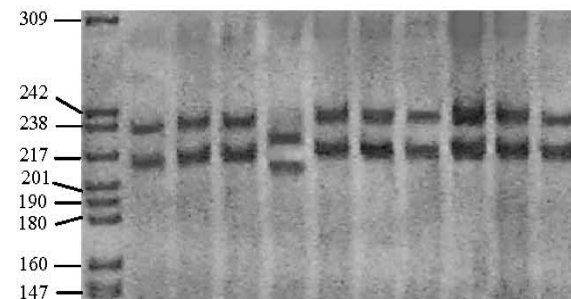


Fig. 4: PAGE of PCR product of Sol44 locus

significantly higher than that of other genotypes ($p < 0.05$); the differences of wool yield between genotype AD and CE was 78 g at age of one year.

Table 2: Allele frequencies at 4 microsatellite loci

Locus	Allele frequencies
Sat4	A (0.255), B (0.255), C (0.490)
Sat13	A (0.216), B (0.078), C (0.480), D (0.078), E (0.049), F (0.098)
Sol33	A (0.373), B (0.216), C (0.059), D (0.294), E (0.059)
Sol44	A (0.098), B (0.118), C (0.373), D (0.412)

Table 3: Heterozygosity and polymorphism information content of Wan line Angora rabbits

Locus	n	H	PIC
Sat4	51	0.630	0.559
Sat13	51	0.699	0.692
Sol33	51	0.721	0.705
Sol44	51	0.668	0.613
Average	51	0.680	0.642

Table 4: Least squares means and standard errors for wool yield of different genotypes of 4 microsatellite loci in Wan line Angora rabbit

Locus	Genotypes	n	Wool yield (g)
Sat4	AA	13	330.77±59.366 ^a
	BB	13	345.77±52.988 ^a
	CC	25	314.60±47.520 ^a
Sat13	AC	22	336.82±54.128 ^a
	BD	8	323.75±75.392 ^a
	CC	6	305.00±39.875 ^a
	CE	5	314.00±36.469 ^a
	CF	10	326.00±45.019 ^a
Sol33	AA	8	313.75±32.923 ^a
	AD	22	354.77±58.278 ^b
	BB	7	291.43±22.678 ^a
	BD	8	330.63±34.891 ^b
	CE	6	276.67±33.862 ^a
Sol44	AD	10	334.50±45.853 ^a
	BD	12	321.67±53.908 ^a
	CC	9	341.67±51.962 ^a
	CD	20	319.00±57.207 ^a

The different superscripts within the same column differ significantly ($p < 0.05$)

DISCUSSION

Gene heterozygosity of colony can be measured by use of polymorphism of molecular genetic markers. Heterozygosity is also known as gene diversity. The average gene heterozygosity of a colony is the optimum parameters to measure the variation of a colony (Bin *et al.*, 1999). The average gene heterozygosity can reflect the variation level of the genetic structure approximately. The average gene heterozygosity of Wan line Angora rabbits at 4 microsatellite loci was 0.680 and indicated the colony with high genetic diversity. In this study, the average gene heterozygosity of Wan line Angora rabbits was higher than that of JIRONG rabbit (Chun-Mei *et al.*, 2005) and lower than that of Angora rabbit (Jin *et al.*, 2006). The reason may be caused by using of the different rabbit species, microsatellite markers and the number of microsatellite loci.

Polymorphic Information Content (PIC) is a better indicator which can measure the polymorphism of gene fragment. When the $PIC > 0.5$, indicated the locus of high

polymorphism; When $0.25 < PIC < 0.5$, indicated the locus of medium polymorphism and when $PIC < 0.25$, indicated the locus of low polymorphism. Meanwhile polymorphic information content relate to the availability and efficiency of the locus, the polymorphic information content is higher, the proportion of heterozygous is greater and could provide more genetic information. In this study, the average polymorphism information content of Wan line Angora rabbits was 0.642 and the polymorphic information content at each microsatellite locus was higher than 0.5, indicated they were high polymorphic loci and that are same to the results of Angora rabbit (Jin *et al.*, 2006).

The tests analyzed by least square equation, showed that the relationship was significant between Sol33 locus and wool yield at age of one year. Perhaps, Sol33 locus linked with the major gene which control rabbits wool yield. At present, there are not literature about the research of rabbits, but there are many reports about the research in other species. Van Kaam *et al.* (1999) indicated the relationship was significant between LEI166 microsatellite locus and the 48 day old weight. Van Kaam *et al.* (1998), Tatsuda *et al.* (2000) and Tatsuda and Fujinaka (2001) indicated that maybe there were some QTLs in chicken's chromosome 1 which could control the weight of chickens. Qun-Lan *et al.* (2005) reported that the relationship was significant between ADL278, LEI166, MCW 222 microsatellite loci which were located in the 3rd and 8th chromosome and the weight of Luyuan chickens at age of 12 weeks. Gen-Bao *et al.* (2005) indicated the relationship was significant between some microsatellite locus in the 13th chromosome and pork quality of Taihu pigs. Ming-Xing *et al.* (2005) reported that there were different genotypes of six microsatellite loci could help Beijing Holstein cow to resistance mastitis. These results indicated that microsatellite marker assisted breeding livestock as a choice of molecular marker and could accelerate animal breeding progress. In this study, five genotypes were detected at Sol33 microsatellite locus of Wan line Angora rabbits, which genotype of BD and AD individual's wool yield were significantly higher than the other three genotypes which did not have gene D. This may indicated that the gene D could synergize wool yield of Wan line Angora rabbits, but the study of 51 Anhui rabbits could not detect DD genotype of the individual. Therefore, whether the gene D could synergize wool yield of Wan line Angora rabbits still need further study.

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