

African Journal of Biotechnology Vol. 10(42), pp. 8523-8528, 8 August, 2011
Available online at <http://www.academicjournals.org/AJB>
DOI: 10.5897/AJB10.2717
ISSN 1684-5315 © 2011 Academic Journals

Full Length Research Paper

Screening of seven microsatellite markers for litter size in Xinong Saanen dairy goat

Guang-qin Zhu, Yi-hong Cui, Yu-xuan Song, Jian-gang Wang and Bin-yun Cao*

College of Animal Science and Technology, Northwest A&F University, Yangling, 712100, P. R. China.

Accepted 10 June, 2011

Seven microsatellite markers OarAE101, BM1329, OarHH55, BM143, BMS2508, OarAE129 and OarFCB11 closely associated with high reproduction trait in sheep were analyzed for polymorphisms in Xinong Saanen dairy goat. The results indicated that there were high genetic polymorphisms at six microsatellite loci. The number of effective alleles (N_e), polymorphism information content (PIC) and average heterozygosity (H_e) were the highest at OarFCB11 and the lowest at OarAE129 in Xinong Saanen dairy goat. The analysis of the effect of the six polymorphisms microsatellite loci on the litter size of Xinong Saanen dairy goat indicated that these polymorphisms microsatellite loci had positive effect on the litter size.

Key words: Microsatellite markers, Xinong Saanen dairy goat, genetic polymorphism, litter size.

INTRODUCTION

Microsatellites, also known as simple sequence repeats, are long stretches of a short (1 to 6 bp) tandemly repeated DNA unit, such as the motif CAA repeats 20 times. Microsatellites are common throughout eukaryotic genomes and their length are often highly polymorphic, making them powerful markers for use in genetic mapping (Weber, 1990; Dietrich et al., 1994; Dib et al., 1996; Roder et al., 1998), population genetics (Jarne and Lagoda, 1996; Di Rienzo et al., 1998; Thuillet et al., 2002) and determination of kinship (Queller et al., 1993).

Reproductive traits, which are under the control of multiple genes, are economically important traits in live-stock. To improve the fecundity, also known as litter size, is of special meaning in the selection of animals with high reproductively, especially for the animals raised by people as economic source. Litter size is a character with very low heritability, about 0.1 (Sun et al., 2010). Therefore, it is hard to use normal breeding methods to improve litter size and the marker-assisted selection may accelerate the goat breeding process.

Dairy goat farming is significant to the economics of the western China with characteristics of under-development and poverty. According to the comparative genomics, 5 microsatellite loci OarAE101, BM1329, OarHH55, BM143,

BMS2508 (Lord et al., 1996; Vaiman et al., 1996; Ihara et al., 2004) that are linked to the fecundity gene *FecB* in the sheep, also in goat and cattle chromosome 6, microsatellite locus OarAE129 at goat chromosome 7 (Penty et al., 1993; Vaiman et al., 1996) and OarFCB11 at goat chromosome 2 (Vaiman et al., 1996) which are closely associated with the high reproduction trait in sheep were selected to obtain useful molecular markers for Xinong Saanen dairy goats selection and breeding through markers-assisted selection (MAS).

MATERIALS AND METHODS

Animals and genomic DNA isolation

All procedures involving animals were approved by Qianyang Xinong Saanen goat breeding center in Shaanxi Province, China. A total of 196 Xinong Saanen female goats were examined in this study. Those goats which are in the 5 to 6 years old had the complete record of 1 to 4 parity birthed litter size. Five milliliters blood per goat were collected aseptically from the jugular vein and kept in a tube containing anticoagulant ACD (citric acid: sodium citrate: dextrose – 10:27:38). All samples were delivered back to the laboratory in an ice box. The genomic DNA was extracted from white blood cells using standard phenol-chloroform extraction protocol (Joseph and David, 2002; Wu et al., 2009).

