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Research Article

Investigation of the Relationships Between Wool Quality and Microsatellite in Hybrids of Australian Merino and Chinese Merino

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Abstract

This work was carried out to analyze of effects of twelve microsatellite loci and genetic heterozygosity on wool fineness and natural length, which are important indicators for evaluating of wool quality. 131 individuals from F2 and F3 generations of Australian Merino and Chinese Merino sheep (Xinjiang military reclamation type) mating were used as experimental subjects. Five loci on chromosome 1 and seven loci on chromosome 6 were determined as related with fineness and natural length of wool. The results showed that 3 loci significantly associated with wool fiber diameter (WFD), and 4 loci were significantly related to wool natural length (WNL). WFD increased by approximately 0.2%-2.5%, and WNL decreased by 2%-10.93%, and also heterozygosity increased by 0.05 in the range of 0.5 to 1.0. These results could partially explain the molecular mechanism of heterosis for sheep wool quality. Provide theoretical support for the effective exploitation and utilization of this precious resource.

Keywords: *Wool fiber diameter, Wool natural length, Microsatellite, Heterozygosity, Sheep*

Avustralya Merinosu ve Çin Merinosu Hibritlerinde Yün Miktarı İle Mikrosatellit Arasındaki İlişkinin Araştırılması

Öz

Bu çalışma, yün kalitesini değerlendirmede önemli belirteçler olan yün inceliği ve doğal uzunluğu üzerine on iki mikrosatellit bölgenin ve genetik heterozigotluğun etkilerini incelemek amacıyla yapılmıştır. Avustralya Merinosu ve Çin Merinosu eşleşmesinden (Xinjiang askeri ıslah tipi) F2 ve F3 jenerasyonlarından 131 koyun deneysel materyal olarak kullanıldı. Kromozom 1'de beş bölge ve kromozom 6'da yedi bölge yün inceliği ve doğal uzunluğuyla ilişkili olarak belirlendi. Elde edilen sonuçlar 3 bölgenin anlamlı derecede yün teli çapı ile 4 bölgenin de anlamlı derecede yün doğal uzunluğu ile ilişkili olduğunu gösterdi. Yün teli çapı yaklaşık %0.2-%2.5 artarken ve yün doğal uzunluğu %2-%10.93 azaldı ve heterozigotluk 0.5 ile 1.0 aralığında 0.05 kadar arttı. Bu sonuçlar koyun yün kalitesinde heterozisin moleküler mekanizmasını kısmen açıklamaktadır. Çalışma ile bu değerli kaynağın etkili yayılımı ve kullanımı konusunda teorik destek sağlanmıştır.

Anahtar sözcükler: *Yün lifi çapı, Yün doğal uzunluğu, Mikrosatellit, Heterozigotluk, Koyun*

INTRODUCTION

Xinjiang is an important sheep production base in China, and the fine-wool sheep number ranks first in the country. China Merino sheep (Xinjiang Army-type) has also become one of the most famous fine wool sheep in the world [1]. In the breeding system of fine-wool sheep, when the wool has a fineness of more than 80, it can be grouped into the same type for breeding [2,3]. However, in the present situation, it is difficult to improve or maintain the wool quality of existing high-quality fine wool sheep, either in normal breeding or in the application of high-quality new breeding technology [4]. The key problem is that there are few studies on the genetic methods of controlling sheep wool fiber traits. Without understanding this genetic relationships, improving and enhancing high-quality fine-wool sheep can easily be counterproductive, leading to quality degradation [5,6]. To improve this imminent problem, it is necessary to study the genetic relationships related to the wool production traits of Chinese Merino sheep.

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Microsatellites (simple repeat sequences or short simple tandem sequences) are a very popular molecular genetic marker [7]. They consist of a core sequence of 1-6 bp and a flanking sequence specifically located at a certain position on a chromosome. Studies have shown that microsatellites have been widely used in paternity testing of large domestic animals, such as pigs [8,9], horses [10], sheep [11] and cattle [12], and in poultry [13] and fish [14]. Because microsatellites are highly polymorphic and are widely used in the construction of genetic linkage maps [15]. In marker-assisted selection of livestock, microsatellites inferred genotypic values from phenotypic values by changing the population level and using the established sheep resource families to locate quantitative trait loci (QTL) related to reproduction [16], carcass [17], meat quality [18], etc., near some microsatellites. The studies have shown that WFD and WNL is an important indicator of economic value. Its variation accounts for 61% of total wool profit [19]. However, it is necessary to study the molecular genetic basis of wool characteristics, which provide theoretical support for the effective exploitation and utilization of this precious resource.

