

Original Article

Genetic variability of six indigenous goat breeds using major histocompatibility complex-associated microsatellite markers

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The present study aimed at analyzing the genetic variability of indigenous goat breeds (*Capra hircus*) using the MHC-associated microsatellite markers *BF1*, *BM1818*, *BM1258*, *DYMS1*, and *SMHCC1*. The following breeds were included: Chinese Xuhuai, Indian Changthangi and Pashmina, Kenyan Small East African (SEA) and Galla, and Albanian Vendi. To examine genetic variability, the levels of heterozygosity, degrees of inbreeding, and genetic differences among the breeds were analyzed. The mean number of alleles ranged from nine in the Galla to 14.5 in the Vendi breed. The mean observed heterozygosity and mean expected heterozygosity varied from 0.483 in the Vendi to 0.577 in the Galla breed, and from 0.767 in the SEA to 0.879 in the Vendi breed, respectively. Significant loss of heterozygosity ($p < 0.01$) indicated that these loci were not in Hardy-Weinberg equilibrium. The mean F_{IS} values ranged from 0.3299 in the SEA to 0.4605 in the Vendi breed with a mean value of 0.3623 in all breeds ($p < 0.001$). Analysis of molecular variance indicated that 7.14% and 4.74% genetic variation existed among the different breeds and geographic groups, whereas 92.86% and 95.26% existed in the breeds and the geographic groups, respectively ($p < 0.001$). The microsatellite marker analysis disclosed a high degree of genetic polymorphism. Loss of heterozygosity could be due to genetic drift and endogamy. The genetic variation among populations and geographic groups does not indicate a correlation of genetic differences with geographic distance.

Keywords: genetic variability, indigenous goat breeds, major histocompatibility complex, microsatellites

Introduction

Native goat breeds (*Capra hircus*) are often well-adapted to harsh local environmental conditions and therefore represent important genetic resources for conservation and breeding programs [6,18]. In many countries, indigenous goats are among the most important species of livestock [6,37]. These native breeds did not undergo extensive artificial selection by humans and are generally well-adapted to semi-arid or even arid conditions, especially in developing countries throughout the world [18]. Therefore, goats are a reasonable alternative to breeding of cattle in many regions of the world. These adaptive characteristics for harsh environmental conditions contribute to a growing interest in indigenous species for conservation and breeding programs. It is a declared aim of the Food and Agriculture Organization of the United Nations (Rome, Italy) to preserve the genetic diversity of domestic animals [18].

Previous works have shown that microsatellites are useful for assessing genetic diversity. There are several studies using these markers to analyze the genetic diversity of indigenous goat breeds, but only a few studies explored major histocompatibility complex (MHC)-associated microsatellites [2,7,13,16,24,39]. A recent study of MHC gene-associated microsatellite loci including those of bovine, ovine, and goat breeds revealed convergent evolution, which was interpreted to be the result of similar selective pressures, acting in these different ruminant species [9]. Microsatellite loci associated with genes of the MHC complex can provide highly polymorphic molecular markers potentially associated with resistance against disease and can therefore be useful for conservation and breeding programs [19,28,31].

The genetic characterization of indigenous goat breeds using informative molecular markers is the primary step in developing conservation and breeding programs. In the present study, the genetic variability of six indigenous goat

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breeds from Albania (Vendi goat), China (Xuhuai goat), India (Changthangi and Pashmina goat), and East Africa (Small East African and Galla goat) was analyzed. These breeds were chosen because they are subjected to conservation programs due to their special socio-economic value in local human communities and their population structures are poorly understood [18]. The question of inbreeding within the populations and degree of genetic variation among these breeds from different continents was also addressed. The five markers used in this study, *BF1*, *BM1818*, *BM1258*, *DYMS1* and *SMHCC1*, are all well-characterized in bovine and ovine breeds, but with the exception of *BM1818* have been rarely used in previous studies of goat breeds [9,11,14,24,34,39]. Due to their close association with MHC genes, these markers might be of interest for breeding and conservation programs to improve the animal's immune response and other important traits. Therefore, this study aimed to analyze the degree of polymorphisms in markers from these indigenous goat breeds.

Material and Methods

Animal samples and DNA extraction

DNA probes extracted from blood samples of 404 goats (*Capra hircus*) were a kind gift from the lab of Dr. Hermann Geldermann (University of Hohenheim, Germany). Among these probes, 60 were from the Xuhuai, 87 from the Changthangi, 62 from the Pashmina, 60 from the SEA, 61 from the Galla and 74 from the Vendi goat breed. For the animals used in our study, we knew neither the pedigree structure of single herds nor the degree of interbreeding between herds within a single breed. Because of this, only one female and one non-castrated male were sampled in each heard to limit the collection of samples from related animals. Samples were taken from individuals more than 2 years old. It is important to point out that the samples were derived from animals in small herds raised extensively for use by local human populations without being exposed to intense artificial selection pressures.