Information of primer sequences and 7 microsatellite loci

Primers were selected based on the Boroola sheep data and

*Corresponding author. E-mail: caobinyun@yahoo.com.cn. Tel: +86-029-87092102. Fax: +86-029-87092164

Table 1. Data of seven microsatellite loci.

| Locus | Primer sequence (5'→3') | Resource | Annealing temperature (°C) |
|----------|--|----------|----------------------------|
| OarAE101 | F:TTCTTATAGATGCACTCAAGCTAGG R:TAAGAAATATATTTGAAAAAGTGATCTCCC | Sheep | 63 |
| BMS1329 | F:TTGTTTAGGCAAGTCCAAAGTC R:AACAACGCAGCTTCATCC | Sheep | 62 |
| OarHH55 | F:GTTATTCCATATTCTTCTCCATCATAAGC R:CCACACAGACAACATAAAACCCAGC | Sheep | 64 |
| BM143 | F:ACCTGGGAAGCCTCCATATC R:CTGCAGGCAGATTCTTTATCG | Sheep | 63 |
| BMS2508 | F:TTTCTGGGTTTACAAAATGCTC R:TTTCTTAGGGGAGTGTGATTC | Sheep | 54 |
| OarAE129 | F: AATCCAGTGTGTGAAAGACTAATCCAG R: GTAGATCAAGATATAGAATATTTTCAACACC | Sheep | 64 |
| OarFCB11 | F: GGCCTGAACTCACAAGTTGATATATCTATCAC R: GCAAGCAGGTTCTTTACCACTAGTAGCACC | Sheep | 65 |

synthesized by Sangon Biotech Company, Shanghai, China. The information of primer sequences and the amplified loci are included in Table 1. Five microsatellites (OarAE101, BM1329, BM143, OarHH55 and BMS2508) linked with fecundity gene on the 6th chromosome, one microsatellite (OarFCB11) linked with production gene on the 2th chromosome and one microsatellite (OarAE129) linked with production gene on the 7th chromosome were used.

PCR conditions

The 12 µl PCR amplification system contains 50 ng genomic DNA, 10 pM each primer, 1.2 µl dNTPs (0.25 mM each), MgCl₂ (4.0 or 3.0 mM) and 0.5U *Taq* DNA polymerase (MBI). The cycling procedure is as follows: 4 min at 95°C, 35 cycles at 94°C for 30 s, annealing at 54 to 65°C corresponding to 7 different primer pairs for 1 min, 72°C for 1.5 min, with a final extension at 72°C for 10 min. Polymorphisms of 7 microsatellite loci were detected by 12% PAGE (82×82×1.0 mm) using constant voltage (180 V) for 2.0 to 2.5 h. Silver staining was used to detect DNA fragment (Ji et al., 2007).

Statistical analysis

The average heterozygosity (*He*), the effective numbers of alleles (*Ne*) and the polymorphism information content (*PI*) of 7 microsatellite loci in Xinong Saanen dairy goats were analyzed by the cluster analysis software (this software was developed by Poultry Institute of the Chinese Academy of Agricultural Science).

The relationships and differences between 7 microsatellite loci and litter size in Xinong Saanen dairy goats were analyzed using GLM (general linear model) method, which was performed by SPSS software (version 11.5). Linear model:

$$Y_i = \mu + M_i + e_i$$

Where, Y_i is the litter size trait measured on each of the i^{th} animal; μ

is the overall population mean; M_i is the fixed effects associated with i^{th} genotype and e_i is the random error.

This study only used the average litter size of the first parity to fourth parity. So effects associated with parity and season of birth (spring versus fall) are not matched in the linear model, as the preliminary statistical analyses indicated that season effects did not have a significant influence on variability of traits in the analyzed breed.

RESULTS

The figures are results of electrophoresis at 7 microsatellite loci. We can see that 6 microsatellite loci have polymorphisms in Xinong Saanen dairy goat from figures 1 to 7 except for the BMS2508 microsatellite locus.