Wool fineness and WNL are important indices for evaluating wool quality [20]. That, this study analyzed the effects of twelve microsatellite loci and genetic heterozygosity on wool fineness and natural length to provide a theoretical basis in sheep breeding.

MATERIAL and METHODS

Experimental Animal Establishment of Reference Families and Sample Collection

All procedures involving animals were approved by the Animal Care and Use Committee of Shihezi University, Shihezi, Xinjiang. All animal procedures and experiments were conducted based on the guidelines approved by the Committee of Animal Care and Use at Shihezi University, China. All of the feeding, experimental management and sample collection were conducted by professionals who were highly experienced in conducting these activities to avoid human error and relieve the suffering of the animals.

Chinese Merino sheep (Xinjiang Army) were selected from the Ziniquan breeding sheep farm in Xinjiang, China. All test wool and blood samples were obtained from the Ziniquan breeding sheep farm. To eliminate the influence of other factors, the unified management system of the field was adopted in the study farm feeding and stabling.

Six Australia merino rams were selected according to WFD for 70, 66 and 58 yams and crossed with 160 Chinese Merino ewes having WFD of 70, 66, 64, 60 and 56 yams (diameter 19.57-27.56 μm). The F1 progeny were mated with half-siblings to obtain 131 F2 generation ewes with trait records. Then, from the F2 generation, 6 rams (fineness-selective traits) similar to the fineness of the ancestral rams were selected for semi-sibling mating. Thirteen mating combinations were produced *(Table 1)*, and 70 F3 individuals with trait records were obtained, of which 12 pairs were double lambs.

Wool Fibre Diameter (WFD) and Wool Nature Length (WNL) Assay Method

Three small bundles of wool were randomly taken from a different part of a bundle of hair samples and combined into a bundle. The 0.3 mm wool fiber fragments were cut by a slicer in the middle of the hair bundle, and the process was repeated three times. The chips were made into wool, and the diameter of the fibers was measured under a microscope to at least 300 μm, with a weighted average of wool fineness, that is WFD. Two hundred wool fibers were randomly selected from a bundle of wool samples, and the length in the natural state was measured with a ruler; and the average length of the wool was obtained by averaging the WNL [21].

Microsatellite Genotyping

Blood samples from a total of 173 sheep (6 Australia merino rams, 160 Chinese Merino ewes, and their 7 offspring) were collected from the jugular vein using vacutainer tubes containing EDTA as an anticoagulant. DNA isolation kit (Tiangen, China) was used to extract genomic DNA from blood samples.

Twelve candidate microsatellite loci were selected: BM6506, BM1824, BM6438, ILSTS004, OarDB6 from chromosome 1 and OarAE101, BM 415, BM1329, BM4621, OarHH55, BM143, OarJMP8 from chromosome 6. Primers were synthesized by Shanghai Biotechnology Co., Ltd. and designed according to the literature and Australian Sheep Gene Mapping Web Site *(http://rubens.its. unimelb.edu.cn/-jillm/jill. htm)* [22,23].

Polymerase Chain Reaction (PCR) was performed in a 25 μL

Table 1. *Sheet of thirteen kinds of matiny compose*

Ram (♂) / Ewe (♀)	70 yams (18.78-20 μm)			66 yams (20.22 μm)		580 yams (25.75 μm)	
	n	♀	F3	♀	F3	♀	F3
70 yams (18.78-20.02 μm)	9	3	3 (17.55-18.73 μm)	3	3 (19.09-22.46 μm)	3	3 (15.08-18.91 μm)
66 yams (20.09-21.47 μm)	21	16	16 (17.81-23.58 μm)	3	3 (19.28-21.06 μm)	2	2 (16.88-21.12 μm)
64 yams (21.15-23.01 μm)	19	13	14 (15.99-20.07 μm)	4	4 (18.76-20.59 μm)	2	2 (20.62-22.08 μm)
60 yams (23.10-24.09 μm)	15	9	9 (17.90-21.78 μm)	4	5 (18.59-22.04 μm)	2	2 (25.06-23.66 μm)
56 yams (27.10-28.11 μm)	4			4	4 (21.92-22.64 μm)		

reaction volume containing 2 μL of DNA (50 ng), 1 μL of each primer, 12.5 μL of PCR Mix, and 8.5 μL of water. Loci were amplified using standard PCR cycling conditions of 95°C for 2 min, 36 cycles of 30 s at 94°C, 30 s at the primer sequences and annealing temperatures (T1), and 45 s at 72°C. PCR products were resolved by electrophoresis on standard 10% polyacrylamide sequencing gels and detected by silver staining [11]. The gels were stained with silver to visualize microsatellite loci and allelic patterns and were analyzed by software. T1 of all markers are presented in *Table 2*.

