

Analysis of Genetic Diversity of the Thai Swamp Buffalo (*Bubalus bubalis*) Using Cattle Microsatellite DNA Markers

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ABSTRACT : Recently the numbers of the Thai swamp buffalo (*Bubalus bubalis*), a native species of Thailand, have been rapidly declining, leading to a requirement for conservation programs for this breed. Such studies of the genetic diversity of this species are essential for conservation decisions and to assist the rational implementation of breeding programs. In this study, the genetic diversity of 80 Thai swamp buffalo, randomly selected from seven different research stations of the Thai Department of Livestock Development, were studied using ten cattle microsatellite markers. Polymorphic PCR products were observed at all microsatellite loci, with percentages of polymorphic loci ranging from 80.00 to 100.00%. The population from Payao showed the lowest level of polymorphism. The mean number of alleles per locus was 4.7 with the highest number of alleles being eight (ETH152) and the lowest being three (HAUT27 and ILSTS030). The average unbiased heterozygosity for all seven populations was 0.61 and varied between 0.5314 (Samui) and 0.6798 (Surin). The genetic distance according to Nei's (1972) ranged from 0.0722 to 0.4427. The populations from Surin and Buriram are the closest populations, while populations from Samui and Payao are the most divergent. The information generated by this study will greatly aid in the establishment of effective breeding and conservation programs for the Thai swamp buffalo. (*Asian-Aust. J. Anim. Sci.* 2006. Vol 19, No. 5 : 617-621)

Key Words : *Bubalus bubalis*, Cattle Microsatellites, Genetic Diversity, Thai Swamp Buffalo

INTRODUCTION

The Asiatic water buffalo is divided into two types, the riverine buffalo (2n = 50) and the swamp buffalo (2n = 48) according to ecotypes and geographical distribution (Macgregor, 1941) as well as diploid status. The riverine buffalo have been primarily developed for milk and secondarily for meat and draught, whereas the swamp buffalo have been developed primarily for draught, while meat and milk production are secondary. In Thailand, most buffalo are classified as the swamp type, and are usually dark gray but may also be black and have long, gently curved horns. Traditionally, the Thai swamp buffalo were raised by small farm holders for multipurpose roles, complementary with their crop production (Indramangala, 2001). Buffalo are native to Thailand and are well-adapted to poor conditions, especially in the northeastern part of the country.

A recent survey suggested that there are about 170 million head of buffalo in the world (Food and Agriculture Organization of the United Nations, 2004), and roughly 97% of them are found in the Asian region. However, while overall buffalo numbers are increasing world-wide at 1.3 percent annually, the numbers are reducing dramatically in Thailand. From 1995 to 2004, the number of buffalo in

Thailand has decreased from 3.7 to 1.49 million head or an average decline of nearly 22% per year (The Department of Livestock Development, Thailand, 2004). As a consequence, the Thai swamp buffalo are not only eligible for, but also require conservation as well as sustainable utilization in the production system (Na-Chiangmai, 2001).

Studies of livestock breeds have primarily focused on estimations of breed relationships (Barker et al., 1997b) in order to manage the conservation and maintenance of breeds (Martin-Burriel et al., 1999). Moreover, knowledge of phylogeny and genetic characterization of native breeds is the first step in conservation programs (Martin-Burriel et al., 1999).

DNA polymorphisms are powerful tools for molecular analysis and are being used to understand evolution and diversity (Navani et al., 2001). Microsatellites or short tandem repeats (STRs) are the DNA markers of choice for genome mapping, genetic dissection of complex traits and genetic diversity studies because of their highly polymorphic nature, co-dominant manner of inheritance and ease of typing (Barendse et al., 1994, 1997; Bishop et al., 1994; Kappes et al., 1997; MacHugh et al., 1997; Kim et al., 2001). At present, microsatellites have been identified and used for genetic study in many organisms including several livestock species such as in native Japanese chickens (Osman et al., 2005), Native Chinese chicken (Chen et al., 2005), and Mafriwal dairy cattle (Selvi et al., 2004). However, only a few genetic studies have been devoted to the Thai swamp buffalo, and no systematic studies have been undertaken to develop polymorphic DNA markers in this species. However, comparative genome studies have

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Received August 4, 2005; Accepted November 16, 2005