The allele frequencies of 6 polymorphisms microsatellite loci

In Xinong Saanen dairy goat, 8 alleles of OarAE101 microsatellite locus were found with fragment size from 109 to 137 bp, the maximum frequency is 0.25 of 114 bp; 7 alleles of OarAE129 microsatellite locus were found with fragment size from 155 to 205 bp, the maximum frequency is 0.3878 of 205 bp; 10 alleles of BM1329 and OarHH55 microsatellite loci were found, with fragment size from 182 to 234 bp and 125 to 170 bp at BM1329 and OarHH55 microsatellite locus, respectively, the maximum frequency is 0.1480 of 225 and 190 bp at BM1329 microsatellite locus and 0.1837 of 165 bp at

Table 2. The allele frequencies and fragment size of 6 polymorphisms microsatellite loci.

| Locus | Alleles (bp) | Frequency | Locus | Alleles (bp) | Frequency |
|----------|--------------|-----------|----------|--------------|-----------|
| OarAE101 | 137 | 0.1276 | OarAE129 | 205 | 0.3878 |
| | 135 | 0.1633 | | 200 | 0.0765 |
| | 132 | 0.0867 | | 180 | 0.0357 |
| | 129 | 0.0459 | | 178 | 0.1276 |
| | 127 | 0.0765 | | 170 | 0.1939 |
| | 116 | 0.1276 | | 165 | 0.1429 |
| | 114 | 0.2500 | | 155 | 0.0357 |
| | 109 | 0.1224 | | | |
| BM1329 | 234 | 0.0459 | OarHH55 | 170 | 0.1224 |
| | 225 | 0.1480 | | 165 | 0.1837 |
| | 223 | 0.1173 | | 160 | 0.0918 |
| | 219 | 0.0918 | | 155 | 0.1020 |
| | 215 | 0.0969 | | 150 | 0.0510 |
| | 197 | 0.0459 | | 145 | 0.1173 |
| | 190 | 0.1480 | | 140 | 0.1633 |
| | 187 | 0.1173 | | 135 | 0.1020 |
| | 185 | 0.0918 | | 130 | 0.0357 |
| | 182 | 0.0969 | | 125 | 0.0306 |
| BM143 | 140 | 0.0408 | OarFCB11 | 215 | 0.0816 |
| | 137 | 0.1071 | | 209 | 0.0663 |
| | 135 | 0.0816 | | 207 | 0.0918 |
| | 132 | 0.2041 | | 205 | 0.0867 |
| | 129 | 0.0612 | | 199 | 0.0612 |
| | 124 | 0.0612 | | 197 | 0.0816 |
| | 120 | 0.1071 | | 190 | 0.0459 |
| | 119 | 0.0459 | | 188 | 0.0663 |
| | 115 | 0.2041 | | 185 | 0.1582 |
| | 110 | 0.1276 | | 180 | 0.0867 |
| 105 | 0.0204 | 160 | 0.0459 | | |
| | | 156 | 0.1276 | | |

OarHH55 microsatellite locus; 11 alleles of BM143 microsatellite locus were found with fragment size from 105 to 140 bp, the maximum frequency is 0.2041 of 132 and 115 bp; 12 alleles were found at OarFCB11 microsatellite locus, which is the most one with fragment size from 156 to 215 bp and the maximum frequency is 0.1582 of 185 bp (Table 2). So, we could see that the degree of variation at OarFCB11 microsatellite locus is maximum and at OarAE129 microsatellite locus is minimum. These 6 microsatellite loci can be used for genetic diversity assessment of Xinong Saanen dairy goat with significant polymorphisms.

Genetic information of the 6 polymorphic microsatellite loci

The average heterozygosity (H_e), number of effective

alleles (N_e), polymorphism information content (PIC) are measured to reflect the genetic variation within groups. N_e , PIC and H_e are all used to evaluate population genetic diversity (Sun et al., 2010).