Statistical Method

Population gene heterozygosity (Heterozygosity, H) is calculated using Nei [24]:

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

Calculation of individual gene heterozygosity Hi: If an individual has m loci, let n be a sample of m loci. In the sampled n individual microsatellite loci, the ratio H1 of the number of heterozygous seats H1 to the number of sampled seats n is the individual gene heterozygosity, and the formula is as follows:

$$H_i = H_l / n$$

Individuals were divided into 10 heterozygosity grades in steps of 0.05 based on individual gene heterozygosity, according to whether the microsatellite locus and WFD are related to the genetic heterozygosity: individual heterozygosity calculated in all 12 loci, 3 individual heterozygosities calculated in relation to the fineness of wool, 9 individuals calculated in neutral seats heterozygosity, 4 individual heterogeneities calculated with WNL, and 8 neutral loci with individual heterozygosity. Classification criteria and classification results are shown in *Table 3, Table 4*.

Table 2. *Primer sequence of nine microsatellite markers and reference annealing*

Gene Name	Primer Sequence	T1/°C
BM6506	GCACGTGGTAAAGAGATGGC AGCAACTTGAGCATGGCAC	50
BM1824	GAGCAAGGTGTTTTTCCAATC CATTCTCCAACTGCTTCCTTG	47
BM6483	TTGAGCACAGACACAGACTGG ACTGAATGCCTCCTTTGTGC	58
ILSTS004	CTTAAAATCTGTCTTTCTTCC TAGTGTGTATTAGGTTTCTCC	54
OarDB6	GACATGACTAAAGCAATTTAGCATGC TGGACTACAGTCCATAGCTCTC	57.5
OarAE101	TAAGAAATATATTTGAAAAAACTGTATCTCCC TTCTTATAGATGCACTCAAGCATGG	63
BM415	GCTACAGCCCTTCTGGTTTG GAGCTAATCACCAACAGCAAG	52
BM1329	TTGTTTAGGCAAGTCCAAAGTC AACACCACAGCTTCATCC	64
BM4621	CAAATTGACTTATCCTTGGCTG TGTAACATATGGGCTGCATC	51
OarHH55	GTTATTCCATATTCTTTCCTCCATCATAAGC CCACACAGAGCAACTAAACCCAGC	66.5
BM143	ACCTGGGAAGCCTCCATATC CTGCAGGCAGATTCTTTATCG	68
OarJMP8	CGGGATGATCTTCTGTCCAAATATGC CATTTGCTTTGGCTTCAGAACCAGAG	67

Polymorphic information content (PIC) is calculated according to the formula provided by Botstein [25]:

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

Pi and Pj are the i-th and j-th allele frequencies in the population, and n is the number of alleles.

The phenotypic mean of microsatellites was analyzed by the generalized linear model (GLM) of SAS (V6.12) software, and multiple comparisons between genotypes were performed using the least significant difference method (LSD) [26].

RESULTS

Wool Phenotypic Traits and Microsatellite Locus Heterozygosity

WFD: 20.96±0.2008 μm, WFL: 10.784±0.8907 cm.

The heterozygosity and PIC of 12 microsatellite loci on chromosomes 1 and 6 are shown in *Table 5*. The heterozygosity ranged from 0.6069 to 0.7965, and the PIC ranged from 0.5258 to 0.8008. This result demonstrates that heterozygosity significantly associates with PIC. The number of alleles reported in the literature and Australian Sheep Gene Mapping Web Site (http://rubens.its.unimelb.edu.cn/ -jillm/jill.htm) were not fully detected in this study.

Microsatellite Heterozygosity Associations with WFD and WNL

BM1824, BM6438, BM6506 alleles at 12 microsatellite loci indicate significant association with WFD, but the remaining 9 loci do not ($P<0.05$, *Table 6)*. Chr1:ILSTS004 and Chr6:OARAE101, OARJMP8, BM143 alleles at 12 microsatellite loci manifest significant relevance to WNL ($P<0.05$, *Table 7)*.

