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Research Article

Study on Population Genetic Characteristics of Qinchuan Cows Using Microsatellite Markers

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Abstract: To evaluate the genetic polymorphisms and to search for available molecular markers for Qinchuan cattle, 90 Qinchuan cows were genotyped with 12 microsatellite markers. A total of 247 alleles were detected, with the number of alleles ranging from 13 (*INRA005*) to 33 (*HEL13*), giving a mean number of 21 alleles per locus. The total and mean effective allele number were 142.6229 and 11.8852, respectively. Mean sampling variance of the allele frequency was 2.6036×10^{-4}. Allele size ranges of the 12 microsatellite loci were different. The observed heterozygosity and expected heterozygosity were from 0.7842 (*INRA005*) to 0.9775 (*BM315*) and 0.7952 (*BM315*) to 0.9446 (*HEL13*), respectively. Mean observed heterozygosity and mean expected heterozygosity were 0.9117 and 0.9047, respectively. Polymorphism information content values were from 0.7653 (*INRA005*) to 0.9420 (*HEL13*), and mean polymorphism information content of the 12 microsatellite loci was 0.8965. All the 12 microsatellite loci were highly polymorphic, which showed that there were rich genetic polymorphisms at these detected microsatellite loci in Qinchuan cows. At the 12 microsatellite loci, the mean fixation index was −0.0076, reflecting that the degree of heterozygote defect at these loci was not high and deviations from Hardy-Weinberg equilibrium were not significant.

Keywords: Qinchuan cattle; microsatellite DNA; polymorphism

In the past 200 years, cattle breed registrations have led to genetic isolation of many cattle breeds. The selection of a few highly productive breeds has caused the decline of numerous other diverse breeds. This is the reason why the evaluation and the preservation of cattle genetic resources have already become a major and common problem that has attracted global concern. The genetic polymorphism and diversity found in the domestic breeds allow farmers to develop new characteristics in response to changes in environment, diseases, or market conditions. Some indigenous breeds often possess special gene combinations and adaptations (such as disease resistance, adaptation to harsh conditions or poor quality feeds, etc.) that are not found in other breeds, so the importance of increasing, maintaining and conserving the genetic diversity in these animals for the future has been recognized[1]. Maintenance of genetic diversity is a crucial basis for the selection of novel characteristics and ensures more accurate selection for higher

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quality products[2]. Gradually attention has been turned in this direction, with every tool including phenotypic parameters and biochemical and molecular genetic techniques having been used to assess the genetic diversity of the animal. DNA-based technologies enable the detection of different polymorphic types. Among those, microsatellites or short tandem repeats (STRs) have been identified in all the eukaryotic species that have been investigated thus far[3,4]. Recently, microsatellite markers have become the mainstay of genetic linkage mapping[5–7], have been used to identify the quantitative trait loci for economic traits[8,9] and to address questions concerning the genetic diversity and evolutionary history of cattle[10,11].

As one of the elite yellow cattle breeds, Qinchuan cattle breed has had a long history of feeding and breeding in China. It is recorded that selecting good cattle to present to the master was written in 800 BC[12]. Qinchuan cattle were mainly used as draught animals during the long history. Since Zhangqian brought back alfalfa seeds from the West in 126 BC by the Silky Road, people began to plant alfalfa for cattle feed on the Guanzhong Plain, the main production area of Qinchuan cattle. This resulted in tremendous advances in the improvement of the Qinchuan cattle, particularly its body size, workability, and individual meat yield. In the long history of selection and breeding, Qinchuan cattle experienced draft type selection, dual type of draft and beef purpose selection and present beef purpose selection. Up to the last decade, although the genetic features of Qinchuan cattle have been extensively examined on body conformation traits[13], chromosome characteristics[14,15], blood protein polymorphisms[16] and mtDNA polymorphisms[17], very little information of microsatellite data is available. The purpose of this study was to uncover the genetic polymorphisms of Qinchuan cattle by examining the microsatellite DNA and to accumulate some basic microsatellite data for quantitative trait loci detection and molecular breeding for the future.

