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Use of Cattle Microsatellite Markers to Assess Genetic Diversity of Thai Swamp Buffalo (*Bubalus bubalis*)

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ABSTRACT : In this study, cattle microsatellite markers recommended for diversity studies of cattle by the EU AIRE 2066 Concerted Action Group were used to study the genetic diversity of 105 Thai swamp buffalo which were randomly selected from eight different research stations of the Department of Livestock Development, Thailand. Of 34 primer pairs, 16 were successfully amplified while the rest showed non-specific amplification. The lowest number of alleles was two while the highest was nine, with an average of 4.7 alleles per locus. The average unbiased heterozygosity for all eight populations was 0.5233, with a low of 0.4772 (Samui) and a high of 0.5616 (Buriram). The genetic distance ranged from 0.0574 to 0.2575. Populations from Lopburi and Buriram showed the closest relationship, whereas Srisagat and Samui were the most divergent. The results generated with the primers recommended by the EU AIRE 2066 Concerted Action Group are at a slight variance from our previous study, possibly as a result of the number of specific amplification products obtained, suggesting that cattle markers may not be optimal for studies of the genetic diversity of the Thai swamp buffalo. (**Key Words** : Genetic Diversity, Microsatellite, Thai Swamp Buffalo)

INTRODUCTION

Microsatellites have been identified and used for genetic studies of many organisms including several livestock species (Selvi et al., 2004; Chen et al., 2005; Osman et al., 2005; Girish et al., 2007) but only a few genetic studies have been devoted to the Thai swamp buffalo (Triwitayakorn et al., 2006). Moreover, no systematic studies have been undertaken to develop polymorphic DNA markers specific to this species. However, comparative genome studies have 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, cattle microsatellite markers have been randomly selected to study the genetic diversity of the Thai swamp buffalo (Triwitayakorn et al., 2006) riverine buffalo (Navani et al., 2001), the Asian water buffalo (Barker et al., 1997a, b) and the African buffalo (Van Hooft et al., 2000). In this study we applied cattle microsatellite markers that have been approved for diversity studies of cattle by the EU AIRE 2066 Concerted Action Group and recommended by the MoDAD program (FAO), to analyze the genetic variation and diversity of the Thai swamp buffalo from eight locations in Thailand. The results of this study were compared with a previous study in order to evaluate the results obtained.

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MATERIALS AND METHODS

Experimental animals

A total of 105 Thai swamp buffalo (*Bubalus bubalis*) were randomly selected from eight research stations of the Department of Livestock Development, Thailand, located in Payao, Lopburi, Buriram, Srisagat, Surin and Suratthani provinces, Samui island and Akha Tribe which is a hill tribe folk who live in Maechan District, Chiangrai Province. Most samples used in this study were the same samples used previously (Triwitayakorn et al., 2006) except the samples from Payao and the new additional group from the Akha Tribe. Blood samples were collected from each animal for genomic DNA extraction using QIAamp DNA blood kit (QIAGEN GmbH, Hilden, Germany) according to

Table 1. Characteristics of cattle microsatellite markers approved for diversity studies by the EU AIRE 2066 Concerted Action Group tested in Thai swamp buffalo

Loci	Tm (°C)	No. of alleles	Allelic range[1]	Heterozygosity
D14S15	55	Multiple bands	-	-
D14S16	55	Multiple bands	-	-
D11S26	58	Multiple bands	-	-
D19S10-1	59	Multiple bands	-	-
D19S10-2	60	Multiple bands	-	-
DXS11	55	1-4	200-220	0.7280
D29S7	58	1-3	175-180	0.2275
D10S27	60	1-5	140-150	0.7161
D0S001	58	Multiple bands	-	-
D5S000	60	Multiple bands	-	-
D17S40-1	61	Multiple bands	-	-
D17S40-2	60	1-4	170-175	0.6422
D2S42	63	1-3	150-160	0.4721
D5S54	60	Multiple bands	-	-
D11S59	60	1-8	160-170	0.7923
D25S24	58	1-7	160-170	0.6220
D14S001	60	Multiple bands	-	-
D9S30	58	1-2	130-135	0.4154
D11S62	58	1-8	160-170	0.5969
D10S43	58	1-4	160-170	0.4790
D0S009	61	1-2	200-210	0.1654
D25S20	61	1-3	160-170	0.5222
D10S41	59	Multiple bands	-	-
D11S61	60	1-4	130-140	0.4361
D20S24	59	Multiple bands	-	
D13S17	58	1-7	165-170	0.7169
D21S28	60	1-9	180-200	0.8577
D10S31	60	Multiple bands	-	-
D21S29	58	Multiple bands	-	-
D24S12	60	Multiple bands	-	-
D1S41-1	58	Multiple bands	-	-
D1S41-2	58	Multiple bands	-	-
D13S32	60	1-2	175-180	0.4739
D1S44	59	Multiple bands	-	-