Correlation Analysis Between Heterozygosity with WFD and WNL

The experimental sheep were divided into 10 levels according to the level of heterozygosity. The sample in each of the levels were similar *(Table 3, Table 4)*. In terms of heterozygosity classified by counting 12 microsatellite loci, analysis showed that the value of WFD was significantly different ($P<0.05$) among 10 heterozygosity levels. Heterozygosity and WFD were directly proportional *(Table 8, Fig. 1)*. The WFD of level 2 (average heterozygosity 0.5450) had the highest diameter at 19.90 μm. In terms of heterozygosity

Table 3. *Mean heterozygosity and number of individuals at 10 levels in different classifications*

Level	Classified by 12 Loci		Classified by 3 Loci Associated with Wool Fiber Diameter		Classified by 9 Loci Unassociated with Wool Fiber Diameter	
	Heterozygosity Range/ (mean)	Observe Number/(%)	Heterozygosity Range/ (mean)	Observe Number/ (%)	Heterozygosity Range/ (mean)	Observe Number/ (%)
1	h≤0.5 (0.4605)	6 (4.58)	H≤0.5 (0.4826)	13 (9.93)	H≤0.5 (0.4210)	11 (8.40)
2	0.5<h≤0.55 (0.5450)	7 (5.34)	0.5<h≤0.55		0.5<h≤0.55	
3	0.55<h≤0.6 (0.5886)	10 (7.63)	0.55<h≤0.6		0.55<h≤0.6 (0.5600)	11 (8.40)
4	0.6<h≤0.65 (0.6360)	9 (6.87)	0.6<h≤0.65		0.6<h≤0.65 (0.6250)	7 (5.34)
5	0.65<h≤0.7 (0.6730)	11 (8.40)	0.65<h≤0.7 (0.6639)	66 (50.38)	0.65<h≤0.7 (0.6670)	13 (9.92)
6	0.7<h≤0.75 (0.7340)	24 (18.32)	0.7<h≤0.75		0.7<h≤0.75 (0.7370)	16 (12.21)
7	0.75<h≤0.8 (0.7930)	10 (7.63)	0.75<h≤0.8		0.75<h≤0.8 (0.7790)	16 (12.21)
8	0.8<h≤0.85 (0.8290)	25 (19.08)	0.8<h≤0.85		0.8<h≤0.85 (0.8330)	1 (0.76)
9	0.85<h≤0.9 (0.8810)	8 (6.11)	0.85<h≤0.9		0.85<h≤0.9 (0.8790)	28 (21.37)
10	h>0.9 (0.9580)	21 (16.04)	h>0.9 (0.9226)	52 (39.69)	h>0.9 (1.000)	26 (19.85)

Heterozygosity range/mean and observation number/(%) for ten heterozygosity levels classified by 12 loci, by 3 loci associated with wool diameter or by 9 loci unassociated with wool fiber diameter

Table 4*. Mean heterozygosity and number of individuals at 10 levels in different classifications*

Level	Classified by 12 Loci		Classified by 3 Loci Associated with Wool Natural Length		Classified by 9 Loci Unassociated with Wool Natural Length	
	Heterozygosity Range/ (mean)	Observe Number/ (%)	Heterozygosity Range/ (mean)	Observe Number/ (%)	Heterozygosity Range/ (mean)	Observe Number/ (%)
1	h≤0.5 (0.4605)	6 (4.58)	H≤0.5 (0.3960)	42 (32.06)	H≤0.5 (0.4620)	14 (10.69)
2	0.5<h≤0.55 (0.5450)	7 (5.34)	0.5<h≤0.55		0.5<h≤0.55	
3	0.55<h≤0.6 (0.5886)	10 (7.63)	0.55<h≤0.6		0.55<h≤0.6 (0.5760)	6 (4.58)
4	0.6<h≤0.65 (0.6360)	9 (6.87)	0.6<h≤0.65		0.6<h≤0.65 (0.6250)	12 (9.16)
5	0.65<h≤0.7 (0.6730)	11 (8.40)	0.65<h≤0.7 (0.6670)	8 (6.11)	0.65<h≤0.7 (0.6670)	1 (0.76)
6	0.7<h≤0.75 (0.7343)	24 (18.32)	0.7<h≤0.75 (0.7500)	40 (30.53)	0.7<h≤0.75 (0.7330)	32 (24.43)
7	0.75<h≤0.8 (0.7934)	10 (7.63)	0.75<h≤0.8		0.75<h≤0.8 (0.8000)	4 (3.05)
8	0.8<h≤0.85 (0.8288)	25 (19.08)	0.8<h≤0.85		0.8<h≤0.85 (0.8370)	11 (8.40)
9	0.85<h≤0.9 (0.8785)	8 (6.11)	0.85<h≤0.9		0.85<h≤0.9 (0.8680)	23 (18.32)
10	h>0.9 (0.9578)	21 (16.03)	h>0.9 (1.0000)	41 (31.30)	h>0.9 (1.000)	27 (20.61)

