# Genetic Variation of Four Goat Breeds in Malaysia Using Microsatellite Polymorphism Markers

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## Abstract

The characterisation of animals using PCR-based microsatellite markers is informative, economical and useful to elucidate genetic diversity within and among breeds. The determination of genetic variability in four goat breeds found in Malaysia, namely the indigenous Katjang goat and the exotic Jamnapari, Boer and Savanna goats, was successfully analysed using 30 microsatellite markers. Low levels of allelic variations were found in the four goat breeds. The mean numbers of observed alleles per locus were relatively low with values of 5.43, 5.73, 5.90 and 5.70 in Katjang, Jamnapari, Boer and Savanna goats, respectively. The mean observed heterozygosity was lower than the mean expected heterozygosity for the all four goat breeds. Katjang goats showed the lowest observed heterozygosity with a value of 0.36. The results showed that the Katjang goat population had a high level of inbreeding compared to the other goat breeds.

**Keywords: Katjang, Jamnapari, Boer, Savanna goats, heterozygosity**

## Introduction

There is a need to increase chevon and mutton production in Malaysia to achieve the targeted self-sufficiency rate of 35% by the year 2015. This has generated interest in raising high quality breeds of goat which ultimately will be one of the main factors that determine the success of commercial goat production in Malaysia. Thus, a number of exotic goat breeds have been imported and bred locally to fulfill the demand of the industry (Ariff *et al*., 2010). In the effort to find a suitable goat breed for breeding, the indigenous Katjang goat was neglected. There is still a wide gap between demand and supply of the goat meat in Malaysia and the industry faces many challenges in the supply chain to meet the overall national demand. Therefore special priority must be given to plan and execute strategic breeding programmes.

Advances in biotechnologies have been utilised to aid breeding and selection in the improvement of livestock production. Basically, information on the genetic background of breeds and populations is required for proper breeding programmes. Local databases on adequate genetic characterization of goat breeds and their similarities are lacking. The characterization of animals using PCR-based microsatellite markers is informative and useful for genetic diversity studies (Sunnucks *et al*.,

2001; Kim *et al*., 2004). Microsatellite is an economical and preferred marker to elucidate goat genetic diversity (Saitbekova *et al*., 1999; Takahashi *et al*., 2008). The resulting genetic information would be properly utilised as the basis for breeding, selection and ultimately conservation purposes. Thus, the present study was conducted to evaluate the genetic variability of goat breeds, namely the Katjang, Jamnapari, Boer and Savanna breeds, in Malaysia using microsatellite analysis.

## Materials and Methods

### *Sample Collection and DNA Extraction*

A total of 151 goats of four breeds: Katjang (n=37), Jamnapari (n=34), Boer (n=40) and Savanna (n=40) were used in the study. Jamnapari goats originated from Indonesia meanwhile Boer and Savanna were imported from South Africa and Australia. Blood samples were collected from the Boer and Savanna goats raised at MARDI Research Station, Kluang, Johor, Jamnapari goats sourced from a private farm in Yong Peng, Johor and Katjang goats sampled from smallholders' flocks from several locations in the country. All farms practiced the semi-intensive production system. Genomic DNA was extracted using Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's procedure with minor modifications. The DNA concentration was estimated using NanoDrop ND-1000 Spectrophotmeter (NanoDrop Technologies, Inc., USA).

### *Microsatellite Markers and PCR Conditions*

Thirty microsatellite primer pairs were studied and detailed information on these markers is presented in Table 1. Twenty-six of these are recommended for biodiversity studies by International Society for Animal Genetics (ISAG) and Food and Agriculture Organization (FAO, 2004), and the rest were reported by Martinez *et al*., (2004) and Mainguy *et al*. (2005). The PCR amplification reaction was performed in a total volume of 25 µl in a PTC-200 Peltier Thermal Cycler (MJ Research, USA) or C1000 Thermal Cycler (Bio-Rad Laboratories, USA). The PCR reactions consisted of 1X Buffer, 1.25-1.50 mM $MgCl_2$, 0.25 mM dNTPs (Promega, USA), 0.5 µM of forward and reverse primers (1st Base, Malaysia), 1 U Taq DNA Polymerase (Promega, USA) and 50 ng genomic DNA as a template. The PCR amplification was conducted by initial denaturation at 94°C for 7 min, 40 cycles of denaturation at 94°C for 35 s, annealing for 35 s at the optimised temperature, and extension at 72°C for 45 s. A final extension at 72°C for 7 min was included. The PCR products were electrophoresed on 4% MetaPhor® agarose gel (Lonza, USA) at 90 V for 1.5 to 2 h in TAE as the running buffer, followed by staining of the gel with ethidium bromide and visualizing using AlphaEaseFC Stand Alone Software of the gel documentation system (Alpha Innotech, California).

