Genetic Diversity of Bali Cattle: Cytochrome b Sequence Variation

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Abstract. Livestock productivity in Indonesia is categorized as low compared to the other countries, yet the local Indonesian cattle is potential to develop. This research aimed to describe the genetic variation and phylogenetic profile of Bali cattle compared to their ancestor, i.e. Bos javanicus based on mitochondrial cytochrome b gene. This research was descriptive in which as many as 11 samples from Bali and 18 samples from Lombok were involved. The data, in term of sequencing results, were analyzed by using MEGA 4 version. The research results showed that the highest variation occurred in sample CL_8 (83 nucleotides), while the lowest one was shown by CB_7 (12 nucleotides). Meanwhile, the phylogenetic tree constructed presented that, genetically, Bali and Lombok cattle were categorized in seven big groups in which the closest genetic distance was between BL_1 and Bos javanicus (0.061), whereas the farthest was the distance between BL_8 and Bos javanicus (0.312). The results of this research can be utilized as the basic data for related fields to determine the most appropriate policies, particularly in managing local cattle breeding to obtain superior filial.

Keywords: Bali cattle, cytochrome b gene, genetic, diversity

1. Introduction
Bali cattle become one of the essential sources of livestock in Indonesia. They are considered as the most proper indigenous cattle breed in Indonesia [1]. There are superiorities found in Bali cattle, i.e. high fertility, rusticity, and low calf mortality [2]. Bali cattle are one of the potential local cows that have very good carcasses. Some of the advantages of Bali cattle include having fertility and a high percentage of carcass, low meat fat content, and being able to utilize low-quality feed, as well as providing a fairly good response in feed improvement. This case shows that Bali cattle have the potential and are relevant to be developed in Indonesia in general.

The main problem faced in the development of Indonesian cattle is the low population increase each year as considerable as the low productivity of livestock [3], both as cattle and livestock breeds. However, the need for beef consumption tend to rise every year [4,5]. Notwithstanding that the productivity of beef in Indonesia is relatively low compared to other countries, it actually can be improved either through environmental modifications [6], such as improving the quality of cattle feed [7,8], or changing its genetic quality in which the application of both aspects are highly recommended. Thus,
database aiming, including genetic profiling, based on more than one value per trait to obtain complete information of a particular species, is crucial [9].

Genetic diversity is a genetic variation at the species level which includes biochemical aspects, structure [10], and the nature of organisms that are physically derived from their parents [11] and formed from DNA [12]. Genetic diversity can occur due to changes in the nucleotides of DNA constituents. This change, under certain conditions, can affect the phenotype of an organism [13], which in turn, affects fitness or an individual's performance ability through natural selection [14]. In general, the genetic diversity of a population can occur due to mutations, recombination, or migration of genes from one place to another [15].

The genetic diversity of Bali cattle can be utilized to identify individuals, families, and offspring, as well as to see how close the relationship of Bali cattle is. In addition, genetic diversity is also important for the preservation of Bali cattle because of their rapid, easy, and accurate identification. These conservation efforts are expected to reduce the dependence on imported meat from abroad. Genetic diversity to identify individual offspring can be done by analyzing genetic information contained in mitochondrial DNA. It has been widely used as a molecular marker for the study of population genetics, trace origin, and tracking of several degenerative diseases, aging, and cancer. It also can be used to study phylogenetic precisely based on cytochrome b fragment. There are some advantages of mitochondrial DNA (mtDNA) as a marker. mtDNA has a high copy number [16], even in cells that do not contain a nucleus [17]. The number of copies per cell is around 1,000 - 10,000 so that mtDNA is used to analyze samples with the minimal amounts of DNA or easily-degraded DNA. This case will be really useful in case the analysis of samples with nuclear DNA cannot be done [18]. Moreover, mtDNA has a high rate of polymorphism with an evolutionary rate of about five to ten times faster than nuclear DNA.

It has been known that cytochrome b gene is one of potential markers to identify a particular species [19–24] as well as in taxonomic and phylogenetic studies. Mitochondrial DNA (mtDNA) has a higher copy number compared to nuclear DNA which makes its locus is ideal for analysis from highly degraded DNA. Notwithstanding that there were the advantages found in DNA [16] by some previous researchers [25–28], the observation of genetic diversity of Bali cattle is still needed to complete the limited data gained in recent days due to its difficulties in conducting the observation [29]. Therefore, this study aimed to obtain the information about the genetic diversity of Bali cattle based on the variation found in cytochrome b gene as well as to construct the phylogenetic tree based on the variation found.