The PIC , N_e and H_e of 6 polymorphic microsatellite loci in Xinong Saanen dairy goats is OarFCB11>BM1329>OarHH55>BM143>OarAE101>OarAE129 (Table 3), which show that the PIC , N_e and H_e have a positive correlation in Xinong Saanen dairy goats with the same order.

The litter size effect of the 6 polymorphic microsatellite loci in Xinong Saanen dairy goat

The linear model analysis results show that the 6 polymorphic microsatellite loci have significantly different effects on litter size trait at 0.01 level which the allele 109

Table 3. The average heterozygosity (*He*), effective numbers of alleles (*Ne*) and polymorphism information content (*PIC*) of 6 microsatellite locus in Xinong Saanen dairy goat.

| Parameter | OarAE101 | BM1329 | OarHH55 | BM143 | OarAE129 | OarFCB11 |
|------------|----------|--------|---------|--------|----------|----------|
| <i>PIC</i> | 0.8301 | 0.8781 | 0.8642 | 0.8481 | 0.7373 | 0.8974 |
| <i>Ne</i> | 6.7513 | 8.9925 | 8.1149 | 7.2932 | 4.2913 | 10.5423 |
| <i>He</i> | 0.8478 | 0.8888 | 0.8768 | 0.8629 | 0.7669 | 0.9052 |

Table 4. Least squares means±standard deviation of 6 microsatellites genotypes for litter size effect in Xinong Saanen dairy goat.

| Locus | Genotype/bp | Least square means±SD | Locus | Genotype/bp | Least square means±SD |
|-------------|-----------------------------|-------------------------------|-----------------------------|-------------|-----------------------------|
| OarAE101 | 137/116(50) | 1.6072±0.3119 ^a | BM1329 | 234/197(18) | 2.0444±0.3548 ^a |
| | 135/114(64) | 1.6750±0.3327 ^a | | 225/190(58) | 1.6407±0.3388 ^b |
| | 132/114(34) | 1.7329±0.3623 ^{ab} | | 223/187(46) | 1.7196±0.3591 ^{ab} |
| | 129/109(18) | 2.0300±0.3684 ^b | | 219/185(36) | 1.6200±0.3150 ^b |
| | 127/109(30) | 1.7500±0.2707 ^{ab} | | 215/182(38) | 1.7405±0.2561 ^{ab} |
| OarHH55 | 170/150(20) | 1.7020±0.3564 ^{ab} | BM143 | 140/124(6) | 1.7233±0.2542 ^{ab} |
| | 170/145(28) | 1.6129±0.2376 ^{ab} | | 140/119(10) | 1.7340±0.2526 ^{ab} |
| | 165/145(18) | 1.7644±0.2983 ^{ab} | | 137/120(42) | 1.6929±0.3394 ^a |
| | 165/140(42) | 1.8390±0.3527 ^a | | 135/115(32) | 1.6563±0.3302 ^a |
| | 165/125(12) | 1.5000±0.3536 ^b | | 129/115(48) | 1.6500±0.3069 ^a |
| | 160/140(22) | 1.6182±0.3345 ^{ab} | | 129/110(32) | 1.6619±0.3943 ^a |
| | 160/130(14) | 1.7043±0.3219 ^{ab} | | 124/110(18) | 2.0478±0.3524 ^b |
| 155/135(40) | 1.7465±0.3906 ^{ab} | 119/105(8) | 1.8125±0.2394 ^{ab} | | |
| OarAE129 | 205/178(12) | 2.3250±0.6556 ^{Aa} | OarFCB11 | 215/197(32) | 1.6438±0.3327 ^a |
| | 205/170(12) | 1.8467±0.5.60 ^{ABab} | | 209/188(26) | 1.9946±0.3695 ^b |
| | 205/165(50) | 1.8788±0.2165 ^{ABab} | | 207/184(36) | 1.6889±0.3711 ^{ab} |
| | 200/178(38) | 1.6679±0.1923 ^{ABb} | | 204/180(34) | 1.6088±0.3174 ^a |
| | 200/170(64) | 1.5469±0.1668 ^{Bb} | | 199/157(24) | 1.6967±0.2678 ^{ab} |
| | 200/165(14) | 1.5700±0.3360 ^{Bb} | | 190/160(18) | 1.7222±0.2471 ^{ab} |
| | 180/155(6) | 1.4167±0.2230 ^{Bb} | | 184/157(26) | 1.6477±0.3301 ^a |