### *Genetic Analysis*

Microsatellite banding patterns were scored and compiled as matrix data. Genetic analysis was performed with the assistance of the Genetic Data Analysis (GDA) software (Lewis and Zaykin, 2002) and the POPGENE software version 1.31 (Yeh *et al*., 1999). The CONVERT software version 1.31 (Glaubitz, 2004) was used to convert the matrix data into the required format.

Table 1: Information on microsatellite markers used in the study

| No. | Locus | Origin | Repeat structure | Tm[1](°C) |
|---|---|---|---|---|
| 1 | CSRD247 | Ovine | Na | 58°C |
| 2 | DRBP1 | Bovine | $(TG)_6$ | 58°C |
| 3 | ETOH10 | Bovine | $(CA)_{19}$ | 56°C |
| 4 | ILSTS005 | Bovine | $(nn)_{39}$ | 55°C |
| 5 | ILSTS011 | Bovine | $(TC)_7TTAT(CA)_{11}$ | 58°C |
| 6 | ILSTS029 | Bovine | $(CA)_{19}$ | 53°C |
| 7 | ILSTS087 | Bovine | $(CA)_{14}$ | 57°C |
| 8 | INRA063 | Bovine | $(CA)_{13}(TA)_3$ | 57°C |
| 9 | INRABERN172 | Bovine | $(GT)_{11}$ | 58°C |
| 10 | INRABERN185 | Caprine | Na | 55°C |
| 11 | MAF065 | Ovine | $(CA)_{40}$ | 58°C |
| 12 | MAF70 | Unknown | $(CA)_{38}$ | 69°C |
| 13 | MAF209 | Ovine | $(TG)_2(TC(TG)_4AA(TG)_4$ | 57°C |
| 14 | McM527 | Ovine | $(GT)_{20}$ | 58°C |
| 15 | OarAE54 | Ovine | $(CA)_9CT(CA)_{14}$ | 58°C |
| 16 | OarFC20 | Ovine | $(GT)_{15}$ | 55°C |
| 17 | OarFC48 | Ovine | $(TG)_{11}CA(TG)_3$ | 58°C |
| 18 | P19 (DYA) | Ovine | Na | 55°C |
| 19 | SPS113 | Bovine | Na | 58°C |
| 20 | SRCRSP5 | Caprine | (GT)16 | 58°C |
| 21 | SRCRSP7 | Caprine | Na | 58°C |
| 22 | SRCRSP8 | Caprine | Na | 58°C |
| 23 | SRCRSP9 | Caprine | Na | 58°C |
| 24 | SRCRSP15 | Unknown | Na | 58°C |
| 25 | TCRVB6 | Unknown | Na | 58°C |
| 26 | TGLA53 | Bovine | Na | 58°C |
| 27 | BM1225 | Bovine | Na | 58°C |
| 28 | BM1329 | Ovine | Na | 58°C |
| 29 | OarHH35 | Ovine | Na | 55°C |
| 30 | TGLA122 | Bovine | Na | 57°C |

[1]Tm = annealing temperature; Na = not available;
Loci above the dotted horizontal line are loci recommended by ISAG/FAO

