Assessment of Genetic Diversity on Goat Breeds in Malaysia Using Microsatellite Markers

Amie Marini, A.B.[1,3*], Hifzan, R.M.[2], Tan, S.G.[3] and Panandam, J.M.[4]
[1]Strategic Livestock Research Centre, MARDI, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia, [2]MARDI Research Station, P.O. Box 525, 86009 Kluang, Johor, Malaysia, [3]Faculty of Biotechnology and Biomolecular Sciences, [4]Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
*Corresponding author: amieza@mardi.gov.my

Abstract

Genetic characterisation of goat populations in Malaysia is essential for conservation strategy as well as for genetic improvement. The goat populations in Malaysia comprise the small-framed original Katjang goats and the larger framed exotic goats: the Jamnapari, Boer and Savanna. With continuous importation, these exotic breeds are slowly replacing the indigenous Katjang, which is in danger of extinction if there is no concrete plan to conserve the breed. The aim of this study was to determine the genetic diversity of goat breeds in Malaysia using microsatellite markers for proper utilisation in breeding program. Thirty microsatellite markers were used successfully to amplify caprine genomic DNA using a PCR-based technique. Low levels of number of alleles per locus were found in the Katjang, Jamnapari, Boer and Savanna breeds. The mean number of alleles per locus was 5.69. All four breeds and loci showed significant ($p<0.05$) deviations from HWE except for three loci. The results of the F_{IS} determinations, together with the low number of alleles indicate the occurrence of inbreeding in all four breeds.

Key words: genetic characterisation, indigenous goat breeds, inbreeding

Introduction

The livestock industry faces many challenges in the breed supply chain in meeting the overall national agricultural development plan. Special priority must be given to the plan and to execute strategic breeding programmes. Therefore, the genetic characterisation of goat populations in Malaysia as a genetic resource is essential as a conservation strategy as well as for genetic improvement. The determination of goat populations in Malaysia comprises small-framed animals made up of the original Katjang goats with the addition of larger framed exotic goats such as Jamnapari and Boer. The local indigenous Katjang goats acquired a reasonably high degree of tolerance to the local environment. On the other hand, the Boer, Jamnapari and Savanna which are introduced breeds are well adapted in Malaysia.

In the past few decades, molecular markers have been shown to be an efficient tool in the evaluation of genetic diversity of various populations (Saitbekova *et al.*, 1999). Discovery of the polymerase chain reaction (PCR) had a major impact on research and contributed to the development of various DNA markers. Microsatellite is a preferred marker because it is an economical tool to elucidate genetic diversity. The characterisation of animals using PCR based microsatellite markers is informative and useful at the level of worldwide genetic diversity studies of the world (Kim *et al.*, 2004, Saitbekova *et al.*, 1999). Thus, this study was carried out to evaluate the genetic

diversity of goat breeds in Malaysia using microsatellite markers analysis. Such knowledge could be useful as the basis for the proper utilisation of animals in various goat breeding programmes.

Materials and Methods

Blood samples were collected from four goat breeds, namely Katjang (n=37), Jamnapari (n=34), Boer (n=40) and Savanna (n=40). Katjang goats were collected from smallholders' flocks in Jerantut, Pahang, Kuala Terengganu, Terengganu and Kuala Pilah, Negeri Sembilan, while Jamnapari goats were collected from a private farm in Yong Peng, Johor. The Boer and Savanna goats were collected from the herds in MARDI Kluang Station, Johor. All farms practiced the semi-intensive production system. Genomic DNA was obtained from whole blood samples using Wizard Genomic DNA Purification Kit (Promega). The DNA concentration was measured using ND-1000 NanoDrop Spectrophometer (NanoDrop Technologies, Inc., USA) and ran under 0.7% agarose gel.