The numbers in the brackets are the individuals that belong to the respective genotypes; and different significances are shown with capital and lower case letters at 0.01 and 0.05 levels respectively.

bp at OarAE101, 165 and 140 bp at OarHH55, 124 bp at BM143, 205 bp at OarAE129, the genotype 219/185 bp at BM1329, 209/188 bp at OarFCB11 have positive effects to litter size (Table 4).

DISCUSSION

Genetic diversity analysis of the 6 polymorphic microsatellite loci in Xinong Saanen dairy goat

Polymorphism information content (*PIC*) value is defined as the probability that a given marker genotype of an offspring of an affected parent will allow deduction of the parental genotype at marker locus. The gene locus is highly informative if $PIC > 0.5$, reasonably informative if $0.25 < PIC < 0.5$ and slightly informative if $PIC < 0.25$

(Botstein et al., 1980). According to this theory, the 6 microsatellite loci are highly informative and this result is the same with the polymorphisms at microsatellite loci OarAE101 and BM1329 in small tail Han sheep (Chu et al., 2002) and also the same with the polymorphisms at microsatellite locus OarAE101 in three goat breeds (Boer goat, Taihang goat and Hebei dairy goat) (Zhang et al., 2003).

Heterozygosity (*He*) also known as genetic diversity is defined as the probability that a random individual chosen from the population is heterozygous at a locus (Shete et al., 2000). It is generally believed that it is optimal parameter for genetic variation metric of groups. In 6 polymorphic microsatellite loci in our study, the maximum heterozygosity is 0.8974 of OarFCB11 microsatellite locus and the minimum heterozygosity is 0.7373 of OarAE129 microsatellite locus.

The effective number of allele (N_e) is the reciprocal of purity; it showed the interaction between the allele. The more uniform the alleles distributed in the group, the more close the number of effective alleles to the absolute number of detected allele. In this study, the effective number of alleles (N_e) ranges from 4.2913 (OarAE129) to 10.5423 (OarFCB11) at 6 polymorphic microsatellite loci (OarAE101, BM1329, OarHH55, BM143, OarAE129 and OarFCB11).

The importance of goat and producing ability of female goats

The world goat population amounts to 550 million, 94% of which are found in developing countries. Around the Mediterranean, in Asia and Africa, goats provide milk, meat and, to a lesser extent, wool and hides. Furthermore, in a few European countries, the making of goat cheese is of major interest, motivating the setting of genetic improvement programs for this species. Goat meat is also consumed in many regions of the world (Daniel et al., 1996). There are 135.92 million goats in China and the Chinese indigenous goat breeds are a valuable resource in the world goat population.

The profits of goat production is dependent on litter size and it is very difficult to improve litter size by normal breeding methods since its heritability only about 0.1 (Sun et al., 2010). But the Australian Booroola merino sheep has been clearly known with the fecundity traits. The fecundity gene *FecB* (*Fec*=fecundity, *B*=Booroola) was located on 6th chromosome (Piper and Bindon, 1982; Davis et al., 1982, 1983; Gognosag, 1989), it was pre-cisely located in the area of 10 cM between OarAE101 and BM1329 (Montgomery et al., 1993, 1994; Lord et al., 1998). But it is hard to use normal breeding methods to improve litter size of goat; the maker-assisted selection may accelerate the goat breeding process. However, currently, few molecular researches on litter size of milk goat have been conducted. Litter size of goat is affected by many exterior factors as physiological condition, nutrition level and sperm quality of ram. Phenotypic value of fecundity do not pass down truly, thus, it is not very efficient to use correction between marker and phenotypic value.