**Results and Discussion**

All 30 microsatellite loci were polymorphic and used in the analysis for genetic diversity. Low levels of allelic variations were found in the four goat breeds. The number of detected alleles ranged from three to seven in the Katjang, four to nine in Jamnapari and Boer, and two to ten in the Savanna (Table 2). The numbers of alleles of the four goat breeds were lower than those reported for the Swiss goats (Saitbekova *et al*., 1999), Ethiopian goats (Tesfaye *et al*., 2004), West African Dwarf (Mujibi, 2005) and Sub-Saharan goats (Muema *et al*., 2009). The mean numbers of observed alleles per locus in Katjang (5.43), Jamnapari (5.73), Boer (5.90) and Savanna (5.70) goats were similarly low as the mean number of alleles of 5.86 per locus reported for the Brown Short-Haired goats by Jandurova *et al*. (2004). Barker (1994) suggested that loci with at least four alleles should be used in diversity studies to reduce the standard error of the estimated distance. In the present study only two loci, MAF209 and TGLA122, were found to have less than four alleles in Katjang goats, and locus MAF209 in Jamnapari. Meanwhile, the two loci INRABERN185 and SRCRSP7 had less than four alleles in Savanna goats. The low number of alleles observed could be due to the small sample size, low number of polymorphic loci or the effect of inbreeding (Pandey *et al*., 2006; Maletsanake *et al*., 2013). The number of goats per breed and the microsatellite markers used were more than the minimum sample size of 30 individuals and 25 polymorphic loci suggested by FAO (2004) for genetic diversity studies. This indicated the possibility of inbreeding in the populations studied.

The mean observed heterozygosities in Katjang, Jamnapari, Boer and Savanna were 0.36, 0.43, 0.43 and 0.42, respectively. The values were lower than the mean expected heterozygosity for the four goat populations studied. The Katjang goats showed the lowest observed heterozygosity suggesting higher level of inbreeding in this breed compared to the other breeds.

The genetic variations in the indigenous Katjang goat population used in the present study were similar to those of the indigenous Asian goat populations studied in Chiang Mai, Medan, Bogor and the Philippines (Musuan) with mean observed heterozygosity values of 0.39, 0.42, 0.48 and 0.45, respectively (Barker *et al*., 2001). This indicated that the Asian goat populations were mostly bred from a small number of animals in each population resulting in inbreeding which caused the low genetic variation. Similar to the present study, the observed heterozygosities of South African Boer and Savanna goat populations were lower than the expected heterozygosities (Pieters *et al*., 2009). This could be due to the Boer known to be one of the oldest goat breeds in South Africa that has been intensively selected for various traits (Visser *et al*., 2004) while Savanna goats probably had gene introduction from Boer goats over the years resulting in inbreeding.

Unlike the findings of the present study, medium level of observed heterozygosity have been reported for other goat breeds: 0.55 for Maltese goats from the Mediterranean, 0.53 for Sardinian goats from Italy (Sechi *et al*., 2007) and 0.59 for Kuthi goats from India (Dixit *et al*., 2008). Average values of observed heterozygosity of 0.50, 0.51 and 0.56 were found in Sirohi goats (Verma *et al*., 2007), Gohilwari goats (Kumar *et al*., 2009) and Sub-Saharan goats (Muema *et al*., 2009), respectively. The highest heterozygosity was found in the Chinese goat breeds of Taihang,

Neimonggol, Lianong, Small-xiang and Tibetan goats (Li *et al*., 2002).

The highest value for observed heterozygosity was found in the Savanna goat at locus ILSTS029 while the lowest observed heterozygosity was found for loci ETOH10 and MAF209 in the Katjang goat, locus MAF209 in the Jamnapari goat and locus SRCRSP7 in the Savanna goat. Four loci CSRD247, ILSTS029, INRABERN172 and OARHH35, were different from the other loci as they had higher observed heterozygosities (0.84, 0.79, 0.85 and 0.95) when compared to the expected heterozygosities (0.83. 0.77, 0.77 and 0.89), respectively. However, the locus ILSTS029, in the Boer goat was unique in that the observed and expected heterozygosity were the same (0.87).

