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Practical Applications of Microsatellite Markers in Goat Breeding

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Abstract

To date, the genetic loci associated with disease and economic traits have been identified in livestock based on linkage analysis or genome-wide association studies. These analyses require the use of numerous genetic markers, of which microsatellites have been utilized most extensively because they allow for the easy genotyping of allelic variation at each locus using PCR. In the domestic goat (*Capra hircus*), microsatellite markers are powerful tools for various genetic studies, such as the estimation of intra- and interpopulation genetic diversity, linkage analyses of phenotypic traits, and marker-assisted selection of favorable phenotypes; however, the studies on goats are less extensive than those on other major livestock. The aim of this chapter is to summarize the currently available information on goat breeding using microsatellite markers. In particular, we use various studies, including our own recent work, to illustrate how these markers may be used to identify phenotypic traits.

Keywords: animal breeding, domestic goats, linkage analysis, population structure, quantitative trait locus

1. Introduction

Over the past few decades, several genetic markers have been developed and have contributed to the progress of various biological fields. Microsatellites, which are composed of between one and six nucleotide repeats, are some of the most frequently used genetic markers for genomics [1, 2]. In animal breeding, microsatellite markers have become valuable tools for the estimation of population genetic structure [3–5] and for marker-assisted selection based on the genetic mapping of disease and economic traits [6]. Although single nucleotide polymorphism (SNP)

markers are widely used for genetic studies of livestock, microsatellite markers are still in great demand worldwide because they can be identified using simple detection protocols. In addition, microsatellites have several advantages, such as a high level of polymorphism, a codominant mode of inheritance, and high reproducibility [2].

The domestic goat (*Capra hircus*) was one of the first animals to be domesticated, with domestication occurring approximately 10,000 years ago [7, 8]. The domestic goat is bred worldwide as an important resource for animal products, such as meat, milk, and coat, particularly in China, India, and other developing countries [9, 10]. Despite this economic importance, considerably fewer genetic studies have been conducted on goat breeding than on the breeding of other livestock. This is largely because the genomic information available for goats is scarce and of low quality. In particular, information regarding the number and chromosomal location of goat genetic markers is limited. Although in a previous study we developed a large number of new microsatellite markers [11], these were insufficient for linkage analyses of phenotypic traits. Recently published studies have reported on whole-genome mapping technologies [12] and on the sequencing data of the goat genome obtained by integrating next-generation sequencing [10]. Therefore, it is now easier to conduct linkage analyses for phenotypic traits using these genome resources.

In the following sections, we will critically discuss the information on and advantages and applications of some of the important aspects of microsatellite markers to genetic studies and the breeding of domestic goats, including (1) the development of markers, (2) the characterization of intrapopulation and interpopulation genetic diversity, (3) linkage analyses of disease and economic traits, and (4) marker-assisted selection using microsatellites.

2. Development of microsatellite markers in goats

Several researchers have developed microsatellite markers for goats. In the initial development, several polymorphic microsatellite loci were screened from the goat genomic library using hybridization with an end-labeled microsatellite probe [13–16]. However, many researchers used the microsatellite markers developed in cattle and sheep genetic studies because several markers were available through sequence conservation among the Artiodactyls. Luikart et al. [17] reported that nine microsatellite markers from cattle and five from sheep were useful for parentage testing in goats. Moreover, a large number of microsatellite markers derived from cattle and sheep were used to construct the first goat linkage map [18]. This linkage map was constructed using 612 microsatellite markers, and most were markers from cattle (approximately 80%) and sheep (approximately 18%).