Caprine genomic DNA was genotyped using 30 reproducible and polymorphic microsatellite markers. Twenty-six of the microsatellite markers are recommended for biodiversity studies by the Food and Agriculture Organisation/International Society for Animal Genetics (FAO/ISAG) (FAO, 2004), meanwhile four markers (TGLA 122, OarHH 35, BM1225 and BM1329) were reported by other studies (Martinez *et al*., 2004; Mainguy *et al*., 2005). A total of 25 µl Polymerase Chain Reaction (PCR) reaction of 1 X Buffer, 1.25-1.50 Mm $MgCl_2$, 0.25 Mm dNTPs, 0.5 µlM of primers (forward and reverse), 50 ng genomic DNA and 1 U of Taq DNA Polymerase (Promega, USA) was prepared. The PCR amplification was conducted in a MJ PTC-200 Peltier Thermal Cycler or a Bio-Rad C1000 Thermal Cycler with initial denaturation at 94 °C for 7 min, followed by 40 cycles of denaturation at 94 °C for 35 s, annealing for 35 s at the optimised temperature (Amie Marini *et al*., 2013), and extension at 72 °C for 45 s, and a final extension at 72 °C for 7 min. The PCR products were electrophoresed on 4% MetaPhor® agarose gel (Lonza, USA) at 90 V for 1.5 to 2 h and stained with ethidium bromide. The microsatellite bands were visualised using the AlphaEaseFC Stand Alone Software of the gel documentation system (Alpha Innotech, California). A 25 bp Low Molecular Weight DNA Ladder was used as the standard marker.

The microsatellite banding patterns were scored and analysed based on the co-dominance and diploid method using the Genetic Data Analysis (GDA) software (Lewis and Zaykin, 2002) and the POPGENE software v1.31 (Yeh *et al*., 1999) to calculate the genetic variability and differentiation of the breeds.

Results and Discussion

Level of Heterozygosity

All 30 microsatellite markers were analysed to assess the genetic differentiation in four goat breeds in Malaysia. Low levels of the number of alleles per locus were found in the Katjang, Jamnapari, Boer and Savanna breeds. The mean number of alleles per locus was 5.69 (Table 1). The mean observed heterozygosity was lower than the mean expected heterozygosity for the four goat breeds. The Katjang goat breed had the greatest difference between the observed and the expected heterozygosity values (0.35), while the Savanna goats had the smallest difference of 0.29. Similar genetic variation values were reported by Barker *et al*. (2001) for indigenous Asian goat breeds. This indicates that the Asian goat breeds are mostly bred from a small number of animals

in each breed causing low variations and resulting in the occurrence of inbreeding. The occurrence of inbreeding was further confirmed by HWE and inbreeding analyses.

Table 1: Genetic differentiation in four goat breeds in Malaysia

Breed	A[1]	Ho[2]	He[3]
Katjang	5.43	0.36	0.71
Jamnapari	5.73	0.43	0.71
Boer	5.90	0.43	0.73
Savanna	5.70	0.42	0.71
Mean	5.69	0.41	0.72

[1]A, mean number of alleles per locus; [2]Ho, observed heterozygosity; [3]He, expected heterozygosity

Hardy-Weinberg Equilibrium (HWE)

Based on the exact test, all the four goat breeds showed deviations from HWE ($p<0.001$). In line with the population results, all except three of the polymorphic loci showed significant deviations from HWE (Table 2). Locus OARHH35 in the Jamnapari and Savanna goat breeds and; loci TGLA122 and INRABERN185 in the Savanna goat breed showed non-significant ($p \geq 0.05$) deviations from HWE. Generally, all breeds and loci showed significant ($p<0.05$) deviations from HWE except for these three loci.

Deviations from HWE may be due to many reasons, such as selection, non-random mating, migration and small population sizes. Deviations from HWE at microsatellite loci are as expected for livestock which are subjected to selection practices which cause changes in allele frequencies. This condition had been reported for various livestock studies (Araujo *et al*., 2006; Barker *et al*., 2001). This situation is also faced by goat breeds in Malaysia where small populations of the local indigenous Katjang are found as animals of this original goat breed are scarce. Meanwhile, the introduced goat breeds are subjected to selective breeding for increased productivity. Farmers keep good animals with certain characteristics for breeding purposes, and slaughter or sell off the poor performing animals as meat animals. Thus, only a limited number of selected bucks are used for controlled breeding practices.