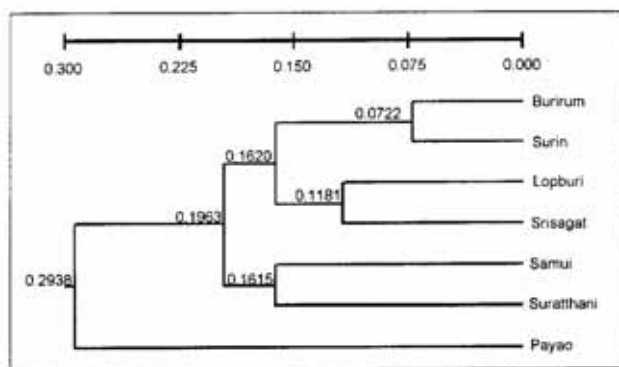


Figure 1. Locations of the Thai swamp buffalo analyzed in this study and the UPGMA dendrogram based on NEI's (1978) unbiased minimum distance showing the genetic relationship between among the seven buffalo population. Populations from Burirum and Surin show the smallest genetic distance (0.0722), while other closely related populations include those from Lopburi and Srisagat (0.1181) and Samui and Suratthani (0.1615). The population from Payao shows the largest genetic divergence (0.2938).

shown that microsatellite primer sequences are often conserved across related species and can be used for the development of markers in related species (Navani et al., 2001). Recently, a genome analysis of riverine buffalo (Navani et al., 2001), and a genetic diversity studies of the Asian water buffalo (Barker et al., 1997a, b) and the African buffalo (Van Hooft et al., 2000) using cattle microsatellite DNA markers have been reported. In addition, microsatellite markers were also used to study of genetic diversity in comparison between sheep and goat population (Sun et al., 2004; Yang et al., 2004). In studies of the genetic diversity of the Asian water buffalo (Barker et al., 1997a, b) swamp buffalo samples collected from different regions of Thailand (Chiang Mai, Kam Paeng Seng, Surin and Hat Yai) and other countries in Asia were investigated using microsatellite and protein-coding loci in order to analyze genetic variation within and amongst populations. In this study we applied cattle microsatellite markers that have previously been used in genetic diversity studies of both riverine and swamp buffalo (Moore et al., 1995; Navani et al., 2001) to analyze the genetic variation and diversity of the Thai swamp buffalo from seven locations of Thailand.

MATERIALS AND METHODS

Sample collection and DNA extraction

A total of 80 Thai swamp buffalo (*Bubalus bubalis*) were randomly selected from seven research stations of the Department of Livestock Development, Thailand located at Payao, Lopburi, Burirum, Srisagat, Surin and Suratthani

provinces and Samui island. Blood samples were collected from each animal and genomic DNA was extracted from blood samples using QIAamp DNA blood kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturers' instructions.

Microsatellite analysis

Ten cattle microsatellite markers, BM1818, BM1824, ETH3, ETH152, HEL13, HAUT27, ILSTS005, ILSTS030, CSSM36 and CSSM46 were used for the study. Polymerase chain reaction (PCR) was performed in a total volume of 20 μ l containing 50 ng of genomic DNA, 10 pmole each of forward and reverse primers, 200 μ M dNTP (Promega), 1 \times PCR Buffer, 1.5 mM MgCl₂, and 1.5 U *Taq* polymerase (Promega). PCR was accomplished by 1 min at 94°C, 1 min at primer annealing temperature, and 1 min at 72°C for 30 cycles. The PCR products were separated on 5% denaturing polyacrylamide gels and were visualized by silver staining according to manufacturer's protocol (Promega). To estimate size of the PCR products, 100 bp DNA standard ladder was loaded in parallel with the samples. The genotypes were scored manually.

Data analysis

The genotypic results of all individuals group according to location were analyzed using TFPGA 1.3 (Miller, 1997). Allele frequencies were calculated followed by calculations of expected heterozygosity based on Hardy-Weinberg equilibrium, the percentage of polymorphic loci (95% criterion) and genetic distance using the same program. A mantel test was used to assess the association between NEI's unbiased (1978) genetic distance matrix and the euclidian distance matrix using TFPGA 1.3.

An UPGMA tree based on NEI's unbiased (1978) genetic distance corrected for small sample size was produced to further examine the relationship between geographic and genetic distance (TFPGA 1.3). To generate increased confidence in the tree constructed by the original data, bootstrapping was performed with 1,000 permutations.