1 Materials and Methods

1.1 Materials

Fresh blood samples were collected from 90 pure Qinchuan cows maintained at the Shaanxi Provincial Qinchuan Cattle Farm and Shaanxi Linwei Qinchuan cattle preservation area and stored at −80℃. The 12 bovine microsatellite markers located on different chromosomes used in this study were from microsatellite data of European cattle breeds (*Bos taurus*). Primers, map positions (Chromosome No.), and annealing temperatures can be found in CaDBase (http://www.pro- jects.roslin.ac.uk/cdiv/markers. html) (Table 1).

1.2 Methods

Genomic DNAs were isolated from whole blood samples as described by Chen *et al*[18]. DNA samples were dissolved in TE solution and stored at −20℃. PCR amplification was performed in 12 μL of the reaction mixture. Each reaction step contained *Taq* DNA polymerase (0.5 U/μL) 1.0 μL, PCR buffer 1.2 μL, $MgCl_2$ (25 mmol/L) 1.5 μL, dNTPs (2.5 mmol/L) 0.75 μL, primers (10 pmol/L) 1.0 μL, template DNA (50 ng/μL) 2.0 μL, and sterilized H_2O 4.55 μL. The temperature profiles were: initial denaturation at 95℃ for 2 min; 35 cycles of denaturation at 94℃ for 30 s, annealing at the optimal temperature of each primer pair for 30 s; and extension at 72℃ for 45 s. Final extension was at 72℃ for 10 min and then samples were held at 4℃. After PCR amplification, 3−4 μL of the amplified PCR products was loaded onto 8% polyacrylamide gel. After 3−4 h of electrophoresis (250 V), the gels were stained with silver nitrate (silver staining) and the fragment sizes were read using the Kodak Digital Science ID Image Analysis Software System.

Effective number of alleles (*Ne*), locus heterozygosity (*h*), mean locus heterozygosity (*H*), sampling variance of allele frequency ($V_{(p_{ij})}$), polymorphic information content (*PIC*), and fixation

Table 1 Information of 12 bovine microsatellites analyzed in this study

Microsatellite loci	Primer sequences (5′→3′)	Chromosome No.	Annealing temperature (℃)
BM1824	F：GAGCAAGGTGTTTTTCCAATC R：CATTCTCCAACTGCTTCCTTG	1	58.5
BM2113	F：GCTGCCTTCTACCAAATACCC R：CTTCCTGAGAGAAGCAACACC	2	56.4
CSSM66	F：ACACAAATCCTTTCTGCCAGCTGA R：AATTTAATGCACTGAGGAGCTTGG	14	61.2
ETH152	F：TACTCGTAGCGCAGGCTGCCTG R：GAGACCTCAGGGTTGGTGATCAG	5	65.9
HEL1	F：CAACAGCTATTTAACAAGGA R：AGGCTACAGTCCATGGGATT	15	54.0
HEL13	F：TAAGGACTTGAGATAAGGAG R：CCATCTACCTCCATCTTAAC	11	51.8
HEL5	F：GCAGGATCACTTGTTAGGGA R：AGACGTTAGTGTACATTAAC	21	54.0
HEL9	F：CCCATTCAGTCTTCAGAGGT R：CACATCCATGTTCTCACCAC	8	51.8
INRA005	F：CAATCTGCATGAAGTATAAATAT R：CTTCAGGCATACCCTACACC	12	58.5
TGLA126	F：CTAATTTAGAATGAGAGAGGCTTCT R：TTGGTCTCTATTCTCTGAATATTCC	20	58.8
TGLA227	F：CGAATTCCAAATCTGTTAATTTGCT R：ACAGACAGAAACTCAATGAAAGCA	18	54.0
BM315(215)	F：TGGTTTAGCAGAGAGCACATG R：GCTCCTAGCCCTGCACAC	5	65.0

index (*F*) were calculated using the following equations:

$$N_e = 1/\sum_{i=1}^{m} p_i^2 \quad h=1-\sum_{i=1}^{m} p_i^2,$$

$$H=\sum_{i=1}^{m} h/L \quad V(p_{ij}) = p_i(1-p_{ij})/[2(n-1)],$$

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

$$F=(f_{\text{expected}} - f_{\text{observed}})/f_{\text{expected}}.$$

Where, *m* is the allele number of a microsatellite locus; *pi*, *pj* the frequency of the *i*th and *j*th allele of a locus; *pij* the frequency of the *i*th allele of the *j*th locus; *L* the number of loci, and f_{expected} and f_{observed} are the expected frequency and the observed frequency of heterozygote.