All these activities contributed to deviations from HWE. Similar disequilibria were also found in Tswana goats in Bostwana (Maletsanake *et al*., 2013), Agew, Abergelle and Gumuz goats in Ethiopia (Hassen *et al*., 2012), Ghana dwarf, Togo dwarf, Senegal dwarf and Mossi goats in West Africa (Missohou *et al*., 2011) and Kanniandu goat breed in India (Thilagam *et al*., 2006).

Table 2: Exact tests of Hardy-Weinberg Equilibrium for the polymorphic loci in four goat breeds

No	Locus	P value			
		Katjang	Jamnapari	Boer	Savanna
1	CSRD247	0.0000	0.0000	0.0000	0.0003
2	DRBP1	0.0000	0.0000	0.0000	0.0000
3	ETH10	0.0000	0.0000	0.0000	0.0000
4	ILSTS005	0.0000	0.0000	0.0000	0.0003
5	ILSTS011	0.0000	0.0000	0.0000	0.0000
6	ILSTS029	0.0000	0.0000	0.0309	0.0009
7	ILSTS087	0.0000	0.0000	0.0000	0.0000
8	INRA063	0.0000	0.0000	0.0000	0.0000
9	INRABERN172	0.0000	0.0000	0.0000	0.0000
10	INRABERN185	0.0000	0.0022	0.0000	0.0534
11	MAF065	0.0000	0.0000	0.0000	0.0000
12	MAF209	0.0000	0.0000	0.0000	0.0000
13	MAF70	0.0006	0.0066	0.0059	0.0000
14	McM527	0.0000	0.0000	0.0000	0.0000
15	OARAE54	0.0000	0.0147	0.0013	0.0006
16	OARFCB20	0.0000	0.0000	0.0103	0.0000
17	OARFCB48	0.0000	0.0038	0.0347	0.0300
18	P19DYA	0.0000	0.0003	0.0000	0.0000
19	SPS113	0.0000	0.0000	0.0000	0.0000
20	SRCRSP15	0.0000	0.0000	0.0000	0.0000
21	SRCRSP5	0.0000	0.0000	0.0000	0.0000
22	SRCRSP7	0.0000	0.0000	0.0000	0.0000
23	SRCRSP8	0.0000	0.0003	0.0000	0.0000
24	SRCRSP9	0.0000	0.0000	0.0000	0.0000
25	TCRVB6	0.0000	0.0000	0.0000	0.0000
26	TGLA53	0.0000	0.0000	0.0000	0.0000
27	BM1225	0.0000	0.0000	0.0000	0.0000
28	BM1329	0.0000	0.0000	0.0000	0.0000
29	OARHH35	0.0225	0.1441	0.0203	0.1916
30	TGLA122	0.0000	0.0003	0.0013	0.1156
HWE p- value for each population		0.0000	0.0000	0.0000	0.0000

Loci above the dotted horizontal line are those recommended by FAO

Inbreeding and Genetic Differentiation

The inbreeding coefficients (F_{IS} values) showed heterozygote deficiencies occurring in all the four goat breeds which indicated inbreeding. All loci found positive F_{IS} values except of six loci. A positive F_{IS} value indicates an excess of homozygotes, while a negative value shows deficit in heterozygosity. Loci CSRD247 and OARHH35 were found in Katjang and Jamnapari, respectively with negative F_{IS} values, while loci ILSTS029 and INRABERN172 in the Boer and loci ILSTS005 and ILSTS029 in the Savanna also had such negative values.

The Wright's F-statistics values estimated for each of the 30 polymorphic microsatellite loci are summarized in Table 3. These positive values indicated heterozygote deficit with a mean F_{IS} value of 0.43. Thus, the results of $F_{IS,}$ together with the low number of alleles indicated the occurrence of inbreeding in all four breeds. This may be due to non-random mating. This situation probably occurred because indigenous breed conservation, an important subject, has never been seriously considered before in Malaysia. In recent years, very little effort had been focused on the value of indigenous animals and their conservation. This could be due to the national strategy of increasing meat production through the importation of large numbers of exotic animals. The indigenous Katjang goats were kept mostly by small farmers, resulting in mating of related animals which caused the low genetic variability as most of the farmers were not aware or concerned with proper breeding practices. The inbreeding coefficient value (F_{IS}) for the Katjang goat breed was higher than those of the other goat breeds. The Boer and Savanna goats sampled from the MARDI farm also showed inbreeding which could be due to the small number of foundation animals used as bucks for developing the Boer and Savanna goat breeds in Malaysia.

