{Original paper}

Genetic diversity and relationships between indigenous and the original Thai meat goat population based on microsatellite analysis

Suwit ANOTHAISINTHAWEE¹, Takao OISHI², Takashi AMANO³, Koh NOMURA²

¹ Bureau of Animal Husbandry and Genetic Improvement, Department of Livestock Development, Thailand

² Faculty of Agriculture, Tokyo University of Agriculture, Japan

³ Professor emeritus, Tokyo University of Agriculture, Japan

ABSTRACT

A total of 149 goats from two Thai indigenous breeds (northern and southern), two exotic breeds (Anglo-Nubian and Boer), and an original Thai meat goat population were genotyped with 26 microsatellite markers. The average number of alleles observed was 9.3. The observed and expected heterozygosity (H_O and H_E) for the population ranged 0.52-0.71 and 0.60-0.66, respectively. The H_O and H_E of southern Thai indigenous goats (0.52 and 0.62, respectively) were lower than those of the northern Thai indigenous goats (0.67 and 0.63, respectively). Analysis of Nei's genetic distances and Bayesian-model-based clustering grouped the two indigenous goat breeds together and separated them from the two exotic breeds. A combined dendrogram obtained by adding Asian indigenous goat populations showed that the northern Thai indigenous goats are closest to Bangladesh Black Bengal, the dendrogram of which was located in the major southeastern Asian indigenous goat clade with southern Thai indigenous goats. The original population (a hybrid with 50% Boer, 25% Anglo-Nubian, and 25% southern Thai indigenous ancestry) was located close to Boer and Anglo-Nubian; it formed a separate clade from that of the Thai indigenous goats. Genetic characterization of these genetic resources provides essential information for conservation and breeding programs.

Key words: Genetic diversity, Genetic resource, Thai indigenous goat

INTRODUCTION

In compliance with the 1992 Convention on Biological Diversity (CBD 1992), Thailand has established a national action plan for sustainable conservation of biological diversity from 1998 to 2002. The purposes were to strengthen the capacity for sustainable use of environment and natural resources as well as to implement measures for the conservation of biological resources (Khumnirdetch 2002). There is a need for proper description and evaluation of the existing genetic resources and for identifying breeds to be used for improving indigenous goats. Information on goat-breeding improvement is needed for the optimum production of meat, milk, and skins. Indigenous goats, although less productive than exotic breeds, are well adapted to low-input extensive production systems, enabling them to survive under harsh conditions and making them important resources for smallholders.

Of the ten goat breeds present in Thailand, two are indigenous and eight are exotic. One of the indigenous

breeds is the northern Thai, referred to as "Bangala", which has a large but thin body, long pendulous ears, and a straight face profile. The Bangala may be of Bangladeshi or Indian origin or it may be a crossbreed introduced from Myanmar many years ago. The other is the southern Thai native breed, which is small with short upright ears and similar to the Katjang goat found throughout southeast Asia. Exotic goats are widely raised in Thailand. Anglo-Nubian, imported to Thailand in 1982, has long, pendulous ears and a decidedly Roman nose. It is a relatively large and dual-purpose (milk and meat producing) breed with a great capacity for adaptation to hot climates. The Boer goat is a popular meat breed owing to its rapid growth, excellent meat quality, and

Correspondence: Dr. Koh Nomura, Laboratory of Animal Genetics and Breeding, Faculty of Agriculture, Tokyo University of Agriculture, Funako 1737, Atsugi, Kanagawa 243-0034, Japan. (E-mail: meeme@nodai.ac.jp)

high fertility (Malan 2000). This breed originated in South Africa and was first introduced to Thailand in 1996 by the Department of Livestock Development (DLD). The DLD has initiated a research project to develop a Thai meat goat breed that can be reared in the humid tropical climate of the southern area. It has been developed from three-way crossing (50% Boer x 25% Anglo-Nubian x 25% southern Thai indigenous) and, with a rapid growth rate, is deemed suitable for meat production on a smaller scale. At present, this original Thai meat goat population is in the F_1 generation and is producing F_2 kids (Anothaisinthawee *et al.* 2010).

