GENETIC STRUCTURE OF FOUR BOVINE POPULATIONS IN THE PHILIPPINES USING MICROSATELLITES

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ABSTRACT

This study evaluated polymorphism of 11 microsatellite markers in four local genetic groups of cattle. Batanes cattle which has never been studied using microsatellite was evaluated for its genetic distance from the Ilocos cattle while Brahman and Holstein-Sahiwal were also included as the government uses these two breeds in their insemination program. PCR products for each marker were analyzed using POPGENE v32. Results showed that 55% (Fst=0.5501) of the genetic variation is due to the differences between populations while the remaining 45% is due to individual variation. The Fst value also indicates that there were very great differences from population to population using the range proposed by the geneticist Sewall Wright. The constructed phylogenetic tree based on Nei's genetic distance using the modified neighbor joining procedure of PHYLIP v3.5 showed the admixture of Brahman and Holstein-Sahiwal having them grouped in the same clade. Batanes and Ilocos cattle were grouped in a different cluster showing that they have descended from a single parental population. This would presumably address the claim that Batanes and Ilocos cattle are genetically distant from other groups and still exist despite the artificial insemination program of the government using Brahman and other imported breeds. The knowledge about the genetic structure of this population supports the development of conservation programs for the smallholder farmers.

Key words: cattle, microsatellites, Philippines, population genetics

INTRODUCTION

It is reported that almost one breed of domestic species disappeared each month within the period of 2000-2006 (FAO, 2008) all over the world. Around 20% of the reported breeds are classified at risk (FAO, 2007). Thus, there is a need to develop strategies for the conservation of local animal genetic groups. This needs consideration of multiple factors involved in biology of animals, agro- ecology of the environment, husbandry system of the animals, purpose of rearing and affordability of the owners duly to be addressed (Bayer *et al*., 2001). This marks the necessity to study the population structure of native animals and molecularly characterize them to employ conservation efforts and genetic improvement programs.

Genetic improvement programs are anchored on the studies of populations as baseline information of considering a particular genetic group to be unique from other existing populations. Traditionally, blood groups are used to evaluate parentage and genetic variability among populations. This has been expanded with the use of microsatellite

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markers and software to analyse these genetic groups. Several local cattle populations has been studied such as Ilocos, Siquijor, Batangas, and Philippine Bali to understand the structure and provide a knowledge of the ancestry of these genetic groups using microsatellites. These four local genetic groups are found to be different from one another with the Philippine Bali as the most genetically distant from the other groups (Aquino *et al*., 2006). Other populations of cattle such as in Cebu, Negros Occidental and Bohol are found to be genetically variable using albumin, transferrin esterase, acid phosphatase and malate dehydrogenase (Doydora, 2006).

The result of this study provides baseline information of the parentage of the two local animal genetic groups and evaluated their genetic distance from the imported breeds used for upgrading purposes.

MATERIALS AND METHODS

The blood samples used in this study were collected from Northern Luzon wherein 55 heads of cattle belong to four different groups, all found in Northern Luzon: 15 heads of Batanes cattle from Vujos, Batanes; 15 heads of Ilocos cattle from San Emilio, Ilocos Sur; 15 heads of Brahman and 10 heads of Holstein-Sahiwal from Tuguegarao City, Cagayan. The one ml of blood collected from the jugular vein of the animals were stored in Whatman FTA (Flinders Technology Associates) cards (Sigma-Aldrich Co., Singapore).

Polymerase Chain Reaction (PCR) primers used as prescribed by FAO for microsatellite studies are presented in Table 1. PCR was performed using a programmable thermal cycler (BIOTEK Applied Biosystems, GeneCo, Foster City, CA, USA) in a volume of 15 µl containing the DNA template (about 10-100 ng), 1 U Taq DNA polymerase (Vivantis, Revongen Corp. Center, Selangor Darul Ehsan, Malaysia), 1X PCR Buffer, 2.5 mM dNTPs, 10 pmol of each primer and 1.0-2.0 mM of MgCl2. PCR profile for the analysis of individual samples were as follows: 5 min at 95°C; 35 amplification cycles of 30 sec at 95°C, 30 sec at the specific annealing temperature for each primer pair, 30 sec at 72°C; 10 min at 72°C. PCR of the template DNA extracted from Ilocos and Batanes cattle were carried out with 40 amplification cycles. Two µl of PCR product from each sample were diluted in 40 µl of distilled water and were denatured for five minutes using the thermal cycler. A mixture of Hidi (1000 µl) and 500 Liz (10 µl) was prepared and 10 µl of the mixture were placed in each ABI tube. Two µl of the diluted and denatured PCR products were added having a total volume of 12 µl of each sample for ABI sequencing machine analysis. The results of the analysis were read using the software GeneMapper version 3.0 (Thermo Fisher Scientific, Waltham, MA, USA).

Allele frequencies were computed using the F-STAT program and Weir and Cockerham (1984) estimate of the F-statistics. An exact test for deviations from Hardy Weinberg equilibrium (HWE) due to heterozygote deficiency with 50,000 Monte Carlo Markov chain (MCMC) repetitions and dememorization steps was performed by the GENEPOP program (Raymond and Rousset, 1995). The weighted Reynold's distance (Dr. Reynolds *et al*., 1983), a distance measure based on Fst values, was used to construct an unrooted tree based on the neighbor-joining method (Saitou and Nei, 1987), with 1000 resampling on each locus (Felsentein, 1995) using POPULATIONS program (Langella, 2002). Multivariate statistical analysis (Carvalli-Sforza *et al*., 1994) using correspondence analysis of allelic frequencies as performed with GENETIX program (Belkhir *et al*., 2004). The STRUCTURE program (Pritchard *et al*., 2000) was used to infer population structure, by assuming an ancestry model from four populations with correlated allele frequency for a burn-in period of 1,000,000 simulations and 50,000 MCMC repetitions after burn-in.