The mean F_{IT} and F_{ST} values of 0.46 and 0.06, respectively measured the degree of differentiations within and among breeds. The F_{ST} value indicated a lack of genetic differentiation among the goat breeds. The overall deficit of heterozygotes across subpopulations (F_{IT}) amounted to 46% and the genetic differentiation among subpopulations (F_{ST}) was low (6%). Although the total genetic variability could be recognised as differences among subpopulations, evidence for moderate genetic subdivisions in goat populations in Malaysia was detected.

Similar mean F_{IS} values were reported by Barker *et al.* (2001) for 11 indigenous Southeast Asian goat populations. The F_{IS} estimates were very high and were contrary to those of most studies of livestock (Canón *et al*., 2000). Strong inbreeding in Southeast Asian native goat populations was also reported by Davendra and Nozawa (1976) and this was due to small population size, inbreeding and genetic drift. However, heterozygote deficiencies could also be due to microsatellite null alleles or scoring bias (Maletsanake *et al*., 2013; Thilagam *et al*., 2006), and these are generally difficult to differentiate (Christiansen *et al*., 1974). Microsatellite studies may be influenced by scoring bias against heterozygotes for some loci during allele screening using electrophoresis, but not for all loci.

Table 3: F-statistics values averaged over microsatellite loci in four goat breeds

Locus	Overall F_{IS}	F_{IT}	F_{ST}
1225	0.55	0.58	0.08
BM1329	0.24	0.25	0.02
CSRD247	0.17	0.19	0.02
DRBP1	0.69	0.70	0.01
ETH10	0.84	0.85	0.06
ILSTS005	0.43	0.47	0.06
ILSTS011	0.52	0.54	0.04
ILSTS029	0.22	0.30	0.11
ILSTS087	0.62	0.71	0.24
INRA063	0.69	0.70	0.03
INRABERN172	0.19	0.20	0.01
INRABERN185	0.55	0.64	0.20
MAF065	0.28	0.32	0.06
MAF209	0.87	0.87	0.04
MAF70	0.12	0.12	0.00
McM527	0.35	0.38	0.05
OARAE54	0.29	0.30	0.01
OARFCB20	0.48	0.48	0.01
OARFCB48	0.29	0.41	0.16
P19DYA	0.33	0.35	0.02
SPS113	0.30	0.36	0.08
SRCRSP15	0.78	0.78	0.00
SRCRSP5	0.38	0.43	0.07
SRCRSP7	0.85	0.88	0.20
SRCRSP8	0.51	0.52	0.03
SRCRSP9	0.34	0.36	0.02
TCRVB6	0.53	0.53	0.01
TGLA53	0.55	0.55	0.01
BM1225	0.55	0.58	0.08
BM1329	0.24	0.25	0.02
OARHH35	0.12	0.16	0.05
TGLA122	0.21	0.22	0.02
Mean	0.43	0.46	0.06
Bootsrap Upper	0.51	0.53	0.08
Bootsrap Lower	0.35	0.39	0.04

Loci above the dotted horizontal line are those recommended by FAO

Conclusion

There are some genetic variations present in the Katjang, Jamnapari, Boer and Savanna goat breeds in Malaysia. The results of this study indicated the occurrence of inbreeding and low genetic variabilities in all four goat breeds. Therefore, implementation of appropriate breeding programmes and strategies are necessary to avoid loss of genetic diversity, particularly in the indigenous Katjang goat breed.