Heterozygosity range/mean and observe number/(%) for ten heterozygosity levels classified by 12 loci, by 4 loci associated with wool natural length or by 8 loci unassociated with wool natural length

Table 5. *Locus number, gene frequency, heterozygosity and polymorphism information content(PIC)*

Chromosome	Microsatellite	Allele Number	Gene Frequency	Heterozygosity	PIC
1	BM1824	4	0.1311, 0.0410, 0.0074, 0.0164	0.7377	0.7877
1	BM6438	5	0.1271, 0.0085, 0.0593, 0.0254, 0.0085	0.7712	0.8008
1	BM6506	4	0.1951, 0.0488, 0.0244, 0.0325	0.6992	0.6762
1	ILSTS004	5	0.0708, 0.0177, 0.0177, 0.0354, 0.0619	0.7965	0.7646
1	OarDB6	6	0.0964, 0.0175, 0.0175, 0.0175, 0.0351, 0.0088	0.7472	0.7185
6	OarAE101	5	0.1774, 0.0081, 0.0242, 0.0403, 0.0161	0.7339	0.7274
6	OarHH55	5	0.0645, 0.0645, 0.0242, 0.0161, 0.0403	0.7955	0.7769
6	OarJMP8	4	0.1145, 0.1985, 0.0153, 0.0076	0.6641	0.6123
6	BM4621	5	0.0756, 0.0672, 0.0756, 0.0252, 0.0084	0.7480	0.7234
6	BM143	3	0.1536, 0.1536, 0.0859	0.6069	0.5258
6	BM415	5	0.1111, 0.0171, 0.0171, 0.0256, 0.0513	0.7778	0.7699
6	BM1329	4	0.1750, 0.0250, 0.0167, 0.0583	0.7250	0.6097

classed by arithmetic heterozygosis of 12 microsatellite loci, analysis indicated that the value of WNL was significantly different (P<0.05) among 10 heterozygosity levels. Heterozygosity and WNL were inversely proportional *(Table 9, Fig. 2)*. The WNL of level 7 (average heterozygosity 0.7934) was the longest, at 11.588 cm.

Table 6. *The genotypic variance analysis of WFD at three microsatellite loci*

Loci	BM1824		BM65106		BM6438	
Genetype	**n**	**Fiber Diameter (X±Sx)**	**n**	**Fiber Diameter (X±Sx)**	**n**	**Fiber Diameter (X±Sx)**
AA	15	19.76±0.5605^{BCc}	23	20.48±0.4235^{A}	14	20.87±0.5385Aab
AB	0		3	20.53±0.4265^{A}	11	18.72±0.5399Bc
AC	44	20.96±0.3130^{ABb}	67	20.83±0.2465^{A}	42	20.53±0.2806Ab
AD	11	19.16±0.5402^{CDcd}	2	20.73±1.5350^{A}	10	20.49±0.8028Ab
BB	5	18.18±0.8821^{Dd}	1	24.46±0.0000	1	24.72±0.0000
BC	12	20.98±0.4683^{ABb}	0		0	
BD	5	22.26±0.7865^{Aa}	0		3	20.99±1.1875Aab
BE	0		0		2	21.99±0.4100Aa
CC	7	20.83±0.5417^{ABb}	2	17.17±0.3900^{B}	5	21.83±1.2218Aa
CD	2	20.38±3.7950^{ABbc}	4	17.12±0.9159^{B}	10	20.61±0.4772Aab
DD	1	21.47±0.0000	4	20.41±1.5077^{A}	3	21.79±1.6404Aa
EE	0		0		1	24.90±0.0000

Significant differences (P<0.05 or P<0.01) are shown using different letters in columns, as shown in Table 5