Location of markers and genotyping

The present study included five microsatellite markers all located on chromosome 23 in the region of the MHC gene loci (Table 1). Corresponding flanking regions of microsatellites were sequenced in goats and compared with cattle (*Bos taurus*) and sheep (*Ovis aries*) to determine their correct identities within or close to the MHC gene regions. The microsatellites were amplified with the GeneAmp PCR system 9600 (Perkin Elmer, USA), applying different specific annealing temperatures (Table 1). Analysis of amplified markers was performed using an ABI3100 sequencer combined with GeneMapper 3.0 software (Applied Biosystems, USA).

Table 1. Names and structures of the five microsatellites localized on chromosome 23 along with the sequences of corresponding primers and their annealing temperatures

Name	Repetitive sequence	Primer sequences	Annealing temperature (°C)
<i>BF1</i>	CA	CAACGGTCTGCAACCGAATTACC CAATCCGTGGGTTGGAACACAA	58
<i>BM1818</i>	GT	AGCTGGGAATATAACCAAAGG AGTGCTTTCAAGGTCCATGC	58
<i>BM1258</i>	GT	GTATGTATTTTCCCACCCTGC	56
	GA	GTCAGACATGACTGAGCCTG	
<i>DYMS1</i>	CA	TCCTGGGGATTCCCAATACC CATAGAAGTCTTCACTGGTG	52
<i>SMHCC1</i>	CA	ATCTGGTGGGCTACAGTCCATG GCAATGCTTTCTAAATTCTGA	55

Statistical analysis

The numbers of alleles per locus and their frequencies were computed with the PHYLIP (version 3.6) package of software [17]. Exact tests for deviations from Hardy-Weinberg equilibrium (HWE), including expected heterozygosity (H_E) and observed heterozygosity (H_O), have been carried out with the ARLEQUIN (version 3.0) [15] and GENEPOP (version 1.2) program [15,32]. The program performs a probability test using a Markov chain (1,000 batches, 5,000 iterations, 10,000 de-memorization, 10,000, batches 1,000, iteration per batch 1,000). Analysis of molecular variance (AMOVA) was computed with the ARLEQUIN program [15]. Standard genetic distances were computed using the TFGPA program (version 1.3) [25]. The F -statistics, including F_{IS} and F_{ST} , were calculated using the method of Weir and Cockerham [38] with the GENEPOP program (version 1.2) [32]. Significance of the F_{IS} and F_{ST} values was estimated from 10,000 bootstrap replicates; as usual, the F parameters were considered significantly different from zero when their corresponding 95% confidence intervals did not include zero.

Results

The mean number of alleles ranged from nine in the Galla to 14.5 in the Vendi breed with an average of 10.36 alleles for all five loci in the six breeds (Table 2). The lowest number of four alleles (*BF1*) was found in the Galla and Xuhuai breeds whereas the highest number of 16 alleles (*BM1258*; *DYMS1*) was found in the Vendi breed (Table 2). The allele sizes ranged from 90 bp (*BM1258*) in the SEA breed to 276 bp (*BM1818*) in the Vendi breed. The lowest (0.0027) and highest (0.4886) allele frequencies were

Table 2. Numbers of alleles and significant differences of expected (H_E) and observed (H_O) heterozygosity for the microsatellite loci in the different breeds

		Xuhuai	Pashmina	Changthangi	Vendi	Galla	SEA	Mean
Number of alleles								
Highest		12	13	13	16	12	12	
Lowest		4	8	5	13	4	6	
Mean		9.25	10.00	10.20	14.50	9.00	9.20	10.36
H_O and H_E								
<i>BF1</i>	H_O	0.457	0.480	0.273	0.367	0.439	0.273	0.382
	H_E	0.775	0.674	0.690	0.861	0.752	0.721	0.746
<i>BM1818</i>	H_O	0.796	0.561	0.685	0.632	0.796	0.473	0.657
	H_E	0.880	0.880	0.871	0.869	0.872	0.780	0.859
<i>BM1258</i>	H_O	0.350	0.526	0.416	0.489	0.350	0.509	0.440
	H_E	0.810	0.866	0.845	0.890	0.805	0.783	0.833
<i>DYMS1</i>	H_O	0.612	0.607	0.663	0.444	0.617	0.729	0.612
	H_E	0.874	0.873	0.885	0.897	0.872	0.753	0.859
<i>SMHCC1</i>	H_O	—	0.630	0.673	—	0.684*	0.509	0.624
	H_E	—	0.872	0.898	—	0.871*	0.800	0.860
Mean values	H_O	0.554	0.561	0.542	0.483	0.577	0.499	
	H_E	0.835	0.833	0.838	0.879	0.834	0.767	

The asterisk (*) indicates pairs of values of H_E and H_O , which did not show a significant difference ($p < 0.01$). Alleles undetected in a specific population are indicated by a dash (—). The last two rows show the mean values of heterozygosity for the single breeds over all markers analyzed. SEA: Small East African.