Knowledge of genetic variation within and among different breeds is important for understanding and developing endogenous economic traits of breeds, optimizing breeding strategies, and regulating germplasm conservation (Yeo *et al.* 2000). Several analyses of genetic diversity at the breed and population level have used microsatellite markers whose genomic locations have been defined by genetic mapping and which are highly polymorphic, distributed across the entire genome, and neutral with respect to selection. Microsatellite markers have been used to analyze genetic variations in cattle (Mateus *et al.* 2004), sheep (Stahlberger-Saitbekova *et al.* 2001), pigs (Martínez *et al.* 2000), and goats (Barker *et al.* 2001; Cañón *et al.* 2006). Genetic diversity in farm animals is required to meet the current production requirements in diverse environments, to allow sustained genetic improvement, and to facilitate rapid adaptation to changing breeding objectives (Notter 1999). Only few studies have analyzed the molecular genetic variations in Thai goat populations to determine their position in the global goat diversity (Barker *et al.* 2001).

The objectives of the present study were to investigate the polymorphism in a set of goat genomic microsatellites and to quantify the genetic diversity of two indigenous, two exotic goat breeds and an original Thai meat goat population (G_0 and F_1) using microsatellite markers.

MATERIALS AND METHODS

Sample collection and microsatellite markers

Total 149 blood samples were collected from six goat populations. Two Thai native goat breed samples were collected from smallholders within the original area of the respective population, i.e., southern Thai indigenous goats

from Yala, Songkla, and Trang provinces ($n = 25$); northern Thai indigenous goats from Tak and Pitsanulok provinces ($n = 25$); Anglo-Nubian ($n = 25$), Boer ($n = 25$), and the original three-way cross goat (50% Boer, 25% Anglo-Nubian, 25% southern Thai indigenous goats) from an elite herd (G_0 , $n = 25$); and F_1 progeny of the G_0 goat ($n = 24$) collected from DLD livestock research stations. Pedigree information was used to sample unrelated animals. The blood samples were collected, treated with 4% EDTA, and stored at -70°C . DNA was extracted from blood using a standard phenol-chloroform extraction method (Sambrook *et al.* 1989).

PCR reactions were performed using a 10- μl final volume containing 200 μM of dNTPs, 1.5 mM of MgCl_2 , 2.5 pmol/ μl of each primer, 1 U of Taq DNA polymerase, 0.05 μg of genomic DNA, and 10X magnesium-free PCR buffer containing 50 mM Tris-HCl, 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, and 1% Triton[®] X-100. PCR profiles were as follows: initial denaturation for 4 min at 94°C , 35 cycles each of 30 s at 94°C , 30 s at annealing temperature of 45°C – 65°C (depending on the primers used), and 1 min at 72°C , followed by a final extension step at 72°C for 15 min. PCR products were resolved on the ABI-PRISM[®] 3100-Avant Genetic Analyzer (Life Technologies Japan). Allele sizes were determined relative to the GeneScan[™] -400HD internal lane size standard and scored using the GeneMapper[™] 3.5 software. We used 26 microsatellite markers (listed in Table 1) that were previously selected using tests for marker neutrality and linkage disequilibrium (Nomura *et al.* 2012).

Statistical analyses

For each population, allele frequencies, mean number of alleles (MNA), and observed (H_O) and expected (H_E) heterozygosities were calculated with Microsatellite Toolkit (Park 2001). Allelic richness (AR) per population (i.e., the corrected mean allele number reflected in the standardized sample size), F statistics (F_{IS} , F_{IT} , and F_{ST}) for each locus, and overall population and pairwise F_{ST} values were calculated and their significance was tested using FSTAT version 2.9.3.2 (Goudet 2002).

Pairwise D_A genetic distances (Nei *et al.* 1983) were calculated from allele frequencies and used to depict phylogenetic relationships using the neighbor-joining (NJ)

Table 1. Allele size and number of alleles for each 26 loci across the six Thai goat populations