Although some microsatellite markers from cattle and sheep can be utilized for goats, few goat genetic markers have been identified for use in genetic studies. Therefore, we sought to develop new microsatellite markers that are derived from the goat genome. Many methodologies for obtaining microsatellite loci have been reported. The most common method is the combination of cloning small genomic fragments and hybridization with an end-labeled oligonucleotide probe, as mentioned above [13–16]. We also used this approach but found it ineffective,

probably because microsatellite repeats are less abundant within the goat genomic sequence. This lack of repeats indicates that the enrichment of genomic sequences, including microsatellites, is indispensable to the efficient isolation of the sequences. Although enrichment strategies have been developed for microsatellite screening, we used the protocol described by Glenn and Schable [19]. This protocol is based on linker ligation-mediated PCR using a unique SuperSNX linker. The amplification of DNA using the SuperSNX linker primer is biased against producing small PCR products, and PCR products obtained after enrichment can be cloned directly without contaminating a large proportion of the small DNA fragments [19]. We succeeded in developing 260 novel microsatellite loci using hybridization with biotinylated microsatellite oligonucleotide (TG)₁₂ or (AG)₁₂ probes [11]. These developed markers were composed of two types of repeat motifs containing interrupted DNA sequences: 15 markers contained compound repeats such as (CA)_n and (AT)_n, and 243 were composed of simple repeats such as dinucleotide motifs (239 markers), trinucleotide motifs (two markers), tetranucleotide motifs (one marker), and heptanucleotide motifs (one marker). These results were the most efficient of the protocols we used. We recommend this protocol for the isolation of DNA fragments, including microsatellites, if the genome sequence is not available.

In 2013, the ~2.66-Gb genome sequence of a female Yunnan black goat was reported [10]. The genome project is ongoing. The sequence can be downloaded from the Goat Genome database [20]. Moreover, the potential microsatellite sequences can be easily identified using web-based software such as MicroSAteLLite [21], and design primers can then be used to amplify the genomic region including microsatellites.

3. Characterization of intrapopulation and interpopulation genetic diversity in goats

Intrapopulation and interpopulation genetic diversity in livestock is required to produce food in diverse environments, allow sustained genetic improvement, and rapidly respond to changing breeding objectives [22]. In addition, intrapopulation and interpopulation genetic information is occasionally required to establish a novel strain that exhibits a similar phenotype. Microsatellite markers are often used to estimate genetic diversity because they have higher polymorphism and reproducibility than other genetic markers.

Several studies have analyzed genetic diversity in Asian goat populations. Goats are an important livestock in Asia. There are large numbers of individuals, populations, and breeds of goats in Asia [23]. Moreover, archeological analysis has revealed that the ancestor of domestic goats was initially domesticated in the Iranian Zagros Mountains [7], in the high Euphrates valley, and in Southeastern Anatolia [24], which is located in Western Asia. Wei et al. [25] investigated the genetic diversity of 40 goat populations in China using 30 microsatellite markers and revealed that the average number of alleles ranged from 4.33 to 8.23 and the expected and observed heterozygosity (H_E and H_O) ranged from 0.5070 to 0.7378 and from 0.4336 to 0.6730, respectively. Wei et al. [25] also reported that the Chinese goat population could be divided into at least four genetic clusters using phylogenetic analysis. In India, Rout

et al. [26] investigated microsatellite-based genetic diversity in seven Indian goat breeds using 17 markers and detected that the average number of alleles ranged from 0.739 to 0.783 and that the H_E of the goats they assessed ranged from 0.739 to 0.783. In addition, the authors also suggested that the seven populations of Indian goats could be classified into distinct genetic groups or breeds using microsatellite markers [26]. Nomura et al. [27] investigated genetic diversity in East Asian indigenous goat breeds derived from Korea, Taiwan, the Philippines, Indonesia, Bangladesh, and Mongolia using 26 microsatellite markers and found that the Mongolian indigenous goat population had higher genetic diversity than the other populations. Moreover, Nomura et al. [27] also revealed that Shiba goats, which were established as a small experimental breed in Japan, exhibited lower genetic diversity, indicating that this breed is composed of genetically homogeneous individuals.