2 Results

2. 1 Detection of microsatellite polymorphisms

After amplification, PCR products were first examined with 1% agarose gel, and if the amplification was satisfactory, 3–4 μL of the samples were loaded onto 8% nondenaturing polyacrylamide gel for further analysis (Figs. 1 and 2).

2. 2 Alleles and allele frequency distribution

All 12 microsatellite loci were polymorphic in the Qinchuan cow population (Fig. 3 and Table 2). Total 247 alleles were detected from the 12 microsatellite loci examined. The number of alleles per locus ranged from 13 (*INRA005*) to 33 (*HEL13*), giving a mean number of 21 alleles per locus. The

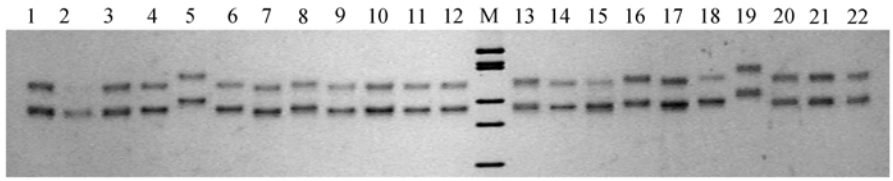

Fig. 1 8% PAGE electrophoresis of PCR products at microsatellite *TGLA126* locus in Qinchuan cows
M: DNA marker pBR322 DNA/*Msp* Ⅰ; 1−22: samples.

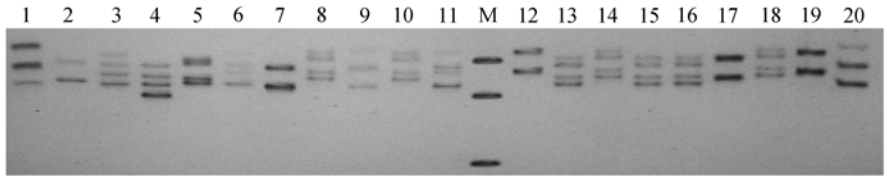

Fig. 2 8% PAGE electrophoresis of PCR products at microsatellite *HEL9* locus in Qinchuan cows
M: DNA marker pBR322 DNA/*Msp* Ⅰ; 1−20: samples.

Table 2 Allele numbers, allele size ranges, most frequent alleles and their frequencies, and effective allele numbers (*Ne*) **of 12 microsatellite loci in Qinchuan cow population**

Loci	Allele number of Qinchuan cattle	Allele number in European cattle breeds	Common allele number in Qinchuan cattle and European cattle breeds	Sampling variance of allele frequency	Number of effective alleles	Allele size (bp)		Most frequent alleles	
						Minimum	Maximum	Alleles	Frequencies
BM1824	17	9	5	3.0057E-04	11.0392	183	219	189	0.1534
BM2113	19	10	7	2.7226E-04	12.5800	128	166	144	0.1280
CSSM66	24	14	13	2.1569E-04	12.7143	179	231	183	0.1517
ETH152	22	11	11	2.3863E-04	15.1783	185	235	215	0.1037
HEL1	15	9	9	3.3460E-04	9.3740	101	129	107	0.1854
HEL13	33	9	9	1.6082E-04	18.0578	142	220	186	0.1200
HEL5	17	10	8	2.9873E-04	10.3962	141	187	161	0.1970
HEL9	22	13	11	2.3792E-04	14.5699	153	191	155	0.1098
INRA005	13	4	3	3.4358E-04	4.8818	139	171	147	0.2819
TGLA126	17	9	8	2.8510E-04	7.2809	113	147	121	0.1778
TGLA227	21	15	15	2.4103E-04	10.1275	67	111	87	0.2031
BM315	27	15	0	1.9536E-04	16.4230	108	174	124	0.0988
Total	247	128	107	–	142.6229	–	–	–	–
Means	21	10	9	2.6036E-04	11.8852	–	–	–	–

total effective allele number and mean effective allele number per locus were 142.6229 and 11.8852. The mean sampling variance of allele frequency at the 12 microsatellite loci was 2.6036×10^{-4}, which was very low, indicating that the allele frequency sampling estimation at each microsatellite locus was accurate and did reflect the genetic characteristics of the Qinchuan cattle.