Locus	Chromosome	Allele size (bp)	Number of allele
BM3205	1	204-241	15
TCRVB6	1	213-251	11
INRA040	2	225-247	11
OarFCB20	2	81-117	9
ILST029	3	149-179	12
MAF70	4	130-154	11
ETH-10	5	200-210	6
BM143	6	91-113	6
INRABERN192	7	172-194	6
MCM527	7	151-169	8
ILST005	10	173-185	5
CSSM030	13	159-169	5
BM2934	14	77-101	14
MAF065	15	115-149	12
HUJ614	16	150-182	9
MAF209	17	98-108	4
INRABERN185	18	261-281	7
HAUT14	18	150-170	10
BM1818	23	249-289	12
P19(DYA)	23	172-193	12
INRABERN172	26	234-256	9
CSSM043	27	233-257	7
RM044	29	84-104	9
SR-CRSP08	unknown	174-246	14
OarCP34	unknown	107-121	8
SR-CRSP26	unknown	126-148	11

algorithm (Saitou and Nei 1987) as implemented in Population version 1.2.30 (Langella 1999). Bootstrap resampling ($n = 1000$) was performed to evaluate the robustness of the phylogenetic clusters. The generated tree was visualized using NJ plot program (Perriere and Gouy 1996). The population structure and degree of admixture were inferred using Structure version 2.1 (Pritchard *et al.* 2000). Structure uses Bayesian clustering analysis of multilocus genotypes to assign individuals to populations, estimate individual admixture proportions, and infer the number of parental populations (K) for a given sample. In a Bayesian paradigm, the estimate of $\text{Ln Pr}(G|K)$ is a direct indicator of the posterior probability that genotypes fall into K clusters given the observed genotypic data (G). To obtain a representative value of K for data modeling, we performed 20 independent runs for each value from 1 to 10 with burn-in and MCMC iterations of 30,000 and 50,000, respectively, with default settings assuming an admixture model, followed by ΔK statistics (Evanno *et al.* 2005). The results of independent runs were aligned using ClumpP version 1.1.2 (Jakobsson and Rosenberg 2007) and a graphical display was generated using Distruct (Rosenberg 2004).

Table 2. Diversity parameters in six Thai goat population

Population	Abbreviation	n	TNA ¹	MNA ²	AR ³	H _E ⁴	H _O ⁵	F _{IS} ⁶
Thai meat goat G ₀	TMG ₀	25	158	6.08	6.04	0.6598	0.7077	-0.074 *** ⁷
Thai meat goat F ₁	TMF ₁	24	149	5.73	5.73	0.6587	0.6683	-0.015
Anglo-Nubian	AN	25	149	5.73	5.68	0.6019	0.5646	0.063 **
Boer	BO	25	157	6.03	5.99	0.6624	0.6308	0.049 *
Northern native	NN	25	139	5.35	5.32	0.6282	0.6738	-0.074 ***
Southern native	SN	25	144	5.53	5.49	0.6156	0.5169	0.163 ***

¹TNA = total number of alleles; ²MNA = mean number of alleles; ³AR = allelic richness based on 24 individuals per population; ⁴H_E = Expected Heterozygosity; ⁵H_O = Observation Heterozygosity; ⁶F_{IS} = average inbreeding coefficients; ⁷*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

RESULTS AND DISCUSSION

Genetic diversity

The 26 microsatellite markers amplified 243 alleles, with frequencies ranging from 4 at MS MAF209 to 15 at BM3205 with an average of 9.3 alleles per locus. The genetic variations expressed as MNA, AR, H_E, and H_O for each population are presented in Table 2. The respective values for the Thai northern and southern indigenous populations are 5.4 and 5.5 for MNA and 0.63 and 0.62 for H_E; these are similar and relatively high, respectively, to the results of a previous study of southeast Asian indigenous goat using the same microsatellite markers (Nomura *et al.* 2012). The F_{IS} value of southern Thai indigenous goats is significantly positive, most likely because of inbreeding, while that of northern Thai indigenous goats is significantly negative owing to an excess of heterozygotes. This result may reflect an influx of goats from Myanmar to northern Thailand and their admixture with Thai indigenous goats. High genetic diversity observed in a breed can be explained by overlapping generations, mixing of populations from different geographical locations, natural selection favoring heterozygosity, or subdivision accompanied by genetic drift (Toro and Mäki-Tanila 2007). The effect of these factors is more pronounced when the effective population size is very large. In the Boer breed, the H_E was the highest (0.66). The wide distribution of this high-producing breed across the country may lead to the enrichment of different alleles in different environments. As expected, the H_O of the three-way crosses goats (TMG₀) was highest (0.71) and consequently the F_{IS} was significantly negative ($P < 0.001$).