In this study, we demonstrate the structural analysis of intrapopulation genetic diversity in goats. We analyzed native Korean and Japanese Saanen breed populations using microsatellite markers. The native Korean population used in this study is a closed herd due to more than 20 years of assortative mating [9]. By contrast, the Japanese Saanen population, which was established by mating native Japanese goats and European Saanen breeds, constitutes a large proportion of the dairy goats in Japan. **Table 1** shows general information on the genetic diversity and differentiation of the native Korean and Japanese Saanen breeds. The average number of alleles (N_A) was 3.09 and 4.82 in the native Korean and Japanese Saanen breeds, respectively. The average expected heterozygosity (H_E) and observed heterozygosity (H_O) in the native Korean breeds were 0.48 and 0.45, respectively. In the Japanese Saanen breed, the H_E and H_O were 0.64 and 0.57, respectively. Although within-population inbreeding (F_{IS}) of the native Korean breed was lower than in the Japanese Saanen breed, both the N_A and H_E/H_O in the native Korean breed indicate that the native Korean breed has lower genetic diversity than the Japanese Saanen breed. Therefore, the loss of genetic diversity in the native Korean breed through assortative mating was confirmed using this microsatellite-based analysis. In contrast, genetic differentiation between the native Korean and Japanese Saanen breeds was indicated by the among-population genetic differentiation (F_{ST}), which was highly significant ($P < 0.001$). In the STRUCTURE analysis [28], $K = 2$ was the most appropriate number of partitions [mean $\ln P(D) = -480.2$], indicating that the native Korean and Japanese Saanen breeds are clearly distinguished by large genetic differentiation (**Figure 1**).

Strain	N_A (SD)	H_E (SD)	H_O (SD)	F_{IS}	F_{ST}
Native Korean ($n = 9$)	3.09 (1.30)	0.48 (0.25)	0.45 (0.30)	0.035	0.34**
Saanen ($n = 9$)	4.82 (1.33)	0.64 (0.17)	0.57 (0.31)	0.107	

N_A , average number of alleles; H_E , expected heterozygosity; H_O , observed heterozygosity; F_{IS} , population inbreeding coefficient; and F_{ST} , population genetic differentiation.
** $P < 0.01$.

Table 1. Intra- and interstrain genetic diversity in native Korean and Japanese Saanen goat breeds calculated using polymorphisms of 11 microsatellite markers.

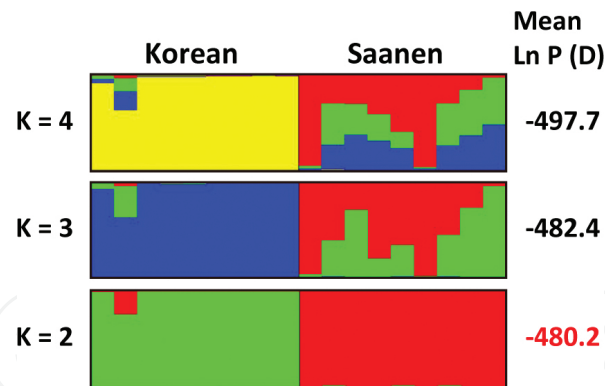


Figure 1. Clustering assignments based on the genotypes of 11 microsatellite markers in native Korean and Japanese Saanen goat breeds using STRUCTURE software ver. 2.3.4.

Figure 2 shows the results of the structural analysis of the interpopulation genetic diversity of the native Korean and Japanese Saanen populations, including four native goats [Indonesian, Mongolian, Bangladeshi, and Japanese (Shiba)] and a wild goat (Bezoar). A neighbor-joining tree, which was constructed based on the genetic distance among the individuals, was calculated using GenAlEx ver. 6.5 [29]. Similar to the results obtained using F_{ST} and STRUC-TURE, the native Korean and Japanese Saanen populations were distinctly clustered into different clades in the neighbor-joining tree (**Figure 2**). Therefore, our simple analysis using closed goat populations as a model demonstrates that microsatellite markers are a useful tool for estimating intrapopulation and interpopulation genetic diversity.