At every microsatellite locus, allele size range was distinctive. And at every locus, there was a most frequent allele. At *INRA005* and *TGLA227*, the most frequent allele was 147 and 87, which had an allele

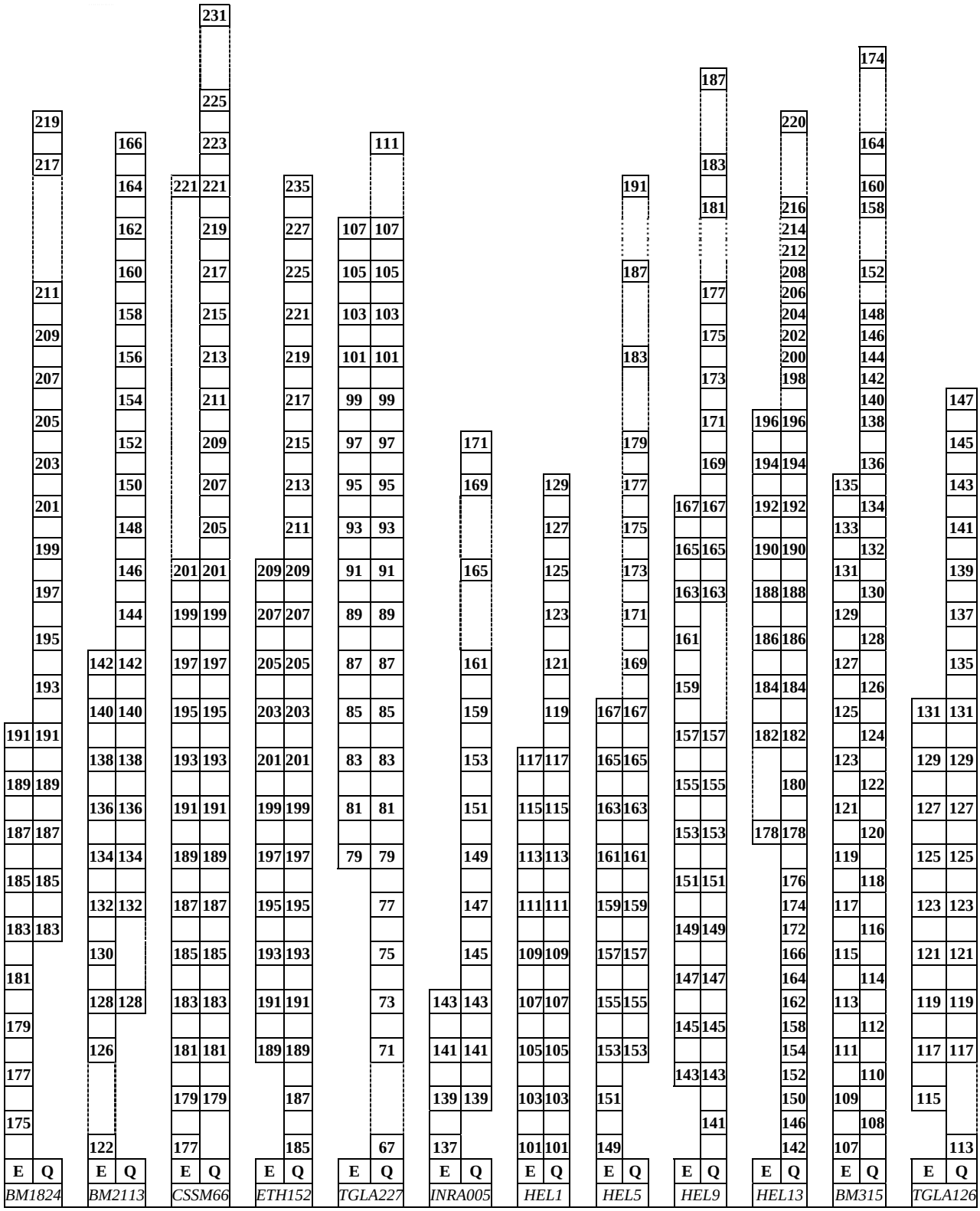

Fig. 3 Alleles of 12 bovine microsatellites in Qinchuan cattle (Q) **mapped against allele identified in European cattle breeds** (E)

The numbers in the figure mean the alleles with different sizes (bp).

frequency of 0.2819 and 0.2031, respectively.

