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A new high-frequency allele of the BM2113 locus in the Yunnan mithun population

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ABSTRACT. The BM2113 locus was amplified in Yunnan mithun (*Bos frontalis*) from the southwest mountains of China. It showed a high degree of polymorphism with a total of 12 alleles. The 121-bp polymorphic allele of the BM2113 locus that accounted for 37.1% of homozygotes was the predominant allele with a frequency of 58.57%, identified as mithun-specific for *Bos* species in Yunnan mithun. The polymorphism information content value was high with a mean of 0.6170, the expected and observed heterozygosity was moderate with values of 0.6427 and 0.6000, respectively, and the BM2113 locus was under Hardy-Weinberg equilibrium (P = 0.2897) in the Yunnan mithun population. This study elucidated the genetic diversity, multi-origin, specific alleles, and characterization of mithun.

Key words: Yunnan mithun (*Bos frontalis*); BM2113; Polymorphism

INTRODUCTION

Mithun (*Bos frontalis*), which is also known as gayal and Dulong cattle by the Dulong tribe in Yunnan, China, is a species of *Bos* that is under a semi-management and rare status in the forests of Southeast Asia. In Yunnan, Dulong cattle is native to the Nujiang River and Gaoligongshan Mountains. It has a small population size, about 3000, with phenotypes that are similar to those of the Asian gaur. For example, it has a distinct spinous dorsal ridge, wide forehead, short-wide ears, and dark brown coat with white stockings (Lekagul and McNeely, 1977, 1988; Kakampuy et al., 2007; He et al., 2009). However, Yunnan mithun underwent introgression and genetic erosion from domestic cattle (*Bos taurus* and *Bos indicus*) based on microsatellite loci and mitochondrial DNA markers with multi-origin (Gou et al., 2010; Qu et al., 2012).

The BM2113 locus was designated and deposited in GenBank (accession No. M97162; Sunden et al., 1993); it exhibits Mendelian inheritance, contains an $(AC/GT)_{20}$ microsatellite, revealing a high degree of polymorphism in bovid species, and is located on chromosome 2. To shed light on the polymorphism of the BM2113 locus in the Yunnan mithun population, we scanned it to provide information to facilitate genetic diversity, genetic components, and phylogeny studies of mithun.

MATERIAL AND METHODS

Animals

Thirty-five mithuns (*Bos frontalis*, 6 males and 29 females) were from Phoenix Mountains, Jiumudang Farm of Yunnan Province, China, including one additional sample of frozen semen (He et al., 2009).

Polymerase chain reaction (PCR) and genotyping

Total DNA was extracted according to a previous study (Qu et al., 2006). The following primers were used: forward primer, 5'FAM-GCTGCCTTCTACCAAATACCC-3'; and reverse primer, 5'-CTTCCTGAGAGAAGCAACACC-3' (Sunden et al., 1993). PCR was performed in a 10-µL volume containing 20-50 ng/µL total DNA, 1.0 pmol each primer, 0.125 U *Taq* polymerase, 0.1 mM each dNTP, 1.0 mM $MgCl_2$, and 1.0 µL 10X PCR Buffer. The PCR procedure involved initial denaturation at 94°C for 4 min; 25 cycles of 30 s at 94°C, 40 s at 54°C, and 30 s at 72°C; and a final extension at 72°C for 10 min. PCR products were diluted and mixed with LIZ 500 size standards according to the procedure for microsatellite markers and were electrophoresed on an ABI PRISM 3730 DNA analyzer (ABI, USA) (Qu et al., 2006, 2012). Allele scoring was performed using the GENEMAPPER 3.0 software.

Data extraction and statistical analysis

Genotyping data of mithun (*Bos frontalis*) was extracted from the GENEMAPPER software 3.0. Genotypes of the locus were collected in an Excel file and then converted to a computable file format through Excel Microsatellite Toolkit Version 3.1 (http://animalgenom-

ics.ucd.ie/sdepark/ms-toolkit/index.php). Allelic frequencies, mean number of alleles per locus, and exact test for Hardy-Weinberg (H-W) equilibrium were calculated using the Genepop computer package (Version 3.4; Raymond and Rousset, 1995). The polymorphism information content (PIC) for loci was calculated according to Botstein et al. (1980).

RESULTS AND DISCUSSION

The genotypes of 35 mithuns (*Bos frontalis*) from Yunnan Province, China, showed high polymorphism and a total of 12 alleles as shown in Figure 1. The allele 121 bp was the predominant allele with a value of 58.57% (Figures 1 and 2), and it was identified as mithun-specific among the bovid species because 37.1% were homozygous for the allele 121 bp. Liao et al. defined it as specific-allele 120 bp (63.2%) in mithun from Yunnan Province (Liao et al., 2008; Zhang et al., 2008); however, there were distinct discrepancies between this report and that from Tian et al. (2011), who used silver staining. Allele 121 bp was absent in European cattle breeds (MacHugh et al., 1997), Brazilian Gir (Bicalho et al., 2006), Vietnamese cattle and wild gaur (Nguyen et al., 2007), Indian Kangayam cattle (Karthickeyan et al., 2009), Colombia Brahman cattle (Novoa and Usaquén, 2010), Uruguayan Creole cattle (Armstrong et al., 2013), Mongolian and Russian yak (Xuebin et al., 2005), Swiss yak (Nguyen et al., 2005), and Chinese yak (Liao et al., 2008; Zhang et al., 2008). No detailed data were shown for other bovid species in the previous studies on the BM2113 locus (Kantanen et al., 2000; Ritz et al., 2000; Kim et al., 2002; Zhang et al., 2007a,b, 2011; Mao et al., 2008; Wang et al., 2008; Rivière-Dobigny et al., 2009; Li and Kantanen, 2010; Azam et al., 2012). Herein, the PIC value was high and had a mean of 0.6170, the expected and observed heterozygosity was moderate with values of 0.6427 and 0.6000, respectively, and the BM2113 locus was in H-W equilibrium (P = 0.2897) in the Yunnan mithun population. This study revealed the genetic diversity, multi-origin, specific alleles, and characterization of mithun.