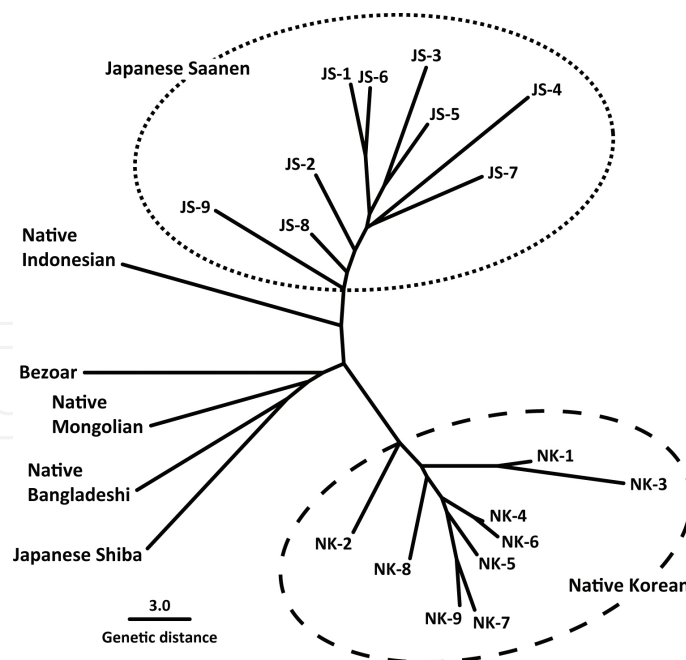


Figure 2. Neighbor-joining tree constructed based on genetic distance calculated using the genotypes of 10 microsatellite loci in domestic and wild goats. The genetic distance among 23 individuals was calculated using GenAlEx ver. 6.502, and the phylogenetic tree was constructed using the neighbor-joining method with the PHYLIP package. NK, native Korean and JS, Japanese Saanen breeds.

4. Linkage analysis of disease and economic traits using microsatellite markers in goats

In animal breeding, it is important to exclude deleterious traits and to select desirable traits. Linkage analysis is a powerful method for identifying the traits associated with disease and the productivity/quality of animal products. Microsatellite markers have provided the genotyping for linkage analysis.

Polled intersex syndrome (PIS) is the most profound genetic disorder in goats and results in the absence of horns in males and females and sex reversal that exclusively affects XX individuals [30]. The sex reversal of XX individuals leads to a reduction of milk production and reproductive efficiency in farmed goats. Based on the development of genomic tools such as microsatellite markers, the identification of the causative genetic locus for PIS has become possible. The PIS locus was mapped to the ~1 centimorgan (cM) region of CHI1q43 on goat chromosome 1 by linkage analysis using microsatellite markers and comparative genomic analysis [31, 32]. An 11.7-kb deletion of this genomic interval was detected in PIS individuals and was shown to be the causative mutation for PIS in goats [33]. Recently, Boulanger et al. [34] demonstrated that the loss of *FOXL2*, which is encoded in this genomic interval, causes an XX female to male sex reversal in the goat.