Table 3. Mean deficit of heterozygotes within the breeds

	Xuhuai	Pashmina	Changthangi	Vendi	Galla	SEA	Mean
F_{IS}	0.4605	0.3302	0.3639	0.3482	0.3408	0.3299	0.3623
Xuhuai		0.0547	0.0371	0.0176	0.0555	0.1261	
Pashmina	0.2420		0.0118	0.0692	0.0508	0.1560	
Changthangi	0.2053	0.1387		0.0509	0.0359	0.1548	
Vendi	0.1592	0.2665	0.2281		0.0314	0.1288	
Galla	0.2461	0.2325	0.1980	0.1881		0.1468	
SEA	0.3506	0.4002	0.3949	0.3567	0.3842		

The lower half of the data (shadowed) and the upper one represent standard genetic distance and F_{ST} ($p < 0.001$) between breeds, respectively.

detected at the loci *BM1258* and *BF1* in the Vendi and SEA breed, respectively. Despite the existence of only six exclusive alleles over all loci, the allelic composition showed considerable differences between individual breeds with many alleles shared by only two breeds mainly at the *BF1*, *DYMS1*, and *SMHCC1* loci.

The values of H_E and H_O for all five loci are summarized in Table 2. The mean H_E values over all five markers ranged from 0.767 in the SEA to 0.879 in the Vendi breed (Table 2). The mean H_O values varied from 0.483 in the Vendi to 0.577 in the Galla breed. A rather uniform loss of heterozygotes, with a corresponding increase of homozygotes, was observed for all genotypes within each

locus analyzed, demonstrating that significant differences detected between H_O and H_E ($p < 0.01$) clearly indicate deviations from HWE with the exception of those found at the *SMHCC1* locus in the Galla breed (Table 2). Loss of heterozygosity was confirmed by elevated F_{IS} values ranging from 0.3299 in the SEA to 0.4605 in the Xuhuai breed ($p < 0.001$), and a mean value of 0.3623 for all six breeds (Table 3).

Standard genetic distances varied from 0.4002 between Pashmina and SEA, and 0.1387 between Pashmina and Changthangi (Table 3). The pair-wise comparison between breeds revealed F_{ST} values ranging from 0.0118 between the Pashmina and Changthangi to 0.1560 between the

Pashmina and SEA pairs (Table 3). High F_{ST} values of all pairs with SEA (Table 3) were due to the distinct allelic composition of this breed compared to the others, most obviously at the *BF1* and *DYMS1* loci (data not shown). The AMOVA results revealed that 7.12% of the total variation significantly different from zero, resulted from differences among the breeds ($F_{ST} = 0.071$; $p < 0.001$), whereas 92.86% of the variation was attributed to differences within each breed (Table 4). When the breeds were structured according to four geographical regions, China, India, East-Africa, and Europe, 4.74% of the variation significantly different from zero was detected between these groups ($F_{ST} = 0.047$; $p < 0.001$) and the remaining 95.26% resulted from differences within each group (Table 4).

Discussion

The microsatellite markers of the present study have disclosed a high degree of genetic polymorphism. Microsatellites *BM1818* and *BM1258* flank the MHC class I and IIa gene regions [8]. The *BF1* locus is within the *BF* gene (MHC class III region), which encodes a factor of the complement [21]. The locus of *DYMS1* is within the *DYA* gene of the MHC class IIb region [10]. The *SMHCC1* locus is within a gene of the MHC class I region [20].

The indigenous breeds examined in our study showed a considerable degree of genetic diversity within each population based on their allele numbers, high levels of heterozygosity, and genetic variance. The mean number of alleles varied between nine in the Galla and 14.5 in the Vendi breeds, and ranged from 7.3 (*BF1*) to 12.5 (*BM1258*) for the microsatellite loci. The high number of alleles detected in the present study may be influenced by

sampling many different herds and could also be due to a higher polymorphic degree of the MHC associated microsatellites. Previous studies of indigenous breeds of goat revealed generally lower numbers of alleles. Recent studies of 18 African and seven Indian breeds showed mean allele numbers in the range from 3.8 to 5.5 and 8.1 to 9.7, respectively [27,33]. In a study of 11 indigenous goat breeds from different regions of Asia, including 25 markers the number of alleles varied from 4.3 to 5.9 [7]. In two studies, each of 8 and 12 native breeds from China, including 18 and 26 microsatellite markers, the number of alleles varied from 4.0 to 13.0 and from 5.2 to 7.7 [23,39].

