

## COMPARATIVE STUDIES ON BLOOD PROTEIN POLYMORPHISM OF BALI, MADURA, JAVA AND FILIAL ONGOLE CATTLE IN INDONESIA

Sutopo<sup>1</sup>, Edy Kurnianto<sup>1</sup>, and Takashi Amano<sup>2</sup>

### Abstract

Twenty-five loci of blood protein polymorphism were used to investigate the genetic structure of Indonesian cattle. Ten loci were found polymorphic, which can be sorted into six specificity alleles belong to *Bali* cattle. The specificity alleles of *Bali* cattle should be from an independent domestication event. A phylogenetic tree of the polymorphic loci was constructed by using the UPGMA method. All populations were significantly different ( $P < 0.01$ ). One distinct group of taurine cattle cluster separately and distantly from Indonesian cattle. Zebu and *Bali* were the major origins of cattle populations in Indonesia. *Madura* cattle could more influence by *Bali* cattle than Fillial Ongole did. To asses further the pattern of within and between populations, a principal component analysis (PCA) was performed. The first principal component accounts for 41.89% of the variation clearly distinguishes the taurine and *Bali* cattle. The second principal component, which also differentiates the taurine and zebu groups, summarizes 21.35% of the variation. The third principal component describes 6.95% of the variation and clearly separates the Indonesian cattle and taurine populations. This study contributes to the knowledge of the genetic structure and one of them may potential threat of extinction.

Key words: Indonesian cattle, Polymorphic loci, Genetic structure

### Introduction

Cattle are one of the most economically important domestic animals in the world. Their origin, genetic diversity, conservation and sustainable utilization have received close attention for a long time. Because of their tremendous economic and cultural importance many effort have focused on the classification of cattle by assigning animals to breed group but relatively little is known about the early origin of these populations. Breed assignments have often based on subjective data and information gleaned from local sources, especially in the developing countries where interbreed morphological differences are not very distinct. Consequently, there exist the need for a more objective system of breed classification, which would ultimately lead to a better use of cattle genetic resources. Such a system would also facilitate

<sup>1</sup> Faculty of Animal Science Diponegoro University, Tembalang-Semarang 50275-Indonesia.

<sup>2</sup> Tokyo University of Agriculture, Tokyo-Japan.

the future conservation of endangered breeds enabling more rational decisions to be made regarding conservation priorities (FAO, 1993).

The *Banteng* inhabits in the forest of Indonesia and some areas of southern Asia are believed to be ancestor of the *Bali* cattle which is native cattle raised in *Bali* island of Indonesia (Payne and Rollinson, 1973). The main difference between the *Banteng* and *Bali* cattle is one of size, the former are heavier than *Bali*. There is no information about other feral herds or individuals, but some Javanese cattle are said to exist in Java Island (Sutopo *et al.*, 2001). Java cattle possess a number of significant characteristics to *Madura* and *Bali* cattle, notably high fertility, minimal fat deposition and ability to thrive in hot humid environment. *Madura* cattle are thought to be derived from crosses between *Bali* or *Banteng* and either Indian or Javanese cattle (Payne and Rollinson, 1976). Coat colours of the *Madura* are generally brown, but the partial Balinese patterns are also existence. The Filial Ongole is undoubtedly believed to be a direct descendant of the Ongole, one of the Indian Zebu breed (Sutopo *et al.*, 2001).

### Materials and Methods

Blood samples were collected from a total of 225 cattle of *Bali* (49 animals), *Madura* (57 animals), Java (21 animals), and Filial Ongole (98 animals) on three islands in Indonesia. Every attempt was made to ensure each population sample was representative. A strict, distributed sampling strategy was employed and farmers were questioned about the origins and familial relationships of individual animals. In addition, a number of samples were taken from related species of *Bos* (European taurine). These were Japanese Holstein (30 animals) and Denmark Friesian (30 animals). The samples were analysed for polymorphisms at each loci using previously-described methods (Sutopo *et al.*, 2001). The degree of genetic variability was estimated by the proportion of polymorphic loci (Ppoly) (Nozawa *et al.*, 1978) and the average heterozygosity per individual ( $\bar{H}$ ). The average heterozygosity was calculated using a version of the DISPAN computer package (Institute of Molecular Evolutionary Genetics, Pennsylvania State University). From the matrix of the genetic distance values, a phylogenetic tree (Saitou and Nei, 1987) rooted with additional other breed of cattle and *Banteng* (Namikawa *et al.*, 1983) were drawn by the unweight-pair group method (UPGM) of clustering in numerical taxonomy (Sneath and Sokal, 1973). Principal component analysis (PCA) was also used to confirm the displacement of the cattle surveyed (Cavalli-sforza *et al.*, 1994).

### Results and Discussion

To compare the allelic polymorphisms in the *Bali*, *Madura*, Java and Filial Ongole cattle in Indonesia, the polymorphisms of 25 blood proteins were examined.