[1] Allelic range was estimated using 100 bp standard DNA ladder.

the manufacturers' instructions.

Microsatellite analysis

A total of 34 microsatellite loci which were approved for diversity studies of cattle by the EU AIRE 2066 Concerted Action Group (Table 1) were used to analyze individual samples. Polymerase chain reaction (PCR) was performed according to Triwitayakorn et al. (2006) in a total volume of 20 μl containing 50 ng of genomic DNA, 10 pmole each of forward and reverse primers, 200 μM dNTP (Promega), 1×PCR Buffer, 1.5 mM $MgCl_2$, and 1.5 U *Taq* polymerase (Promega). PCR was accomplished by 1 min at 94°C, 1 min at primer annealing temperature (Table 1), and 1 min at 72°C for 30 cycles. The PCR products were separated on 5% denaturing polyacrylamide gels and a 100 bp DNA standard ladder was loaded in parallel with the samples in order to estimate sizes of the PCR products. The gels were visualized by silver staining according to Sambrook and Russell (2001).

Data analysis

The genotypes were scored manually. The genotypic results of all individual groups were analyzed as described in Triwitayakorn et al. (2006) using TFPGA 1.3 (Miller, 1997) according to location.

RESULTS AND DISCUSSION

A total 16 of the 34 tested microsatellite primers were successfully amplified. The number of alleles per locus ranged from 2 (D9S30, D0S009 and D13S32) to 9 (D21S28) with an average of 4.7 (Table 1). Unbiased heterozygosity of each locus varied from 0.1654 (D0S009) to 0.8577 (D21S28) as shown in Table 1. The unbiased heterozygosity for all eight populations varied between 0.4772 (Samui) and 0.5616 (Buriram) with an average of