Genetic differentiation and relationships among breeds

All pairwise genetic differences quantified by F_{ST} estimates were significant ($P < 0.05$) except for the value of 0.02 between the TMG₀ and its F₁ progeny TMF₁. As their paternal line is Boer, TMG₀ and TMF₁ are estimated to be closest to the Boer breed with F_{ST} values of 0.06 (Table 3).

Table 3. Pairwise F_{ST} values (upper diagonal) and D_A genetic distances (lower diagonal) between six Thai goat populations

Population	TMG ₀	TMF ₁	AN	BO	NN	SN
Thai meat goat G ₀		0.019 ^{NS}	0.085	0.055	0.157	0.119
Thai meat goat F ₁	0.041		0.091	0.058	0.148	0.114
Anglo-Nubian	0.139	0.151		0.154	0.150	0.150
Boer	0.092	0.099	0.222		0.174	0.153
Northern native	0.246	0.239	0.218	0.268		0.116
Southern native	0.163	0.166	0.207	0.228	0.190	

^{NS} = not significant at $P \geq 0.05$

Fig. 1. Neighbor-joining tree based on 26 microsatellites in six goat populations in Thailand and Asian indigenous goats from seven countries.
A: Japanese Shiba, B: Korean indigenous, C: Taiwanese indigenous, D: Japanese Saanen, E: Philippine indigenous, F: Indonesian indigenous, G: Indonesian Etawa, H: Bangladesh Black Bengal, I: Indian breed from Bangladesh, J: Mongolian indigenous. The numbers on the nodes indicate the bootstrap values (%) obtained from 1000 replications.

The calculated breed relationships may be visualized with a NJ phylogenetic tree based on D_A . To identify the genetic relationships of the goat populations, microsatellite data from 19 populations from seven Asian countries, used in our previous study (Nomura *et al.* 2012), were added to those of the six Thai populations and a NJ tree was constructed (Figure 1). The Thai meat goats TMG₀ and TMF₁ and the Boer breed form a cluster with a 90% bootstrap value and then associate with Anglo-Nubian with a 70% bootstrap value. The two Thai indigenous breeds lie in the southeast Asian indigenous goat clade, away from the Boer and Anglo-Nubian branches. The northern Thai indigenous goats are located closer to Bangladesh Black Bengal (86% bootstrap value) than to the southern Thai

Fig. 2. Genetic structures of six Thai goat populations based on Bayesian analysis of microsatellite genotypes. K is the number of clusters modeled. The population abbreviations are shown in Table 2. Each animal is represented by a single vertical line divided into K colors. Black lines separate populations whose names are indicated above the diagram.

indigenous goats.

A Bayesian model built with the program Structure (Pritchard *et al.* 2000) was used to examine the relationship of the six Thai goat populations (Figure 2). The analysis did not clearly return genetic clusters with maximum likelihood ($\ln \Pr[G|K]$) and reached a likelihood plateau for $K > 5$. The ΔK values obtained were 323.7 at $K = 2$, 7.5 at $K = 3$, 2.1 at $K = 4$, and 1.1 at $K = 5$. The maximum ΔK at $K = 2$ corresponds to the separation of the two Thai indigenous breeds (blue) from the exotic breeds including the original Thai meat goat G₀ and F₁ (red). At $K = 3$, Anglo-Nubian (orange) was separated from the exotic breed cluster. At $K = 5$, Anglo-Nubian (orange), northern Thai indigenous (blue), and southern Thai indigenous (yellow-green) were dominated by single colors, whereas Boer consisted of a mixture of red and green. The color proportions of TMG₀ and TMF₁ were similar to those of Boer, but included more Anglo-Nubian (orange) and southern Thai indigenous (yellow-green) colors than Boer.

In conclusion, both indigenous Thai goats are genetically located in the southeast Asian goat clade, with small but significant differentiation from each other ($P < 0.05$), and separated from the exotic breeds. The two Thai indigenous goat populations should be conserved, independent of each other, as valuable genetic resources in Thailand. This information is important for meeting the demands of future breeding programs as well as for formulating effective conservation strategies for genetic diversity within breeds. It was stated that even small gains in

efficiency and low proportions of the genes from a conserved stock would bring profit for the nation and create genetic diversity to maximize the future economic efficiency of livestock.

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