2. Methods

2.1. Sample Collection
As many as 28 samples were obtained from two groups of Bali cattle, i.e. from Baturiti, Tabanan District, Bali Province, and Lingsar, Lombok Barat District, Nusa Tenggara Barat Province. The whole blood, as much as 10 mL, was taken from jugular venous by using venoject which then was preserved in a tube containing 4 mM EDTA as anticoagulant.

2.2. DNA Extractions
The DNA was extracted from blood using Nucleospin Blood kit; then, the extracted DNA was assessed qualitatively using electrophoresis on 6% agarose gel. The loaded DNA was stained using ethidium bromide and the DNA quality was determined using UV transilluminator; white its quantity was measured using UV-Vis Spectrophotometer. The DNA stocks were then adjusted into working concentration, i.e. 50 ng/μL in 1 x TE solution (pH. 7.5) for the PCR process.

2.3. PCR Amplification and Sequencing
A pair of cytochrome b primer was designed to amplify the targeted gene. The sequences of the primers were cyt-B F = 5’- TAG GAC GTA TCC TAT GAA TGCT - 3’ and cyt-B R= 5’- ACA AAT
CCT CAC AGG CCT ATTC - 3'. The thermal profile consisted of an initial cycle of 94 °C for 5 minutes, denaturation of 94 °C for 1 minute, annealing of 55 °C for 1 minute, extension of 72 °C for 2 minutes, and post extension of 72 °C for 5 minutes. The reaction volumes were 25 μL which consisted of 2 mM MgCl2, 200 μM dNTPs, 1× reaction buffer, 0.2 μM of each primer, and 1 unit of the fast start. The DNA sequencing was done at 1st Base Laboratory, Malaysia in which the processes were done based on Elmer’s method by using ABI PRISM DNA Sequencer [30].

2.4. Data Analysis

The DNA sequences obtained were analyzed using MEGA 4 version. The alignment and phylogenetic tree construction referred to the out-group sequence, i.e. Bos javanicus which is believed as Bali cattle ancestor. It was taken from NCBI in which the sequence reference was GBNC_012706.

3. Results and Discussion

As long as 273 bp of the cytochrome b gene sequence, which was placed between 14691 and 14914, was gained from each sample. Every sequence then was compared among the 28 other samples analyzed as well as the out-group (Bos javanicus) obtained from NCBI (sequence reference GBNC_012706). The alignment results showed variation in diverse sites. This variation can be a replacement of one nucleotide with another nucleotide, insertion, or the addition of one or more nucleotides in the gene, as well as the removal or deletion of one or more nucleotides in the gene (Table 1). It can be seen from Table 1 that the highest variation was found in Lombok 8 (as many as 83 nucleotides), while the lowest variation was found in Bali 7 (12 nucleotides). The conserved number of nucleotides for Bali cattle from Bali was 69.64%, while for Bali cattle from Lombok was 47.77%. This case proves that Bali cattle from Bali region were closer to Bos javanicus.

The number of variations proved that Bali cattle, which have been considered as the descendants of bull, have experienced many crosses with other types of cattle. Murtidjo [31] stated, which is in line with Winaya and Rahayu [26], that Bali cattle are the descendant of Bos javanicus (bull) which was successfully defused and experienced rapid development in Bali Island.

Table 1 The cytochrome b nucleotide variation of Bali cattle based on Bos javanicus sequence (GBNC_012706)