Blood protein polymorphisms were found at 10 loci, namely, albumin (*Alb*), transferrin (*Tf*), post-transferrin (*P-tf*), ceruloplasmin (*Cp*), amylase-I (*Am-I*), carbonic anhydrase (*CA*), diaphorase-II (*Dia-II*), hemoglobin- $\alpha$  (*Hb- $\alpha$* ), hemoglobin- $\beta$  (*Hb- $\beta$* ) and peptidase-B (*Pep-B*). The remaining proteins were monomorphic. (A table of allele frequencies for each of the 10 polymorphic loci in each of the cattle populations is available from Sutopo).

Some populations show markedly different allele frequencies ( $P < 0.01$ ) from some loci, in particular *Alb C*, *Tf D2Bali*, *Cp S*, *Am-I C*, *CA A*, *Hb- $\beta$  X Bali* are fixed in *Bali* cattle. Each of these alleles present at high frequency, but this allele is at low or moderate frequencies in all other Indonesian cattle populations.

To obtain the genetic variability in Indonesian cattle populations, the proportion of polymorphic loci and average heterozygosity were estimated. As shown in Table 1, the values of Ppoly of Indonesian cattle were ranged from  $0.400 \pm 0.1000$ , with an exception of *Bali* cattle. This enlarged genetic variability is ascribed to the high-levelled polymorphisms at the loci observed, which can be inferred as resulting from a large-scale gene-flow from the cattle imported from India for improvement of Indonesian cattle performance of the indigenous populations. Based on the Ppoly value the amount of genetic variability of blood protein in the *Madura* cattle on about the same level as in Java and Filial Ongole cattle. As shown in Table 1, average heterozygosity was lower of the *Bali* than those of the other three Indonesian cattle populations. The Ppoly of the *Bali* cattle was also lower than those of *Madura*, Java and Filial Ongole.

Table 1. Proportion of Poly and Average Heterozygosity of the Indonesian Cattle and Taurine Breed

Population	Ppoly $\pm$ S.E	$\bar{H}$ + S.E
<i>Bali</i> cattle	$0.280 \pm 0.0917$	$0.0583 \pm 0.0255$
<i>Madura</i> cattle	$0.400 \pm 0.1000$	$0.2063 \pm 0.0529$
Java cattle	$0.400 \pm 0.1000$	$0.1859 \pm 0.0485$
Filial Ongole	$0.400 \pm 0.1000$	$0.1747 \pm 0.0484$
Japanese-Holstein	$0.200 \pm 0.0816$	$0.0966 \pm 0.0395$
Denmark-Friesian	$0.200 \pm 0.0816$	$0.0840 \pm 0.0362$

In order to confirm the genetic relationships among the Indonesian native cattle of *Bali*, *Madura*, Java and Filial Ongole, we subjected the data on blood protein polymorphisms to phylogenetic analysis. As shown in Figure 1, the clusters of genetic similarities were seen between the *Bali* cattle and *Banteng*, whereas the *Madura*, Java and Filial Ongole cattle likely separated from the former group. Additionally, the Japanese Holstein and Denmark Friesian apparently formed a

cluster as it separated from Indonesian cattle populations. The additional data reported by Namikawa *et al.* (1983) were used for constructing phylogenetic chart based on principal component analysis. A close inspection of the three-dimensional allele frequency shows the main pattern of genetic diversity between Indonesian cattle and taurine breeds. On PC1, clearly distinguishes the *Banteng* group and taurine breed. The second PC also seems to primarily distinguish the taurine and zebu populations. The vertical axis (PC3), seems to separate the Indonesian cattle and taurine breed.

