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The great diversity of major histocompatibility complex class II genes in Philippine native cattle

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ABSTRACT

Bovine leukocyte antigens (BoLA) are extensively used as markers for bovine disease and immunological traits. However, none of the BoLA genes in Southeast Asian breeds have been characterized by polymerase chain reaction (PCR)-sequence-based typing (SBT). Therefore, we sequenced exon 2 of the BoLA class II *DRB3* gene from 1120 individual cows belonging to the Holstein, Sahiwal, Simbrah, Jersey, Brahman, and Philippine native breeds using PCR-SBT. Several cross-breeds were also examined. *BoLA-DRB3* PCR-SBT identified 78 previously reported alleles and five novel alleles. The number of *BoLA-DRB3* alleles identified in each breed from the Philippines was higher (71 in Philippine native cattle, 58 in Brahman, 46 in Holstein × Sahiwal, and 57 in Philippine native × Brahman) than that identified in breeds from other countries (e.g., 23 alleles in Japanese Black and 35 in Bolivian Yacumeño cattle). A phylogenetic tree based on the D_A distance calculated from the *BoLA-DRB3* allele frequency showed that Philippine native cattle from different Philippine islands are closely related, and all of them are closely similar to Philippine Brahman cattle but not to native Japanese and Latin American breeds. Furthermore, the *BoLA-DRB3* allele frequency in Philippine native cattle from Luzon Island, located in the Northern

Abbreviations: MHC, major histocompatibility complex; SBT, sequence-based typing; BoLA, bovine MHC; PCR, polymerase chain reaction; HLA, human leukocyte antigen.

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Philippines was different from that in cattle from Iloilo, Bohol, and Leyte Islands, which are located in the Southern Philippines. Therefore, we conclude that Philippine native cattle can be divided into two populations, North and South areas. Moreover, a neutrality test revealed that Philippine native cattle from Leyte showed significantly greater genetic diversity, which may be maintained by balancing selection. This study shows that Asian breeds have high levels of *BoLA-DRB3* polymorphism. This finding, especially the identification of five novel *BoLA-DRB3* alleles, will be helpful for future SBT studies of *BoLA-DRB3* alleles in East Asian cattle.

Introduction

The major histocompatibility complex (MHC) proteins are cell-surface glycoproteins that bind small peptide fragments derived from host- and pathogen-expressed proteins via proteolysis. MHC molecules are divided into class I (expressed by all nucleated cells) and class II (expressed by antigen presenting cells and lymphocytes). MHC class I is recognized by CD8 positive cytotoxic T cells and MHC class II by CD4 helper T cells. Binding of peptides with MHC molecules initiates acquired immune responses. Therefore, MHC molecule polymorphism results in diverse immune responses (Germain and Jenkins, 2004).

The MHC system in cattle, known as the bovine leukocyte antigen (BoLA) located in chromosome 23, is highly polymorphic and forms an important component of the immune system (Ellis and Ballingall, 1999). The *BoLA-DRB3* gene is the strongest expressed gene with the highest polymorphism level of class II locus in cattle (Aida, 1995), and influences both the magnitude and epitope specificity of antigen-specific T cell responses to infectious diseases. Indeed, 130 *BoLA-DRB3* alleles have been identified in various breeds of cattle by sequencing of cloned genomic DNA, cDNA, or cloned polymerase chain reaction (PCR) products (Aida et al., 1995; Miyasaka et al., 2011; Takeshima et al., 2001, 2002, 2003). These alleles are listed in the Immuno Polymorphism Database (IPD)–MHC database (http://www.ebi.ac.uk/ipd/mhc/bola/index.html). *BoLA-DRB3* polymorphisms are associated with differences in susceptibility to infectious diseases (e.g., bovine leukemia virus-induced lymphocytosis, mastitis, and dermatophilosis), immunological conditions (according to 20 indicator traits of innate and adaptive immunity), and vaccine responses (e.g., foot-and-mouth disease and *Theileria parva*) (Ballingall et al., 2004; Baxter et al., 2009; Dietz et al., 1997a,b; Maillard et al., 2002; Miyasaka et al., 2013; Sharif et al., 1998; Takeshima et al., 2008b).

The difference between MHC molecules in wild and domestic cattle populations is of great interest to evolutionary biologists because of the high levels of polymorphism. In cattle, as well as in other mammals, the allele frequencies of BoLA class II genes vary between different breeds. *BoLA-DRB3* polymorphisms have been studied at the population level in less than 30 cattle breeds, including Jersey, Holstein, Black Pied, Ayrshire, Argentinean and Brazilian Creoles, Japanese Shorthorn, Japanese Black, Hanwoo, Nelore, Brazilian dairy Gir, Ongole, Martinique Brahman, and native breeds from East Asia and Latin America, which showed significant differences in the degree of polymorphism (e.g., 18 alleles were determined from 102 Holstein cattle in Japan and 36 alleles were detected from 113 Yacumeño cattle in Bolivia) (da Mota et al., 2002, 2004; Giovambattista et al., 1996, 2001, 2013; Lee et al., 2012; Miyasaka et al., 2011; Takeshima et al., 2003, 2008a).

The number of Philippine cattle was estimated to be 836,300 head in 2011 (FAOSTAT; http://faostat.fao.org/), with most being raised in backyard farms. Philippine native cattle are predominantly descended from Chinese and Mexican cattle, which were brought into the country by the Chinese and Spanish (Porter and Mason, 2002). It is thought that they originated from the yellow cattle of Southern China, which evolved from the *Bos indicus* in Java and the Brahmin archipelago (Payne, 1970).

Philippine cattle have been characterized using molecular markers for mitochondrial DNA (Watanabe et al., 1989) and by blood group DNA typing (Namikawa et al., 1984); however, the BoLA genes have not been characterized. Therefore, the present study used PCR-sequence-based typing (SBT) to examine the frequency and distribution of *BoLA-DRB3* genes within Philippine native cattle breeds that become highly adapted to living on certain islands. PCR-SBT can identify specific *BoLA-DRB3* alleles at the nucleotide sequence level, allowing the accurate detection of *DRB3* alleles (Lee et al., 2012; Miyasaka et al., 2011,