Acknowledgement

The authors would like to acknowledge the financial support from the Ministry of Science, Technology and Innovation, Malaysia for a research grant through Science Fund project number 05-03-08-SF0139. The authors thank the staff of the MARDI Research Station, Kluang and the Animal Breeding and Physiology Laboratory (Molecular Biology) members for their technical support.

References

Amie Marini, A.B., Mohd. Hifzan, R., Johari, J.A., Tan, S.G. and Panandam, J.M. 2013. Genetic variation of four goat breeds in Malaysia using microsatellite polymorphism markers. Mal. J. Anim. Sci. 16(2): 1-8.

Araujo, A.M., Guimaraes, S.E.F., Machado, T.M.M., Lopes, P.S., Pereira, C.S., Da Siva, F.L.R., Rodrigues, M.T., Columbiano, V.D.S. and Da Fonseca, C.G. 2006. Genetic diversity between herds of Alpine and Saanen dairy goats and the naturalized Brazilian Moxoto breed. Genet. and Mol. Biol. 29: 67-74.

Barker, J.S.F, Tan, S.G., Moore, S.S., Mukherjee, T.K., Matheson, J-L. and Selvaraj, O.S. 2001. Genetic variation within and relationships among populations of Asian goats (*Capra hircus*). J. Anim. Breed. Genet. 118: 213-233.

Canon, J., Checa, M.L., C., Carleos, C., Vega-Pla, J.L., Vallejo, M. and Dunner, S. 2000. The genetic structure of Spanish Celtic horse breeds inferred from microsatellite data. Anim. Genet. 31: 39-48.

Christiansen, F.B., Frydenberg, O., Gyldenholm, A.O. and Simonsen, V. 1974. Genetics of Zoarces populations VI. Further evidence based on age group samples of a heterozygote deficit in the EST III polymorphism. Hereditas 77: 225-236.

Davendra, C. and Nozawa, K. 1976. Goats in South East Asia – their status and production. J. Anim. Breed. and Genet. 93: 101-120.

FAO. 2004. Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans; Measurement of Domestic Animal Diversity (MoDAD): New Recommended Microsatellite Markers, Food and Agriculture Organisation of the United Nations, Rome. Retrieved 1 March 2009, from http://www.fao.org/dad-is/.

Hassen, H., Lababidi, S., Rischkowsky, B., Baum, M. and Tibbo, M. 2012. Molecular characterization of Ethiopan indigenous goat populations. Tropic. Anim. Health Product. 44: 1239-1246.

Kim, K.S., Min, M.S., An, J.H. and Lee, H. 2004. Cross-species amplification of bovidae microsatellites and low diversity of the endangered Korean goral. Heredity 95(6): 521-525.

Lewis, P.O. and Zaykin, D. 2002. Genetic data analysis: Computer Program for the Analysis of Allelic Data, Version 1.1 (d16c). Retrieved 20 May 2010 from http://lewis.eeb.uconn.edu/lewishome/software.html/.

Maletsanake, D., Nsoso, S.J. and Kgwatalala, P.M. 2013. Genetic variations from 12 microsatellite markers in an indigenous Tswana goat flock in South-eastern Botswana. Livestock Research for Rural Develop. 25 (2): article 21.

Missohou, A., Pout, M.R., Nenonene,A., Dayo, G.-K., Ayssiwede, S.B., Talaki, E., Issa and Fané, A. 2011. Genetic diversity and differentiation in nine West African local goat breeds assessed via microsatellite polymorphism. Small Ruminant Research 99: 20-24.

Saitbekova N., Gaillard C., Obexer-Ruff, G. and Dolf, G. 1999. Genetic diversity in Swiss goat breeds based on micosatellite analysis. Anim. Genet. 30: 36-41.

Thilagam, K., Ramammorthi, J., Sivaselvam, S.N. and Karthickeyan, S.M.K. 2006. Kanniadu goats of Tamildanu, India: genetic characterisation through microsatellite markers. Livestock Research for Rural Develop. 18(10): 149-150.

Yeh, F.C., Boyle, T. and Yang, R. 1999. *POPGENE version 1.31*. Microsoft window-based Freeware for population genetic analysis (http://www.ualberta.ca/~fyeh/popgene_download.html).