The mean H_O values in our study ranged from 0.483 in the Vendi to 0.577 in the Galla. Similar mean H_O values, ranging from 0.435 to 0.559 and 0.511 to 0.617, were detected in indigenous African and Chinese breeds, respectively [27,39]. Other studies found higher values of H_O , ranging from 0.739 to 0.738 in Indian breeds, and from 0.617, 0.654 in Chinese breeds [33,40]. In the Spanish Guadarrama and the Indian Mehsana goat breeds, higher mean H_O values of 0.700, 0.941, and 0.650, respectively, were also found [1,36].

The lowest (0.767) and highest (0.879) mean values of H_E were found in the SEA and the Vendi breeds, respectively. Comparison of H_O and H_E revealed a decline in heterozygosity at all microsatellite loci and elevated mean F_{IS} values ranging from 0.3299 in the SEA to 0.4605 in the Xuhuai breed. Several previous studies found lower mean F_{IS} values of 0.230, 0.156, and 0.023 in the Indian Kutchi, Mehsana, Spanish Guadarrama, respectively, and in the range between 0.09 and 0.277 in breeds from different regions of Asia [1,7,13,36]. Heterozygosity of MHC gene loci is considered to be adaptive whereas homozygosity is generally disadvantageous [4,22]. Therefore, it is unlikely that selection would favor distinct homozygote genotypes of the MHC gene-associated microsatellites used in this study. More likely, the loss of heterozygosity may be explained by the division of each breed into small subpopulations causing an increase of homozygotes due to genetic drift and endogamy. Since only two individuals of each herd were sampled, different allele frequencies in the subpopulations could also cause a Wahlund effect. An analysis of population structure of different goat breeds showed that the herds live under similar conditions but in small populations, probably with reduced gene flow between them and increased rates of inbreeding [12,26]. Deviations from HWE, characterized by loss of heterozygotes, were reported in several previous studies of indigenous and naturalized goat breeds [1,5,13,16,29,33,39].

The pair-wise comparison of breeds showed F_{ST} values ranging between 0.0118 and 0.1560, indicating different degrees of genetic differentiation. From all pairs those five ones that included the SEA breed showed high F_{ST} values.

Table 4. Analysis of molecular variance analysis of goat breeds based on microsatellite DNA variation

	Sum of squares	Variance components	Variation (%)
Among breeds	65.736	0.097	7.14
Within breeds	914.197	1.264	92.86
Total	979.933	1.361	
Analysis of geographic groups			
Among groups	38.101	0.065	4.74
Within groups	941.832	1.298	95.26
Total	979.933	1.363	

Analysis was performed unstructured for all six breeds ($F_{ST} = 0.071$) and the breeds were divided into four groups based on geographical location: Small East African and Galla, Changthangi and Pashmina, Xuhuai, and Vendi ($F_{ST} = 0.047$). The variations among breeds and among groups were significant ($p < 0.001$).

The SEA breed was found to have more rare alleles, most obviously at the *BFI* and *DYMSI* loci, with different frequencies compared to the other breeds. Comparable high genetic differentiation with mean values of 0.143 and 0.105 were found in different indigenous breeds from India and in naturalized breeds from Brazil, respectively [7,29]. From all pair-wise comparisons in our study, those ones not including the SEA breed, showed weaker degrees of genetic differentiation. Results of AMOVA analysis found that 7.14% and 4.74% of genetic variation exist among the breeds and among geographic groups, respectively. These results are comparable to genetic differences of 5% found among indigenous African breeds and 6.59% among Indian breeds [27,33]. Higher degrees of genetic variation have been detected in previous studies: 10.5% among indigenous Chinese breeds, 17% among Swiss breeds, 11% among Italian breeds, and 11.49% among Brazilian naturalized goat breeds [3,23,29,35]. Therefore, the genetic variation among the breeds found in this study was not as high as one would expect given the considerable geographic distance separating the breeds from Africa, Asia, and Europe.

The present study indicated that there is no relation between the geographic distance and genetic variation among indigenous goat breeds examined in our analysis. The MHC-associated markers were polymorphic, characterized by high numbers of alleles, and therefore could be useful for future breeding and conservation programs. Our results revealed loss of heterozygotes in all breeds over all five loci. In order to maintain the genetic diversity of each breed, breeding strategies should be implemented that minimize genetic drift within the herds. Genetic diversity and pedigree structures of different herds within the breeds should be studied to determine the levels of endogamy and gene flow rates between the herds.

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