Table 7. *The genotypic variance analysis of WNL at four microsatellite loci*

Loci	OarAE101		OarJMP8		BM143		ILSTS004	
Genetype	**n**	**Natural Length (X±Sx)**	**n**	**Natural Length (X±Sx)**	**n**	**Natural Length (X±Sx)**	**n**	**Natural Length (X±Sx)**
AA	19	11.54±0.5533^{ACa}	14	13.00±0.6404^{Aab}	15	11.13±0.3637^{ABa}	8	10.76±0.6760^{ABb}
AB	12	9.97±0.5425^{BCb}	57	10.83±0.2192^{ABb}	39	10.67±0.3088^{ABab}	22	10.49±0.2988^{ABb}
AC	0		7	13.14±0.3221^{Aa}	13	11.61±0.5283^{A}	19	11.69±0.3259^{Aab}
AD	34	10.46±0.2837^{ABb}	5	10.51±0.8864^{ABb}	0		9	11.86±0.4773^{Aab}
AE	13	10.48±0.5106^{ABb}	0		0		12	11.33±0.6007^{Aab}
BB	1	8.22±0.0000	21	9.89±0.4174^{BC}	19	11.29±0.5018^{A}	1	6.66±0.0000
BC	2	11.73±1.2700^{ACa}	0		15	9.62±0.4129^{Bb}	2	8.52±1.9800^{Cd}
BD	3	12.50±0.8660^{ACa}	6	11.30±0.5626^{ABab}	0		1	7.89±0.0000
BE	1	11.05±0.0000	0		0		1	7.87±0.0000
CC	2	7.94±1.3950^{Bb}	2	9.28±1.3350^{BC}	10	11.66±0.4039^{A}	2	9.16±1.9750^{BCcd}
CD	7	10.81±0.7633^{ABab}	0		0		3	9.39±0.7285^{BCcd}
CE	5	12.60±0.1000^{Aa}	0		0		1	11.28±0.0000
DD	1	12.00±0.0000	1	11.51±0.0000	0		4	12.25±0.5951^{Aa}
DE	7	11.93±0.5051^{ACac}	0		0		8	10.23±0.7222^{ABb}
EE	2	10.36±0.9350^{ABab}	0		0		3	10.21±1.6647^{ABbc}

Table 8. *Effect of genetic heterozygosity on wool fineness in sheep*

Heterozygosity Level	Classified by 12 Loci		Classified by 3 Loci Associated with Wool Fiber Diameter	Classified by 9 Loci Unassociated with Wool Fiber Diameter
	Mean±Std[1]	**PWD[2]**	**Mean±Std**	**Mean±Std**
1	20.79±0.8853^{abc}	20.2128	20.6200±0.5897	20.79±0.8853^{abc}
2	19.90±0.3651^{c}	20.2847		19.90±0.3651^{c}
3	20.43±0.7009^{ab}	20.4734		20.43±0.7009^{ab}
4	20.65±0.3794^{ab}	20.5698		20.65±0.3794^{ab}
5	21.04±0.4657^{ab}	20.6451	21.1500±1.1254	21.04±0.4657^{ab}
6	19.93±0.3017^{c}	20.7692		19.93±0.3017^{c}
7	21.17±0.9182^{ab}	20.8893		21.17±0.9182^{ab}
8	20.21±0.2217^{bc}	20.9625		20.21±0.2217^{bc}
9	21.45±0.7090^{a}	21.0683		21.45±0.7090^{a}
10	21.63±0.8499^{a}	21.2250	21.4800±0.8033	21.63±0.8499^{a}

Sheep wool fiber diameter and their regression value at 10 levels or gene heterozygosity. Different superscripts in the same column differ significantly (P<0.05)

Table 9. *Effect of genetic heterozygosity on wool fineness in sheep*

Heterozygosity Level	Classified by 12 Loci		Classified by 3 Loci Associated with Wool Natural Length	Classified by 9 Loci Unassociated with Wool Natural Length
	Mean±Std	PWNL	Mean±Std	Mean±Std
1	11.074±0.2859^{ab}	11.4662	11.307±0.9637	11.074±0.2859^{ab}
2	11.243±0.8660^{ab}	11.2639		11.243±0.8660^{ab}
3	11.373±0.1000^{ab}	11.1595		11.373±0.1000^{ab}
4	10.836±0.6532^{ab}	11.0460		10.836±0.6532^{ab}
5	11.505±0.4562^{a}	10.9573	10.573±0.8548	11.505±0.4562^{a}
6	10.326±0.8652^{bc}	10.8105	10.678±0.3629	10.326±0.8652^{bc}
7	11.588±0.6521^{a}	10.6690		11.588±0.6521^{a}
8	11.133±0.4516^{ab}	10.5842		11.133±0.4516^{ab}
9	9.492±0.9530^{c}	10.4652		9.492±0.9530^{c}
10	10.127±0.4870^{bc}	10.2753	10.499±0.6591	10.127±0.4870^{bc}