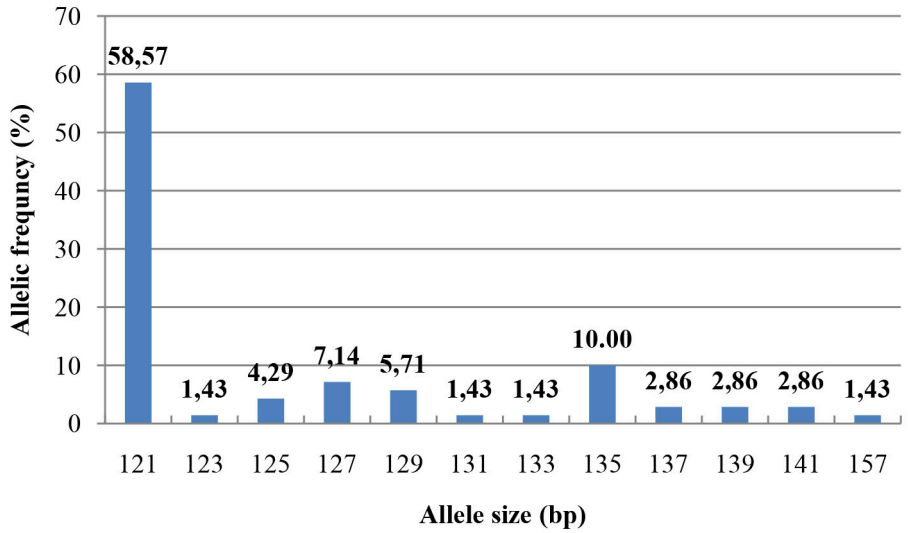

Figure 1. Allelic frequencies of Locus BM2113 in Yunnan mithun population.

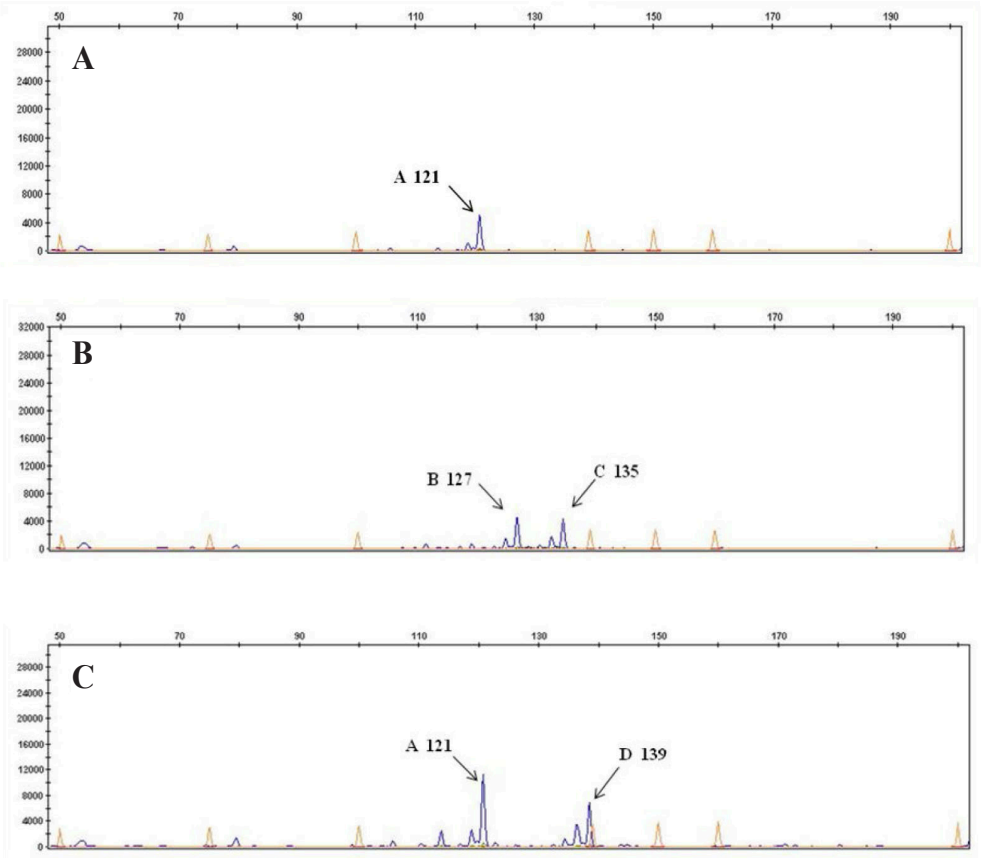

Figure 2. Four alleles of locus BM2113 are shown, A (121 bp), B (127 bp), C (135 bp) and D (139 bp), respectively. Each allele has sharp peaks and contains distinct stutter peaks (blue). **A.** Homozygous presentation of alleles A; **B.** and **C.** heterozygotes with two stutter peaks.

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