The producing ability of the female goat's genetic potentiality on a repeatable character can be obtained by multiple measurement of litter size and it is indeed an important reference to select excellent ewe. The data of producing ability of ewe can be used to evaluate ewe's potentiality on litter size.

Thus, choosing by combining producing ability and special gene (gene type) will play a great role in improving selecting accuracy and breeding progress and is a wonderful and new analyzed thought in repeatable characters with low heritability which is affected by measuring frequencies.

Litter size effect of the 6 polymorphic microsatellite loci in Xinong Saanen dairy goat

In Australian Booroola Merino sheep, the hyper-prolificacy is because of the action of a single autosomal gene (*FecB*) that influences the number of ovulations per estrous cycle in the ewe (Davis et al., 1982; Piper et al., 1985). By genetic analysis of 31 informative half-sib families from heterozygous sires, the results show that the *FecB* locus is situated in the region of ovine chromosome 6 corresponding to the human chromosome 4q22–23 (Mulsant et al., 2001). The microsatellite loci BM1329, OarAE101 and a third microsatellite marker (unpublished) which maps between BM1329 and OarAE101, were used to detect the inheritance of the chromosome region around the *FecB* locus. Genotypes could be determined only when there was a known relationship between the chromosome region, as defined by marker alleles and *FecB*. The proximal half sheep chromosome 6 genetic linkage map that show the order of microsatellite markers and *FecB* is: McM53, OarJLIA, CENPE, BM1329, *FecB*, OarAE101, OarHH55, BM143 and OarJMP36 (Lord et al., 1996, 1998). These microsatellite loci have been used as candidate molecular markers for sheep litter size trait.

In 5 microsatellite loci (OarAE101, BM1329, BM143, OarHH55 and OarHH35) for the litter size of small tailed Han sheep, the OarAE101 is the best one, BM1329 is better than BM143 and OarHH35, no other significant markers were discovered at OarHH55 (Lei et al., 2003). At the OarHH35 locus, the allele of 135 and 125 bp had significant positive effects on litter size in Xiangdong black goat. At BMS2508 locus, the allele of 145 and 93 bp had significant effects on the litter size of Xiangdong black goat and had significant negative effects to the litter size of Xiangdong black goat at the allele of 122 bp. At OarAE101 locus, the allele 107, 109, 111, 119 and 125 bp had positive effects for the litter size in Xiangdong black goat. At BM143 locus, there were positive effects among the allele 104, 106 and 110 bp for the litter size of Xiangdong black goat (Ouyang et al., 2006).

The statistical results revealed different significance between litter size trait and all genotypes of 6 polymorphic microsatellite loci ($P < 0.05$) only at OarAE129 microsatellite locus the results have different significance at 0.01 level. Our study indicate that the allele 109 bp at OarAE101, 165 and 140 bp at OarHH55, 124 bp at BM143, 205 bp at OarAE129, the genotype 219/185 bp at BM1329, 209/188 bp at OarFCB11, have positive effect on litter size trait.

Conclusion

Seven microsatellite markers: OarAE101, BM1329, OarHH55, BM143, OarAE129 and OarFCB11 could be used to study genetics in Xinong Saanen dairy goat

diversity. The result of variance analysis showed that there were extreme significant difference among the different genotypes for the producing ability of litter size in the 6 polymorphic microsatellite loci that have significantly different effects on litter size trait at 0.01 level, while the allele 109 bp at OarAE101, 165 and 140 bp at OarHH55, 124 bp at BM143, 205 bp at OarAE129, the genotype 219/185 bp at BM1329, 209/188 bp at OarFCB11 have positive effects on litter size.

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