Sheep wool natural length and their regression value at 4 levels for gene heterozygosity. Different superscripts in the same column differ significantly (P<0.05)

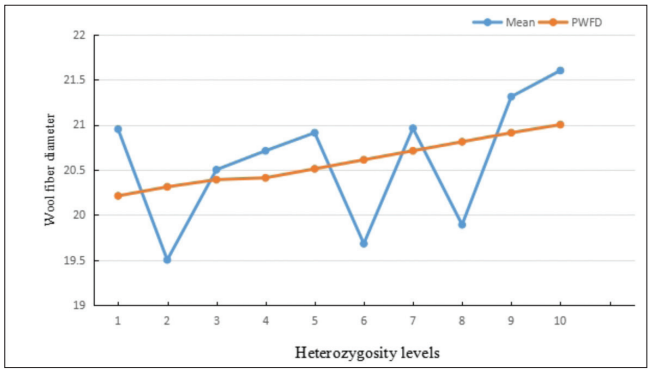

Fig 1. Wool fiber diameter increased as gene heterozygosity increased

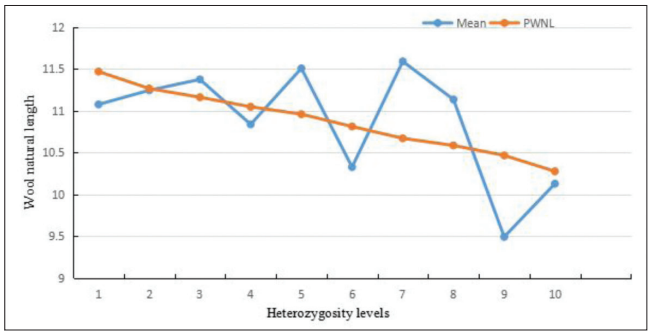

Fig 2. Wool natural length decreased as gene heterozygosity increased

All levels of WFD and WNL between the average heterozygosity of each level were analyzed by regression. The results showed that the regression relation was significant. Regression models were WFD=19.2758+2.3646×Hi (P<0.05), R^2=0.8070, harmony R^2=0.7175.

WNL=12.5691-2.3949×Hi(P<0.05), R^2=0.8592, harmony R^2=0.7725

The average value of WFD was 1.73 μm, which is the difference between the highest value and the lowest value. With heterozygosity increasing by 0.05, the average value of WFD will increase approximately 0.04-0.53 μm, which is 0.2-0.5% of the group WFD average 20.96 μm, except for the 1, 6, and 9 heterozygosity levels. PWFD by the regression model was 1.0122 μm, which is the difference between the highest value and the lowest value. The average value of WNL was 2.096 cm, which was the difference between the highest value and the lowest value. With an increase in heterozygosity of 0.05, the average value of WNL will decrease approximately 0.13-1.179 cm, which is 1.2-10.93% of the group WNL average of 10.784 cm, except for the 7 and 8 heterozygosity levels. The PWNL by regression model is 1.1909 cm, which is the difference between the highest value and the lowest value.

WFD was classified by 3 significantly connected loci and showed heterozygosity mainly distributed in the 1 (h≤0.5), 5 (0.65<h≤0.7), and 10 (h>0.9) levels, and average heterozygosities were 0.4826, 0.6639 and 0.9226, respectively, corresponding to WFD values of 20.62, 21.15 and 21.48 μm, showing that the trend of wool fineness significantly increases with increasing heterozygosity. The difference between the highest point and the lowest was 0.84 cm, which is very close to the difference estimated by all seats.

WNL was classified by 4 significantly connected loci and showed heterozygosity mainly distributed in the 1 (h≤0.5), 6 (0.65<h≤0.7), and 10 (h>0.9) levels, and the average heterozygosities were 0.3960, 0.7500, and 1.0000, respectively, and corresponding WNLs were 11.307, 10.678 and 10.499 cm, respectively. The results clearly show that with the increase in heterozygosity, the WNLs decreased. The difference between the highest value and the lowest value was 0.808 cm, which closely approaches the estimated difference of all loci. The analysis of 9 and 8 neutral loci also showed that the WFD and WNL increased or decreased with increasing heterozygosity.