| No | Sample Code | Nucleotide Position | Variation |
|----|-------------|---------------------|-----------|
| 1  | Bali 1      | 14691, 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898 | 14        |
| 2  | Bali 2      | 14693, 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898, 14903 | 15        |
| 3  | Bali 3      | 1469, 14693, 14694, 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14877, 14898, 14903 | 16        |
| 4  | Bali 4      | 14691, 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898, 14903 | 15        |
| 5  | Bali 5      | 14691, 14692, 14694, 14695, 14696, 14697, 14698, 14670, 14703, 14704, 14705, 14707, 14709, 14710, 14711, 14712, 14713, 14714, 14715, 14718, 14720, 14722, 14726, 14727, 14729, 14734, 14751, 14762, 14763, 14764, 14765, 14790, 14813, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898 | 41        |
| 6  | Bali 6      | 14691, 14694, 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898, 14913 | 16        |
| No. | Location | Positions |
|-----|----------|-----------|
| 7   | Bali 7   | 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898 |
| 8   | Bali 8   | 14691, 14693, 14694, 14696, 14697, 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898 |
| 9   | Bali 9   | 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898 |
| 10  | Bali 10  | 14695, 14696, 14697, 14698, 14699, 14700, 14701, 14702, 14703, 14704, 14705, 14706, 14707, 14708, 14709, 14710, 14713, 14714, 14717, 14718, 14719, 14720, 14721, 14723, 14725, 14726, 14728, 14730, 14731, 14734, 14735, 14736, 14737, 14738, 14739, 14740, 14742, 14743, 14744, 14746, 14748, 14749, 14750, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898, 14903 |
| 11  | Lombok 1 | 14691, 14692, 14693, 14694, 14695, 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14874, 14877, 14898, 14903 |
| 12  | Lombok 2 | 14691, 14692, 14693, 14694, 14695, 14697, 14709, 14715, 14745, 14748, 14751, 14790, 14818, 14823, 14830, 14838, 14841, 14856, 14874, 14877, 14898 |
| 13  | Lombok 3 | 14691, 14692, 14693, 14694, 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14855, 14861, 14863, 14866, 14867, 14868, 14869, 14898 |
| 14  | Lombok 4 | 14709, 14713, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898 |
| 15  | Lombok 5 | 14691, 14692, 14693, 14694, 14695, 14696, 14697, 14698, 14699, 14700, 14704, 14706, 14707, 14708, 14709, 14711, 14712, 14716, 14722, 14723, 14724, 14732, 14744, 14752, 14822, 14827, 14842, 14844, 14860, 14878, 14881, 14902, 14919 |
| 16  | Lombok 6 | 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898 |
| 17  | Lombok 7 | 14691, 14692, 14693, 14696, 14697, 14698, 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898, 14903 |
| 18  | Lombok 8 | 14697, 14698, 14699, 14700, 14701, 14702, 14705, 14706, 14707, 14708, 14710, 14711, 14713, 14714, 14715, 14716, 14717, 14718, 14719, 14721, 14722, 14723, 14724, 14725, 14727, 14728, 14730, 14731, 14732, 14733, 14734, 14735, 14736, 14737, 14738, 14740, 14741, 14742, 14743, 14744, 14745, 14746, 14747, 14748, 14749, 14750, 14752, 14753, 14754, 14755, 14756, 14757, 14758, 14759, 14760, 14761, 14763, 14764, 14765, 14766, 14768, 14769, 14770, 14771, 14772, 14773, 14774, 14775, 14776, 14777, 14778, 14781, 14782, 14785, 14788, 14789, 14790, 14823, 14838, 14841, 14856, 14898 |
| 19  | Lombok 9 | 14691, 14700, 14701, 14709, 14710, 14715, 14746, 14748, 14749, 14750, 14751, 14781, 14818, 14823, 14831, 14841, 14856, 14891 |
It has been widely known that multiple factors influence the variation within individuals. The variation in cytochrome b genes in mitochondrial DNA in Bali cattle is assumed as the results of environmental adaptation of the species. This case is line with Zulkharnaim et al. who proved that the monomorphism occurred in Bali cattle which might be strongly influenced by an extreme condition of the place they live in [27]. In other words, the number of variations in individuals affects the fitness or individual’s performance abilities. The various genes can be caused by mutation. Mutation is a rare change in DNA which ultimately creates genetic diversity. Mutation can be a substitution, deletion or insertion. The higher the variation of a species, the higher the chance of being able to survive is. The characteristics related to fitness such as growth, viability, and fertility can also bring about some types of fluctuations.

The rapid evolution of mtDNA causes such variation. A nucleotide substitution in animal mtDNA that is five to ten times faster than single coffee core DNA is the cause of polymorphism between species (interspecies) or between individuals in one species (intraspecies). Most of the mutations in mtDNA are the mutations of synonyms or those in the codon without changing the protein structure expressed. This case is one of the characteristics of mtDNA which makes the mtDNA reason often used as a study tool in the Science of Genetics Molecular and Systematic Populations, especially phylogeny relationships.

3.1. Phylogenetic Analysis Results
The phylogenetic tree formed is one of the determinations of phylogeny using the cladistic method. Cladistics grouping must have the characteristic that all species share common ancestors and all species originated from common ancestors must be included in the taxon. In this study, a common ancestor of a cow is a bull.