2. 3 Population genetic characteristics of microsatellite loci

Observed heterozygosity, expected heterozygosity, observed homozygosity, expected homozygosity, mean heterozygosity, polymorphism information content (*PIC*), and fixation index (*F*) in Qinchuan cow population were shown in Table 3.

Observed heterozygosity and expected heterozygosity at the 12 microsatellites in Qinchuan cow population were from 0.7842 (*INRA005*) to 0.9775 (*BM315*) and from 0.7952 (*BM315*) to 0.9446 (*HEL13*), respectively. Mean observed heterozygosity and mean expected heterozygosity were 0.9117 and 0.9047, respectively. Polymorphism information content (*PIC*) was from 0.7653 (*INRA005*) to 0.9420 (*HEL13*), and mean *PIC* was 0.8965. *PIC* is a parameter indicative of the degree of informativeness of a marker. Following the criteria of Botstein *et al*[19], in this study, all 12 microsatellite loci appeared to be highly informative (*PIC* > 0.5), According to the selective standard of the microsatellite loci[20], microsatellite loci ought to have at least four alleles to be considered useful for the evaluation of genetic diversity. Based on this criterion, the 12 microsatellite loci used in this study were useful for the evaluation of genetic diversity in Qinchuan cattle. These results imply that abundant genetic polymorphisms exist in the Qinchuan cattle. Of the 12 microsatellite loci, the fixation indices of *BM1824*, *ETH152*, and *INRA005* microsatellite loci were positive, and others were negative, The mean fixation indices was –0.0076, reflecting that the degree of heterozygote defect at these loci was not high and deviations from Hardy-Weinberg equilibrium were not significant.

3 Discussion

The study of genetic polymorphism is the basis for any animal breeding program. The first step in a effective breeding or conservation program is accurate evaluation of available genetic resources, and microsatellite analysis is a well-established tool for measuring the genetic polymorphisms in a population. The microsatellite loci analyzed in this study were

Table 3 Observed heterozygosity, expected heterozygosity and observed homozygosity, expected homozygosity, polymorphic information content (*PIC*), and fixation indices (*F*) in Qinchuan cow population

Loci	Observed heterozygosity	Expected heterozygosity	Observed homozygosity	Expected homozygosity	*PIC*	Fixation indices
BM1824	0.8637	0.9094	0.1363	0.0906	0.9024	0.0503
BM2113	0.9390	0.9205	0.0610	0.0795	0.9149	–0.0201
CSSM66	0.9214	0.9213	0.0786	0.0787	0.9161	–0.0001
ETH152	0.8902	0.9341	0.1098	0.0659	0.9302	0.0470
HEL1	0.9427	0.8933	0.0573	0.1067	0.8839	–0.0553
HEL13	0.9526	0.9446	0.0474	0.0554	0.9420	–0.0085
HEL5	0.9078	0.9038	0.0922	0.0962	0.8965	–0.0044
HEL9	0.9662	0.9314	0.0338	0.0686	0.9272	–0.0374
INRA005	0.7842	0.7952	0.2158	0.2048	0.7653	0.0138
TGLA126	0.8836	0.8627	0.1164	0.1373	0.8501	–0.0242
TGLA227	0.9115	0.9013	0.0885	0.0987	0.8937	–0.0113
BM315	0.9775	0.9391	0.0225	0.0609	0.9358	–0.0409
Mean	0.9117	0.9047	0.0883	0.0953	0.8965	–0.0076