DISCUSSION

China is one of the most developed countries in the world's

textile industry. The demand for wool is very high. Fine wool is the preferred raw material for ideal worsted processing because of its unique advantages in insulation, moisture absorption, elasticity, antistatic and antiwrinkle properties, and so on. Wool traits are an important economic trait in fine wool sheep [27]. The use of molecular markers has become an important phenotypic method, and it have been widely used in genetics and breeding [28]. Wool fineness and the natural length of wool are important factors in determining the economic value of wool. Therefore, it is necessary to understand the effects of genetic heterozygosity on wool fineness and WNL among different microsatellite loci of sheep, laying a foundation for improving the fineness and natural length of wool in sheep breeding.

By analyzing the relevance of 5 loci on chromosome 1 and 7 loci in chromosome 6 to WFD and WNL in sheep, the conclusion that 3 loc: BM1824, BM6506, BM6438 had a significant relation($P<0.05$) with WFD, which suggested that chromosome 1 was an important link block and that the genes or QTL had a greater contribution to WFD. There were 4 loci: ILSTS004 on chromosome 1 and OarAE101, OarJMP8, BM143 on chromosome 6, that had a remarkable connection to WNL. The results showed that the genes or QTLs have key genetic effects on WNL.

The relationship between gene heterozygosity and fiber diameter was analyzed using classification by 12 loci, 3 loci and another 9 loci associated with WFD. The results showed that while the gene heterozygosity increased, the fiber diameter increased. In addition, the gene heterozygosity increased by 0.05, and the diameter increased by 0.2%-2.5% of the average diameter of the group. When the relationship between gene heterozygosity and natural length was analyzed using classification by 12 loci, 3 loci and another 8 loci were associated with WNL, suggesting that while the gene heterozygosity increased, the natural length decreased. When the gene heterozygosity increased by 0.05, the natural length decreased by 1.2%-10.93% of the average length (10.784 μm) of the group. The phenomenon is coincident with heterozygosity involved with traits of the plant. In the heterotic utilization of sheep, it is necessary that the filial generation have proper gene heterozygosity through the appropriate choice of parents. The results showed that of all 12 loci, 3 and 4 loci had similar results. It is more convenient to attain results when using loci associated with traits that analyze the relationship between WFD, WNL and gene heterozygosity [29]. This result could partially explain the molecular mechanism of heterosis of for sheep wool quality.

At present, such as DNA fingerprinting, rapid DNA and protein polymorphism, have been used to explore the heterosis of animals [30,31]. The relationship of gene heterozygosity with heterosis has been studied in wildlife and livestock [33-35]. These studies are based on the group level, the use of heterosis is performed by specified male and female animals in practice. That these studies based on the group level instruct the practical use of heterosis [36]. Gene heterozygosity results in heterosis, the law of which would be easy to analyze at the gene level when the effect of gene heterozygosity on production quality was studied directly [37].

This paper evaluated the gene heterozygosity of the individual and analyzed the wool traits, especially the fiber diameter and natural length of gene heterozygosity of different individuals, The results showed that the gene heterozygosity of the individual would keep some main characteristics that are best suited to make main breeding characteristics by economy of sheep [38]. For example, the sheep had the smallest diameter when the gene heterozygosity was 2 (0.5450) and 6 (0.7340). If the largest diameter was the primary goal of breeding, the gene heterozygosity can be used to help in breeding. We can select a proper ram and ewe according to the individual gene heterozygosity and then obtain sheep of individual gene heterozygosity of 0.5 or 0.7, which would ensure the best heterozygosis effect. This study focused on the analysis the effects of gene heterozygosity on WFD and WNL between different microsatellite marker loci in sheep. Indirect selection and breeding of wool economic traits can be carried out using molecular markers linked to wool traits to improve the accuracy of line selection in sheep breeding. To lay the foundation for (MAS) selection of Chinese Merino (Xinjiang Army) breeding marker-assisted selection, and to accelerate the breeding, genetic analysis and genetic breeding speed of ultrafine lines. It lays a foundation for the separation and cloning of wool fiber trait control genes in the future and has important local characteristics, research value and significance.

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