Table 1. Primer sequences for microsatellite analysis.

Marker Name	Sequence (5' → 3')	Ex. Size
AGLA293	GAAACTCAACCCAAGACAACTCAAG ATGACTTTATTCTCCACCTAGCAGA	237-257
ETH152	TACTCGTAGCGCAGGCTGCCTG GAGACCTCAGGGTTGGTGATCAG	157-169
ILSTS005	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	281-299
ILSTS006	TGTCTGTATTTCTGCTGTGG ACACGGAAGCGATCTAAACG	181-185
ILSTS008	GAATCATGGATTTTCTGGGG TAGCAGTGAGTGAGGTTGGC	179-181
ILSTS013	CTTGATCCTTATAGAACTGG ACACAAAATCAGATCAGTGG	120-126
ILSTS023	TCTAGAAGGCTGGGACTTGG AGATTTCTGAAGTAGGGACC	170-284
ILSTS028	TCCAGATTTTGTACCAGACC GTCATGTCATACCTTTGAGC	128-160
ILSTS033	TATTAGAGTGGCTCAGTGCC ATGCAGACAGTTTTAGAGGG	132-158
ILSTS050	AAATCAGACACCCAGTTTCC GTTTTTCTACACGAGTTGGC	148-160
ILSTS103	CCAGTCTGCCAAAATCTATCG TATCAGGCGTAGTAAACAGC	218-226

Table 2. Nei's original measures of genetic identity and genetic distances based on 11 microsatellite markers in four cattle populations.

Population	Batanes	Ilocos	Brahman	Holstein Sahiwal
Batanes	*			
Ilocos	0.1443	*		
Brahman	0.3091	0.4423	*	
Holstein Sahiwal	0.4744	0.6071	0.2366	*

RESULTS AND DISCUSSION

Differences within and among the four populations were examined by F-statistics for each of the 11 loci. The average F_{is} value of 44% indicates the heterozygote deficiency. Moreover, the mean F_{st} was 55% in the population and would suggest that inbreeding is most likely to have happened within subpopulations and the remaining difference of F_{is} and F_{st} values would indicate of the inbreeding which can be accounted under subpopulation relative to the total population. The F_{it} = 0.1860 shows that there was a minimal inbreeding among individuals within population which was further supported by the Nm value (1.0940) which suggests that there was an effective barrier inhibiting gene flow from population to

population such as selection and geographical isolation.

The F_{st} estimate in all the 11 loci further explains that genetic variability in the different genetic groups was approximately 55%. From the scale of F_{st} values and corresponding population characteristics proposed by Wright (1931), an Fst ranging from 25%-55% indicates a very great genetic differences. This shows that there were relatively high differences among the studied populations showing their divergent evolutionary origins and supporting the classification of Ilocos and Batanes cattle as distinct genetic groups.

The unrooted consensus tree obtained for the four populations using NJ clustering with the D_r distance matrix (Table 2) is presented in Figure 1. As a measure of genetic distance D_r showed that Ilocos and Batanes were the most related population while the least related was Ilocos and HS. Brahman and HS are of the same clade and found to have diverged earlier than Ilocos and Batanes who share a common ancestry as reflected by both genetic groups being classified under a similar clade. These groupings are supported by the fact that the Sahiwal blood of HS has the same origin as that of the Brahman's while Ilocos and Batanes cattle are found in the extreme northern part of the Philippines.

There are four known subdivisions of Philippine native cattle as presented by Parker (1987) using morphological characteristics. Molecular data supports the claim that the two other groups (Ilocos and Batanes) have diverged into different genetic groups from a common descendant. There has been an artificial insemination program using imported breeds such as Brahman and Holstein-Sahiwal. However, Batanes and Ilocos cattle are genetically distant from these groups. Ilocos and Batanes cattle have a very high degree of genetic differences from that of the imported breeds included in the study. Thus, these two local genetic groups cannot be considered as admixtures of Brahman or Holstein-Sahiwal. They may have common ancestors but have diverged into different genetic groups through geographical isolation.

The high genetic variation of the two local animal genetic groups shows the potential of these animals to have diverged from their descendants to adapt to the existing ecotypes of their location. This suggests the need to conserve them as a raw genetic material for breeding purposes and animal genetic improvement programs.

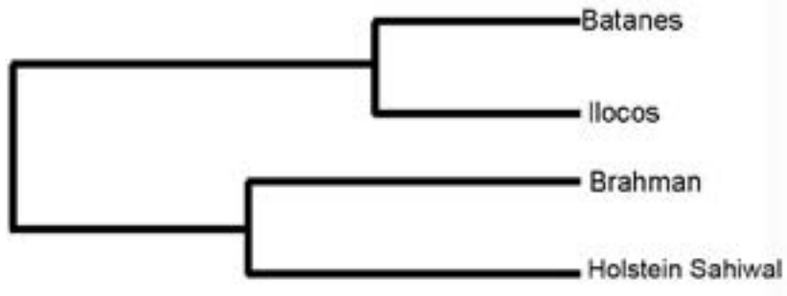

Figure 1. Dendogram showing the genetic relationship among the four genetic groups of cattle populations based on Nei's genetic distance using the modified neighbor procedure of PHYLIP Version 3.5 with a bootstrap of 95%.

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