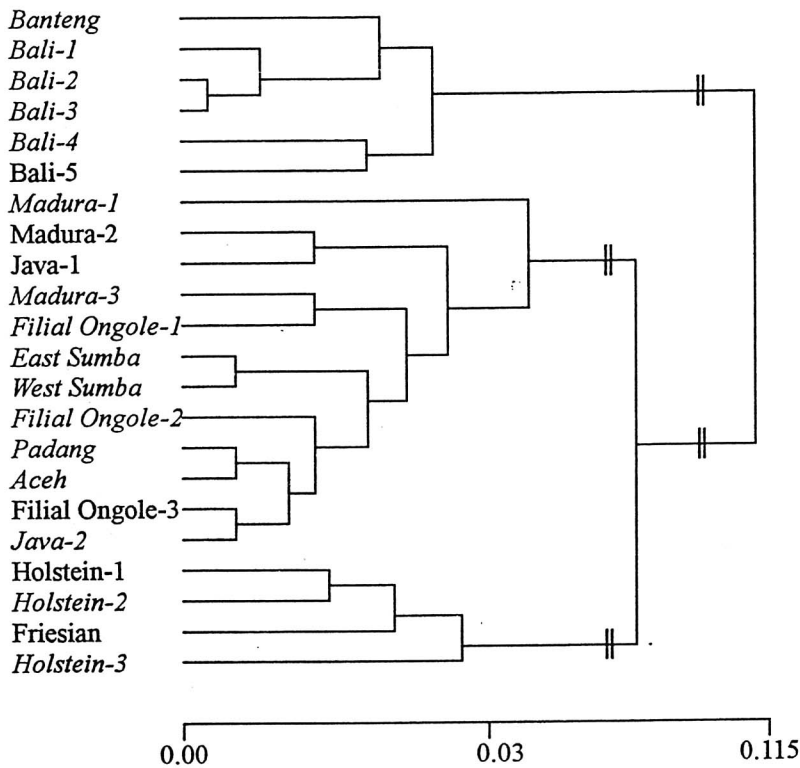


Figure 1. Dendrogram drawn from UPG method based on Nei's genetic distance matrix. Data on the cattle populations shown by italic character were obtained from Namikawa *et al.* (1983)

Detection of protein polymorphisms through the electrophoretic method is a tool for studying genetic variation in population and phylogenetic studies (Baker and Manwell, 1977; Manwell and Baker, 1977). Information from polymorphic loci can

be employed to detect population-specific alleles, to measure of genetic diversity and to evaluate the change in variation in species over time (Kantanen *et al.*, 1995). Also, Identification of novel alleles at loci in a specific population would elucidate the genetic relationships more clearly (Sutopo *et al.*, 2001). Although *Bali* cattle shows lowest genetic variability compared with those of three Indonesian populations (Table 1), but the specific alleles were obtained. It is likely that these allele do actually exist in *Bali* cattle. On the other hand, *Madura*, *Java* and *Filial Ongole* populations sampled should display zebu-specific allele frequencies that they are closer to unity.

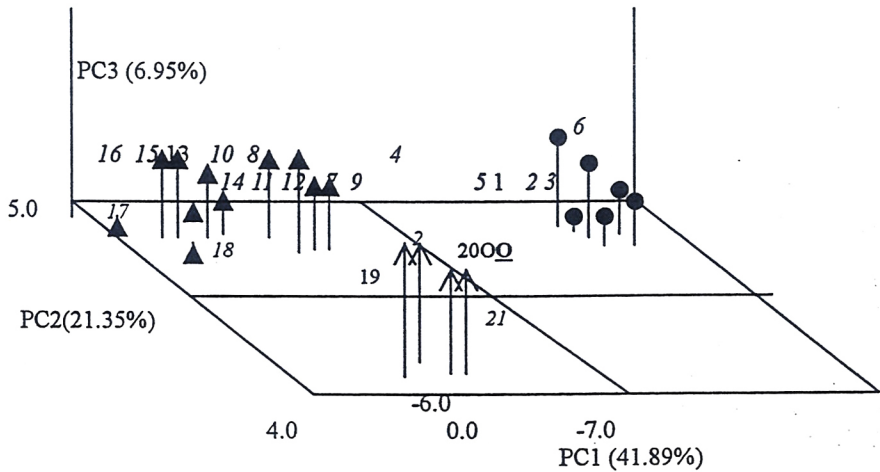


Figure 2. Principal component analysis from transformed allele frequencies in 21 cattle population and *Banteng*. The numbering order code correspond to a population sample: 1-5=*Bali*, 6=*Banteng*, 7-9=*Madura*., 10-11=*Java*., 12-14=*Filial Ongole*., 15=*Aceh*., 16=*Padang*., 17-18=*West/East Sumba*., 19,21,22=*Holstein* and 20=*Friesian*. Data on the cattle populations shown by italic character were obtained from *Namikawa et al.* (1983)

The most widespread view of the origins of domesticated cattle is that the *Bos taurus* and *Bos indicus* subspecies originate from the same early Neolithic domestication centres (Epstein, 1971). Also, phylogenetic analysis of mitochondrial genetic variation in domesticated cattle has underlined the substantial divergence between zebu and taurine breeds (Loftus *et al.*, 1994). In fact, analysis protein polymorphism gave evidence of genetic divergence between *Bali*, zebu and taurine in this study. The phylogenetic trees (Figure 1) showed that the *Bali* cattle were clustered separately from the cattle populations of *Madura*, *Java*, *Filial Ongole* and taurine breeds.

This study shows how blood protein analysis can be used to establish the genetic relationships between populations providing reasonable statistical power for breed assignment, regardless of whether they are closely related or not, allowing their future management to be based on greater knowledge of genetic structuring and relationships between populations. Considering the phylogenetic trees (Fig.1) and PCA diagram (Figure 2), the Indonesian cattle population could be established as *Bali* and *Indicus* breeds from India.

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