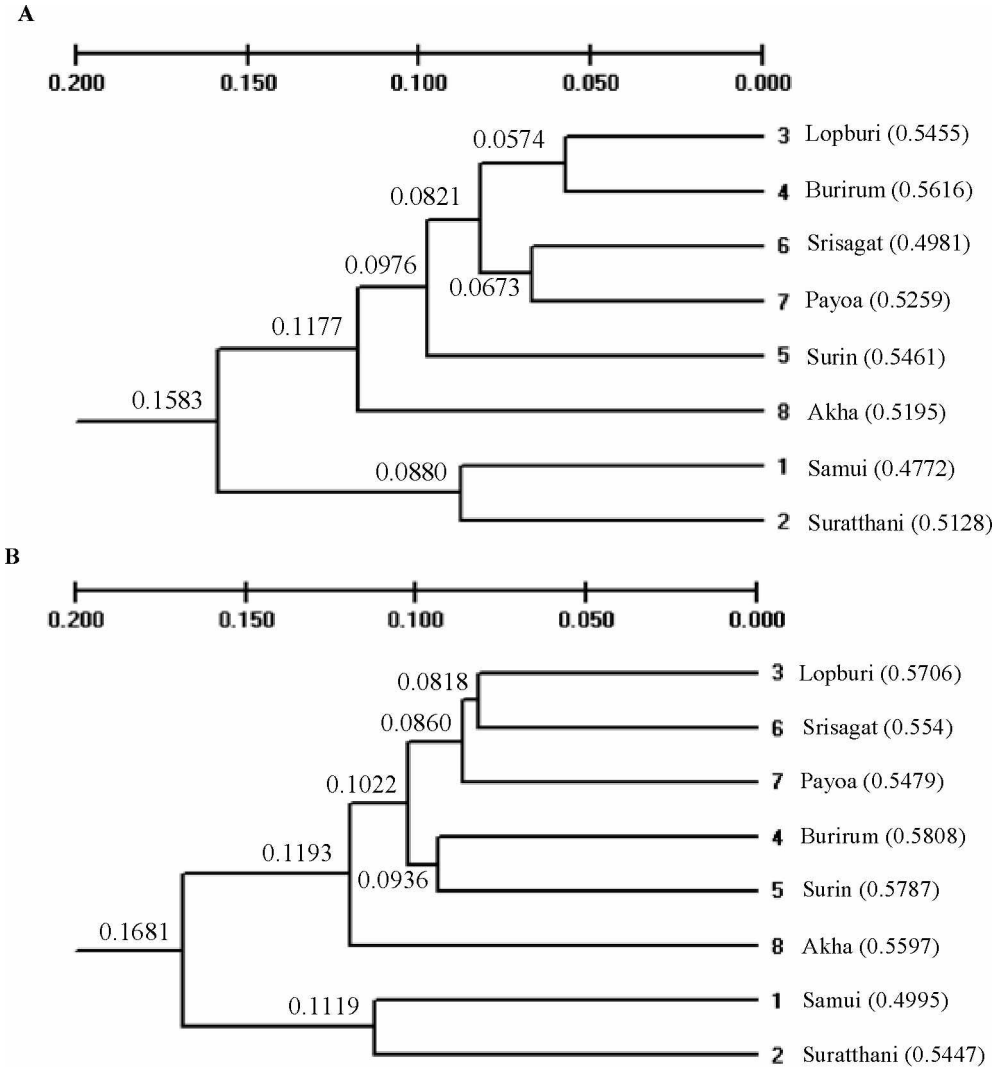

Figure 1. The UPGMA dendogram showing (A) the genetic relationship between among the eight buffalo populations using 16 microsatellite loci for diversity studies of cattle approved by the EU AIRE 2066 Concerted Action Group (B) the genetic relationship between among the eight buffalo populations using 16 microsatellite loci for diversity studies of cattle approved by the EU AIRE 2066 Concerted Action Group and 10 loci from Triwitayakorn et al. (2006).

0.5233. The percentage of polymorphic loci using the 95% criterion varied from 87.50 (Payao) to 100.00% (Suratthani, Lopburi and Akha Tribe). The genetic distance according to NEI's (1972; 1978) ranged from 0.0574 to 0.2575. Considering all distances measured, the closest populations were found to be the populations from Lopburi and Burirum, with the populations from Samui and Srisagat being the most divergent. In support of this analysis, the data with UPGMA also is shown in Figure 1A.

In this study, we found that 16 of 34 (47%) cattle microsatellite markers gave polymorphisms when screened with *B. bubalis*. This similar results was also found by Navani et al. (2002), who reported that 56% cattle microsatellite markers provided polymorphic band patterns when tested with 25 buffalo. Comparing the results of this study to that of our previous study (Triwitayakorn et al., 2006), which used randomly selected microsatellites that were tested for polymorphism in riverine buffalo by Navani et al. (2002), both studies report that the populations from Samui and Suratthani exhibit a close relationship, while the rest of the results are different. The genotype of individuals were re-screened with 26 microsatellite markers, ten from Triwitayakorn et al. (2006), and 16 from this study. The results showed that the populations from Surin and Burirum, Srisagat and Lopburi, and Samui and Suratthani are in the same clusters as reported previously (Triwitayakorn et al., 2006) and as shown in Figure 1B. This indicates that the ten microsatellite markers previously used in the study of genetic diversity of the Thai swamp buffalo have more utility than the microsatellite loci for diversity studies of cattle approved by the EU AIRE 2066 Concerted Action Group. However, only 16 of the 34 (47%) markers that are

recommended by the EU AIRE 2066 Concerted Action Group for genetic diversity analysis could be used in this study which may result in an inaccurate analysis. This suggests that the development of buffalo specific marker will greatly aid genetic diversity studies of the Thai swamp buffalo and other buffalo species.

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