Table 2: Population genetic variation of four goat breeds in Malaysia

| Locus | Katjang | | | | Jamnapari | | | | Boer | | | | Savanna | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Na[1] | Ne[2] | Ho[3] | He[4] | Na | Ne | Ho | He | Na | Ne | Ho | He | Na | Ne | Ho | He |
| CSRD247 | 6 | 5.49 | 0.84 | 0.83 | 6 | 4.11 | 0.62 | 0.77 | 6 | 4.71 | 0.68 | 0.80 | 6 | 3.49 | 0.46 | 0.72 |
| DRBP1 | 7 | 4.28 | 0.24 | 0.78 | 8 | 5.58 | 0.34 | 0.83 | 7 | 4.21 | 0.28 | 0.77 | 7 | 4.95 | 0.15 | 0.81 |
| ETH10 | 4 | 2.81 | 0.00 | 0.65 | 4 | 2.38 | 0.15 | 0.59 | 4 | 2.36 | 0.05 | 0.58 | 4 | 3.49 | 0.21 | 0.72 |
| ILSTS005 | 5 | 2.41 | 0.14 | 0.59 | 6 | 3.27 | 0.29 | 0.71 | 7 | 4.35 | 0.38 | 0.78 | 7 | 4.70 | 0.85 | 0.80 |
| ILSTS011 | 5 | 3.45 | 0.31 | 0.72 | 4 | 2.39 | 0.32 | 0.59 | 4 | 3.09 | 0.49 | 0.69 | 5 | 3.27 | 0.18 | 0.70 |
| ILSTS029 | 7 | 3.78 | 0.38 | 0.75 | 7 | 2.63 | 0.21 | 0.63 | 9 | 7.01 | 0.87 | 0.87 | 10 | 7.94 | 0.95 | 0.89 |
| ILSTS087 | 6 | 4.49 | 0.35 | 0.79 | 9 | 6.45 | 0.55 | 0.86 | 6 | 2.96 | 0.16 | 0.67 | 5 | 1.64 | 0.03 | 0.40 |
| INRA063 | 4 | 2.73 | 0.22 | 0.64 | 5 | 2.78 | 0.26 | 0.65 | 5 | 3.57 | 0.23 | 0.73 | 5 | 2.83 | 0.14 | 0.66 |
| INRABERN172 | 6 | 4.19 | 0.49 | 0.77 | 5 | 3.41 | 0.47 | 0.72 | 7 | 4.19 | 0.85 | 0.77 | 7 | 4.95 | 0.66 | 0.81 |
| INRABERN185 | 6 | 2.96 | 0.41 | 0.67 | 5 | 1.50 | 0.21 | 0.34 | 5 | 3.02 | 0.17 | 0.68 | 3 | 2.38 | 0.20 | 0.64 |
| MAF065 | 7 | 5.15 | 0.70 | 0.82 | 7 | 3.79 | 0.62 | 0.75 | 7 | 4.33 | 0.41 | 0.78 | 7 | 4.69 | 0.55 | 0.80 |
| MAF209 | 3 | 2.49 | 0.73 | 0.80 | 3 | 2.36 | 0.74 | 0.85 | 4 | 2.42 | 0.82 | 0.85 | 4 | 2.18 | 0.64 | 0.81 |
| MAF70 | 6 | 4.66 | 0.00 | 0.61 | 8 | 6.18 | 0.00 | 0.58 | 8 | 6.23 | 0.13 | 0.59 | 6 | 5.07 | 0.18 | 0.55 |
| McM527 | 7 | 3.05 | 0.32 | 0.68 | 6 | 5.47 | 0.48 | 0.83 | 7 | 4.82 | 0.58 | 0.80 | 7 | 5.49 | 0.67 | 0.83 |
| OARAE54 | 5 | 3.48 | 0.38 | 0.72 | 5 | 3.56 | 0.62 | 0.73 | 5 | 3.19 | 0.55 | 0.69 | 5 | 3.73 | 0.51 | 0.74 |
| OARFCB20 | 4 | 2.10 | 0.16 | 0.53 | 4 | 2.06 | 0.26 | 0.52 | 5 | 2.31 | 0.40 | 0.57 | 5 | 1.75 | 0.25 | 0.43 |
| OARFCB48 | 5 | 2.55 | 0.17 | 0.62 | 5 | 2.83 | 0.59 | 0.66 | 5 | 3.37 | 0.58 | 0.71 | 4 | 2.14 | 0.45 | 0.54 |
| P19(DYA) | 6 | 3.86 | 0.59 | 0.75 | 6 | 4.34 | 0.59 | 0.78 | 5 | 3.99 | 0.39 | 0.75 | 5 | 4.27 | 0.48 | 0.78 |