RESULTS AND DISCUSSION

In this study, 80 Thai swamp buffalo from seven locations in Thailand were selected for analysis of their relationship and genetic variation. Blood samples of individuals were collected and used for genomic DNA isolation. A total of ten cattle microsatellite loci were successfully amplified from the genomic DNAs and a total of 47 alleles were detected from the 10 investigated loci. The number of alleles observed at a single locus ranged from three to eight with an average number of 4.7 alleles per locus. The lowest variability markers that showed only

Table 1. NEI'S (1972) genetic distance matrix obtained from the allele frequencies of 10 microsatellite markers, sample size and genetic diversity indices for all sampled populations

Populations (no. of individuals)	Samui	Suratthani	Lopburi	Burirum	Surin	Srisagat	Payao
Samui (10)	-						
Suratthani (10)	0.1615	-					
Lopburi (17)	0.2258	0.1691	-				
Burirum (12)	0.1786	0.2100	0.2002	-			
Surin (10)	0.1835	0.1863	0.1932	0.0722	-		
Srisagat (9)	0.2026	0.2144	0.1181	0.1372	0.1175	-	
Payao (12)	0.4427	0.3790	0.3449	0.2033	0.1736	0.2193	-
Average unbiased heterozygosity	0.5314	0.5944	0.6044	0.6338	0.6798	0.6713	0.5965
Percentage of polymorphic loci	80	100	100	100	100	100	100

Average unbiased heterozygosity calculated from the null homozygote frequencies and percentage of polymorphic loci (95% criterion).

three alleles per loci were HAUT27 and ILSTS030, while ETH152 gave the highest level of diversity with eight alleles. The average unbiased heterozygosity, often used as an indicator of the genetic variability, for all seven populations was 0.61 and varied between 0.5314 (Samui) and 0.6798 (Surin) (Table 1). The percentage of polymorphic loci (using the 95% criterion) varied from 80.00 to 100.00% with the samples from Samui having the lowest degree of polymorphisms.

The genetic distance according to NEI's (1972) ranged from 0.0722 to 0.4427 (Table 1). Considering all distances measured, the closest populations were found to be the populations from Surin and Burirum with the populations from Samui and Payao being the most divergent. In support of this analysis of the data with UPGMA also clustered the populations from Surin and Burirum, as well as those from Suratthani and Samui (Figure 1).

Seven populations of Thai swamp buffalo situated in seven locations in Thailand were characterized using ten cattle microsatellite markers that were previously reported being used in riverine and swamp buffalo (Navani et al., 2001). As expected, buffalo populations are generally most closely related to populations that are geographically near. The exceptions to this are the Srisagat and Lopburi populations are closely related genetically, but distant geographically.

The populations from Surin and Burirum show the closest relationship indicating that they may share common parentage or these ten microsatellite markers are unable to differentiate their genetic diversity. However, since the two stations are located adjacent to each other, the buffalo are more likely to have an opportunity to be exchanged. This explanation may also reflect the relationship between the populations from Suratthani and Samui Island, as, while these two locations are geographically close, there is less chance to cross breed due to the distance between the island and the mainland. Surprisingly, the populations from Srisagat and Lopburi are closely related as compared to the

remaining populations, although these two stations are distant. The results indicated that the buffalo from these two stations may share common parentage due to the animal exchange program between the stations.

The results of this study indicated the expected relationships of the Thai swamp buffalo located in different places. However, according to the tree, the population from Payao that is located in the Northern part of Thailand are isolated from others. This may be due to the fact that these buffalo have the same origin and are bred within the region. Moreover, at the beginning of the establishment of the Payao research station, there was an introduction of buffalo from neighboring countries especially Laos, into the station, thus the buffalo here are diverse from other locations.

Comparing the results of this study with that reported by Barker et al. (1997a), we found four alleles for loci, CSSM036 and CSSM046, while Barker et al. (1997a) reported a total of five alleles for both of the loci. This may be the result of different numbers of swamp buffalo samples used in the two studies. Barker et al. (1997a) collected 25 swamp buffalo from Surin, Thailand while we analyzed ten samples from the Surin station. However, when considering the total numbers of buffalo used in this study (80), a higher numbers of alleles would be expected. The difference in the results may be due to the fact that both of the studies used different sets of swamp buffalo from Surin and that these are diverse in their genetic background.

This present study shows the utility of applying cattle microsatellite markers for the analysis of diversity and genetic relationships of the Thai swamp buffalo in Thailand, and is the first report of the genetic relationship of these populations in the country. Recently, a total of 30 microsatellite markers have been suggested by the EU AIRE 2066 Concerted Action Group for the analysis of genetic diversity in cattle. Therefore, additional microsatellites may be required for future studies. However, the results of the study in the Thai buffalo contribute the knowledge of genetic information of native Thai buffalo.

Due to the crisis of a sharp decline in the number of the Thai swamp buffalo, our data will be useful for the further planning by the Department of Livestock Development, Thailand to establish an effective breeding program and conservation plan for this species.

ACKNOWLEDGEMENTS

This research was supported by the Department of Livestock Development, Bangkok, Thailand, the Institute of Molecular Biology and Genetics, Mahidol University, Thailand and the Thailand Research Fund. We would like to thank the Department of Livestock Development for providing blood samples that were used in this project.

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