The results of the phylogenetic tree construction of Bali cattle population from Bali and Lombok after being compared with a bull (Bos javanicus) are presented in Figure 1. It can be seen from Figure

| No  | Lombok  | Coordinates                      | Distance |
|-----|---------|----------------------------------|----------|
| 20  | Lombok 10 | 14691, 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898 | 14       |
| 21  | Lombok 11 | 14691, 14693, 14709, 14711, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898 | 16       |
| 22  | Lombok 12 | 14692, 14693, 14694, 14695, 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898 | 17       |
| 23  | Lombok 13 | 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898, 14904 | 14       |
| 24  | Lombok 14 | 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898 | 13       |
| 26  | Lombok 15 | 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898 | 13       |
| 27  | Lombok 16 | 14709, 14715, 14748, 14751, 14790, 14818, 14823, 14838, 14841, 14856, 14874, 14877, 14898 | 13       |
| 28  | Lombok 17 | 14751, 14790, 14811, 14823, 14838, 14841, 14856, 14881, 14882, 14883, 14885, 14887, 14888, 14890, 14891, 14892, 14893, 14894, 14895, 14896, 14897, 14898, 14899, 14900, 14901, 14902, 14903, 14904, 14905, 14906, 14907, 14908, 14909, 14910, 14911, 14912, 14913, 14914 | 27       |
1 that, genetically, Bali cattle can be grouped into eight major groups. Group 1 consisted of Bali-Lombok cattle (1,3,4,6,10,11,12,13,14,15,16,17), Bali-Bali (1,2,3,4,7,9), as well as Bos javanicus. In group 1, the Bos javanicus formed a small group with Bali-Lombok 1. Group 2 consisted of a combination of groups I and Bali-Lombok (2,7), and Bali-Bali 8. Bali 8 and Bali-Lombok 7 formed small groups. Group 3 was a combination of Bali-Bali 6 and group 2, Group 4 was a combination of group 3 and Bali-Lombok 5, Group 5 was a combination of group 4 and Bali-Bali 5, group 6 was a combination of group 5 and Bali-Lombok 9, Group 7 was a combination of groups 6 and Bali-Bali 10, while group 8 was a combination of Bali-Lombok 8 and group 7. The highest significance level was in group 2 (98%).

From these results, it can be stated that Bali-Lombok 1 cattle have the closest relationship to Bos javanicus. Moreover, bull (Bos javanicus) is still genetically close to Bali cattle, both from Bali and Bali cattle from Lombok region. Bali cattle from these two regions (Bali and Lombok) and bull form one group and appear to be from the same line [33].

Genetic distance measurement to support proximity between populations with phylogenetic tree indicated that the closest genetic distance to bull (Bos javanicus) was Bali-Lombok 1 cattle with a distance of 0.061 while the farthest distance was Bali-Lombok 8 with a distance of 0.312.

![Phylogenetic tree of Bali cattle from Bali and Lombok Islands](image_url)

**Figure 1.** Phylogenetic tree of Bali cattle from Bali and Lombok Islands

4. Conclusion

As a conclusion of this research, it can be noted that the sequence of cytochrome b gene of mitochondrial DNA from Bali cattle populations showed some variations. To be more detail, the highest variation of the nucleotide sequence of the cytochrome b of mitochondrial DNA was found in Lombok 8 cattle (83 nucleotides), while the lowest variation was found in Bali 7 (12 nucleotides). Moreover, as the phylogenetic tree was constructed, it revealed that, genetically, the both Bali cattle populations (which live in Bali and Lombok) were divided into eight major groups i.e Group 1, in which the members were Bali-Lombok cattle (1,3,4,6,10,11,12,13,14,15,16,17), Bali-Bali
as well as Bos javanicus. Meanwhile, the second group was comprised of all members of the group I, Bali-Lombok (2,7), and Bali-Bali 8. The remain groups were the combinations of the previous big groups: group 3 (Bali-Bali 6 and group 2), group 4 (group 3 and Lombok 5), Group 5 (group 4 and Bali-Bali 5), group 6 (group 5 with Bali-Lombok 9), group 7 (group 6 and Bali-Bali 10), while group 8 was a combination of Bali-Lombok 8 and group 7. The highest significance level was in group 2 (98%). In addition, the closest genetic distance between Bali-Lombok 1 and bull (Bos javanicus) was 0.061 while the farthest distance between Bali-Lombok 8 and Bos javanicus was 0.312.

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