referenced to European cattle breeds proposed by CaDBase. A total of 247 alleles from the 12 microsatellite loci were detected in Qinchuan cattle with the mean allele number per locus being 21, which was considerably higher than the 8.4 reported by MacHugh *et al*. [10] and this probably reflected a bias in the selection of loci in different breeds, which had been preselected for polymorphisms. A total of 107 alleles in Qinchuan cattle shared with European cattle, suggesting that Qinchuan cattle share the same phylogenic origin with European cattle. Compared with the 128 examined alleles found in European cattle breeds, 247 alleles were found in Qinchuan cattle, implying that genetic polymorphisms in Qinchuan cattle were more abundant than European cattle. Most European cattle breeds experienced extensive selection and inbreeding; therefore, some low-frequency alleles at most microsatellite loci might have been lost but are still preserved in Qinchuan cattle. At *BM315* locus, there were no common alleles between Qinchuan cattle and European cattle breeds. Additional studies are needed to explain this result. Genetic and archaeological evidences support at least two domestications events from different wild progenitor aurochs races. *Bos taurus*, also termed taurine cattle, are postulated to have domestic origins in the Near East and Africa, whereas *Bos indicus*, or zebu, arose in India[10,21]. Previous studies[14,15] have shown that Chinese indigenous cattle also originated from European cattle (*B.taurus*) and Indian zebu (*B. indicus*). Several microsatellite alleles in Qinchuan cattle could be interpreted as a result of retention of more alleles from the original ancestor and with fewer alleles being lost in migration and evolution. Moreover, possible introgression of zebu alleles from the zebu to Qinchuan cattle may have contributed to the increased polymorphisms. Allelic size distributions of Indian zebu are possibly distinct from those in taurine animals[10]. It should be noted that, MacHugh *et al.*[10] reported that allele of 191 and 193 bp at locus *ETH152* can be used as the diagnostic alleles for zebu (*B.indicus*), because in their study these two alleles were only found in African zebu (*B.indicus*) and Indian zebu (*B. indicus*), but did not exist in European cattle breeds. However, these two alleles do exist in European cattle breeds in the CaDBase, and they were also found in Qinchuan cattle in this study. This discrepancy possibly resulted from the smaller sampling sizes (30−40 per breed), in which some low-frequency alleles were possibly lost. Because sample size variation has a much greater effect on highly polymorphic loci than on less polymorphic loci [22], to detect more low-frequency alleles at polymorphic loci similar to those in this study, sample size larger than 30−40 microsatellite loci is definitely required.

Genotype data from 12 microsatellites typed in 90 Qinchuan cows displayed a relatively high heterozygosity and *PIC* compared with European cattle breeds [11, 23, 24], and other Chinese cattle breeds [25, 26]. Since the early 19th century, when the concept of breed grew in popularity, many European cattle breeds have become genetically isolated and in most cases their origins could be traced to a small pool of founder individuals. Chinese Holstein has also possibly experienced a similar breeding practice. Simple genetic background and inbreeding contribute to the loss of genetic variation, which is demonstrated as fewer alleles and lower *PIC* [27]. In the case of Qinchuan cattle, during the long history of selection and breeding, crossbreeding and inbreeding in central preservation groups were strictly prohibited and some extensive breeding programs for specialized breeding direction have not been completely undertaken for long periods. Compared with the specialized European cattle breeds, more alleles at most microsatellite loci have been preserved. The abundant genetic polymorphisms affords an opportunity to improve the defects that exist in Qinchuan cattle, such as low daily gain, low dressing percentage and low milk yield, to suffice the needs of the rapidly developing beef industry.

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秦川母牛群体遗传特性的微卫星标记研究

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摘 要：为了从DNA分子水平揭示秦川牛群体遗传多态性和群体遗传结构，寻找可用于秦川牛的微卫星标记，本研究选择了 12 个普通牛（*Bos taurus*）微卫星标记检测了 90 头秦川母牛各微卫星位点的遗传变异及多态性。结果表明，在秦川母牛群体中，12 个微卫星位点共检测到了 247 个等位基因，各位点的等位基因数在 13（*INRA005*）～33 个(*HEL13*)之间，平均每个微卫星位点的等位基因数为 21 个；总有效等位基因数和平均每个位点平均有效等位基因数（*Ne*）分别分为 142.6229 和 11.8852。各位点平均基因频率取样方差($V_{(p_{ij})}$)为 2.6036×10^{-4}。12 个微卫星位点平均观察杂合度(*Ho*)和平均期望杂合度(*He*)在 0.7842（*INRA005*）～0.9775（*BM315*）和 0.7952（*BM315*）～0.9446（*HEL13*）之间。12 个位点平均多态信息含量（*PIC*）在 0.7653（*INRA005*）～0.9420（*HEL13*）之间，平均为 0.8965. 12 个微卫星位点均属于高度多态位点，这表明秦川母牛群体中所检测各微卫星位点具有丰富的遗传多态性，具备较大的选择潜力。12 个微卫星位点的平均固定指数（*F*）为−0.0076，即各位点杂合子的缺陷度不高，即偏离Hardy-Weinberg 平衡的程度不大。

关键词：秦川母牛；微卫星 DNA；多态性

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