| SPS113 | 6 | 2.89 | 0.32 | 0.66 | 6 | 3.49 | 0.35 | 0.72 | 5 | 4.27 | 0.60 | 0.78 | 5 | 3.67 | 0.72 | 0.74 |
| SRCRSP15 | 5 | 2.87 | 0.51 | 0.72 | 4 | 3.28 | 0.56 | 0.73 | 5 | 3.54 | 0.53 | 0.82 | 5 | 3.48 | 0.33 | 0.79 |
| SRCRSP5 | 6 | 3.51 | 0.03 | 0.68 | 7 | 3.59 | 0.03 | 0.51 | 8 | 5.31 | 0.20 | 0.47 | 7 | 4.60 | 0.00 | 0.14 |
| SRCRSP7 | 4 | 3.05 | 0.27 | 0.79 | 4 | 2.01 | 0.50 | 0.78 | 4 | 1.85 | 0.37 | 0.80 | 2 | 1.16 | 0.44 | 0.78 |
| SRCRSP8 | 6 | 4.63 | 0.59 | 0.80 | 5 | 4.27 | 0.62 | 0.82 | 7 | 4.69 | 0.40 | 0.79 | 7 | 4.32 | 0.53 | 0.81 |
| SRCRSP9 | 6 | 4.75 | 0.16 | 0.66 | 7 | 5.28 | 0.29 | 0.71 | 6 | 4.51 | 0.08 | 0.73 | 6 | 4.98 | 0.08 | 0.73 |
| TCRVB6 | 5 | 3.91 | 0.32 | 0.75 | 5 | 4.77 | 0.38 | 0.80 | 5 | 3.67 | 0.33 | 0.74 | 5 | 4.17 | 0.43 | 0.77 |
| TGLA53 | 6 | 4.48 | 0.30 | 0.79 | 8 | 5.12 | 0.47 | 0.82 | 7 | 5.06 | 0.38 | 0.81 | 9 | 6.11 | 0.36 | 0.85 |
| BM1225 | 6 | 3.02 | 0.33 | 0.68 | 8 | 6.08 | 0.32 | 0.85 | 8 | 5.00 | 0.33 | 0.81 | 7 | 4.18 | 0.43 | 0.77 |
| BM1329 | 5 | 2.75 | 0.47 | 0.65 | 5 | 3.60 | 0.71 | 0.73 | 5 | 4.02 | 0.70 | 0.76 | 5 | 4.07 | 0.36 | 0.76 |
| OARHH35 | 6 | 4.53 | 0.65 | 0.79 | 5 | 4.13 | 0.79 | 0.77 | 6 | 3.56 | 0.58 | 0.73 | 6 | 3.64 | 0.67 | 0.73 |
| TGLA122 | 3 | 2.46 | 0.32 | 0.60 | 5 | 3.73 | 0.62 | 0.74 | 5 | 3.38 | 0.58 | 0.71 | 5 | 4.23 | 0.73 | 0.77 |
| Mean | 5.43 | 3.56 | 0.36 | 0.71 | 5.73 | 3.81 | 0.43 | 0.71 | 5.90 | 3.96 | 0.43 | 0.73 | 5.70 | 3.92 | 0.42 | 0.71 |
| Std. Dev. | 1.10 | 0.92 | 0.21 | 0.08 | 1.51 | 1.33 | 0.21 | 0.12 | 1.37 | 1.14 | 0.22 | 0.09 | 1.64 | 1.42 | 0.25 | 0.16 |

[1]Na, observed number of alleles; [2]Ne, effective number of alleles;
[3]Ho, observed heterozygosity; [4]He, expected heterozygosity.
Loci above the dotted horizontal line are loci recommended by ISAG/FAO

## Conclusion

This study showed that there are some genetic variations present in the Katjang, Jamnapari, Boer and Savanna goat breeds in Malaysia. The mean observed heterozygosity was lower than the mean expected heterozygosity for the four breeds which suggested the occurrence of inbreeding and low genetic variabilities. Further investigation and screening of more animals and populations are necessary to ascertain the diversity of goat populations in Malaysia.

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