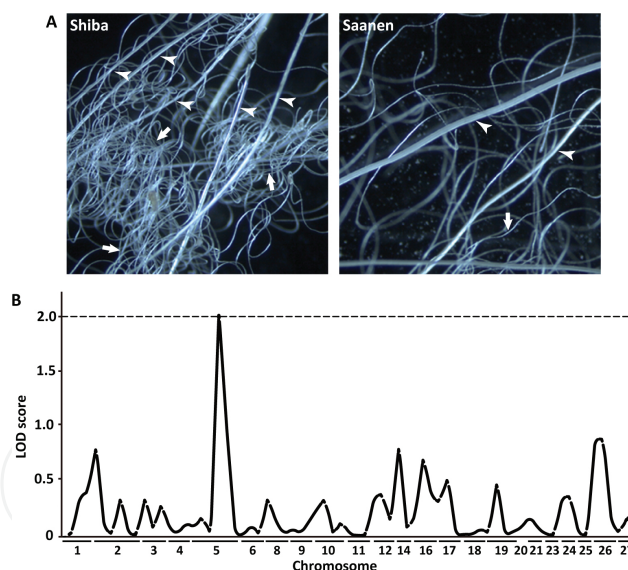


Figure 3. Identification of the genetic locus associated with cashmere productivity using linkage analysis of polymorphic microsatellite markers. (A) Strain differences in cashmere productivity between the Shiba (left panel) and Japanese Saanen breeds (right panel). The Shiba breed produces a higher amount of cashmere than the Japanese Saanen breed. The thin curly hairs and thick straight hairs represent the cashmere (arrows) and the guard hairs (arrowheads), respectively. (B) Genome-wide linkage analysis using 70 microsatellite markers and 35 backcrossed progenies between the Shiba and Japanese Saanen breeds. A suggestive linkage associated with cashmere productivity in the backcrossed progenies was detected on chromosome 5.

In contrast, several economic traits are quantitative traits. Generally, quantitative traits are influenced by polygenes, namely, quantitative trait loci (QTLs). In goat, traits for fecal worm

egg count [35] and for resistance to gastrointestinal nematode infections [36] were detected by QTL linkage analysis using microsatellite markers. Of the goat economic traits, cashmere productivity is one of the most important traits because of the high market value of cashmere. We attempted to detect QTLs for cashmere productivity using linkage analysis based on microsatellite markers in an experimental family generated by backcrossing Shiba and Japanese Saanen populations, which have high and low cashmere productivity, respectively. **Figure 3A** shows the amount of cashmere hair in the Shiba (*left panel*) and Japanese Saanen (*right panel*) populations. Measurement of cashmere production in the 10 F₁ hybrid individuals and 35 backcrossed progeny suggested that a major gene associated with cashmere production had the dominant effect (data not shown). We performed genome-wide genotyping of the backcrossed progeny and detected a suggestive linkage (LOD score = 2.0) on chromosome 5 associated with cashmere productivity. Although our mapping data are preliminary because the sample size and markers are small, a previous study also mapped a QTL associated with cashmere yield in Rayini goats to an overlap region of chromosome 5 [37].

5. Marker-assisted selection using microsatellite markers in goats

Detection of single locus and QTLs associated with disease and economic traits has led to enhanced genetic improvement of livestock through marker-assisted selection [37, 38]. Marker-assisted selection is the indirect selection of animals by linking a marker with a trait but is not based on the trait itself. All genetic markers, such as morphological and biochemical markers, are available for marker-assisted selection. However, detection is difficult for some traits because environmental effects influence these markers. Moreover, these markers cannot genotype all traits, particularly if the traits are controlled by QTLs on multiple chromosomal regions. Molecular markers usually do not have any biological effect and can be used for genome-wide genotyping. Microsatellites are suitable genetic markers for marker-assisted selection because they have a high degree of polymorphism, are abundantly distributed throughout the genome, and can be used as the basis of accurate and simple detection protocols. To date, several economically important QTLs have been reported in goats using QTL linkage analysis [39]. The microsatellite markers linked with QTLs have also been reported. We predict that several phenotypes are commonly found in various goat populations and that the microsatellite markers linked with traits are suitable for marker-assisted selection to establish highly productive goats in several countries.

6. Concluding remarks

Although we described the advantages of microsatellite markers for breeding goats, the applications of microsatellite markers have been discussed in a wide range of genetic studies. Moreover, microsatellite markers are commonly used in other livestock to estimate population structures and detect QTLs. Recently, SNPs have become a popular genetic marker for genetic analyses in humans, mice, and livestock, and several SNP genotyping technologies have been

developed [40–42]. In the future, SNPs may become the main tool for genetic analysis because large-scale genomic sequences will be published for several breeds and populations. However, we predict that microsatellite markers will also be used for genetic studies because the procedure is simple. Above all, we suggest that microsatellite markers are accessible markers for use at a small scale, such as in a laboratory, because of economic concerns such as cost, time, and labor.

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