Phenotype and phylogenetic studies of local cattle in Pacitan District, East Java, Indonesia[1]

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ABSTRACT: Indonesia cattle breed have been varying. Some cattle breeds have been developing greatly in Indonesia. At least three cattle breeds have been developing, they are Bali, Madura, and Ongole-Grade (PO). There is also local cattle in Pacitan District, Indonesia. It has phenotype traits similarity with Madura and PO cattle. The easiest phenotype trait to be observed is the coat color. It can be used to differentiate amongst cattle breed. This research was conducted to evaluate maternal origin of local cattle in Pacitan District based on phenotypic trait and phylogenetic tree. The materials consisted of 28 cattle. They were divided into four breeds, Bali, Madura, PO and local cattle of Pacitan. The coat color of each cattle breed was observed for phenotypic trait. In addition, mt-DNA cytochrome b sequences of cattle were also observed for phylogenetic tree analysis. The results showed that Bali, Madura and Ongole-Grade (PO) have uniform color. The coat color of both Bali and Madura cattle were brown, while PO was white. The coat color of local cattle of Pacitan was varied. They were brown and white color. The phylogenetic tree showed that either Bali and Madura were related to *Bos javanicus*, while PO cattle was related to *Bos indicus*. The local cattle of Pacitan could be divided into two groups. First group (PO1P, PO2P and Pc13 [white color, like-PO breed], Pc9 [brown color, like-Madura/Bali breed]) were related to *Bos javanicus* and second group (Pc11 [brown color, like-Madura/Bali breed]) was related to *Bos indicus*. This results indicated that similarity phenotype trait did not always has similar maternal origin. The majority Pacitan local cattle were closed to *Bos javanicus* "*Banteng*" in maternal origin and it has different phenotype trait.

Key words: pacitan, phenotype, phylogenetic, local cattle

INTRODUCTION

Indonesia cattle breed have been varying. Some cattle breeds have been developing greatly in Indonesia. At least three cattle breeds have been developing, they are Bali, Madura, and Ongole-Grade (PO). There is also local cattle in Pacitan District, Indonesia. Its phenotypic traits is closed to Madura and PO cattle. The easiest phenotype trait to be observed is the coat color. It can be used to differentiate cattle breed.

The original Indonesian cattle is Bali cattle, which was domesticated from the Banteng (*Bos bibos)*, a wild cattle species. This breed is maintained as a pure breed in Bali island. Payne and Hodges (1997) reported that Bali cattle are uniform in type. The other local cattle breed in Indonesia is the Madura cattle breed. Nijman et al. (2003) reported, that formerly, it was considered that Madura cattle originated from cross-breeding between Bali and/or *Bos* (*bibos*) spp, and *Bos indicus* type cattle either in Madura or in Java. Indian *Bos indicus* such as Ongole has another name, Nellore which is classified as Zebu cattle (Blakely and Bade, 1991; Williamson and Payne, 1993). It had been developed in Indonesia by importing from India. The pure Ongole was brought to Sumba island and became the Sumba-Ongole. The Sumba-Ongole at Java had been crossed with the Javanese cattle (*Bos javanicus*) and formed the Ongole-grade (Sosroamidjojo, 1980). Javanese cattle is an indigenous Indonesian cattle which its existence is very hard to be found, but it still can be found in Pacitan and

[1] We are thankful to the head of research center and community services Gadjah Mada University for providing Research Grant, Hibah Bersaing XVI 2009. We also thank to the staff Veterinary Services in Pacitan Regency and Madura Island.

Trenggalek regencies. They are red-brown in coat colour. The red-brown cattle is also white on the *twist* and *metacarpus–phalanx* with smear boundaries (Susilawati et al., 2002).

We examined the sequence of mt-DNA cytochrome b in several breeds which were collected from the difference location for phylogenetic study of local cattle in Pacitan district. Mitochondrial DNA evolves much faster than nuclear (nc) DNA and thus contains more sequence diversity compared to nuclear DNA, facilitating the identification of closely related species (Brown et al., 1996). In addition, maternal inheritance of the mt-DNA generally results in lack of heterozygosity. Mitochondrial DNA of several vertebrates, including mammals, was mainly investigated for evolutionary studies (Wolf et al., 1999). Comparisons of nucleotide sequences of the complete mt-DNA or of single genes (e.g., genes encoding the small ribosomal RNAs 12S and 16S and cytochrome *b* (Esposti et al., 1993), were used to study interspecies and intraspecies relationships of animals in order to establish the molecular phylogeny (Birstein and DeSalle, 1998; Birstein et al., 1998). This research was conducted to evaluate maternal origin of local cattle in Pacitan District based on phenotype trait and phylogenetic tree.

MATERIALS AND METHODS

Sample Collection. Blood and ear tissue were collected from Madura Island (Madura cattle), Pacitan Regency (Java cattle, the mountainous region), Yogyakarta (Ongole-Grade cattle, the slaughter house) and another region (Bali cattle).

DNA Extraction. DNA was isolated from blood samples and tissues by using standard SDS/proteinase K extraction (Sambrook et al, 1989).

Polymerase Chain Reaction (PCR). Amplification of the mtDNA cytochrome b (cytb) gene was carried out in a final volume of 20 µl in 0.5 ml tubes. It was filled by 13.3 µl aquabidest, 2 µl PCR buffer, 1.5 µl $MgCl_2$, 0.1 µl dNTP mix, 1 µl each primer (L14735: AAA AAC CAC CGT TGT TAT TCA ACT A/H15149: GCC CCT CAG AAT GAT ATT TGT CCT CA) as universal cytb internal primer pair, designed by Kocher et al. (1989), 0,5 units of *Taq* DNA polymerase and 1 µl DNA genome. The cycling conditions were as follows: 94°C for 2 minutes for pre-denaturation, 35 cycles of 36 seconds at 95°C, 73 seconds at 51°C, 84 seconds at 72°C and followed by a final extension step of 3 minutes at 72°C and 4°C until the next step. PCR products were examined by electrophoresis through a 1% agarose gel in 10X TBE buffer and stained by ethidium bromide. As size reference, marker (Novagen) was used.

DNA Sequencing. PCR products were separated by electrophoresis on 1% agarose gel in 1X TBE buffer, then the DNA fragment was excised and purified with QIAquick isolation system (Qiagen, USA) and then DNA fragment was inserted into pGEM-T easy vector (Promega, USA) follow the protocol from the company. Recombinant DNA was cloned by using the *E. coli* JM 109. The clones were isolated and purified with Qiagen kit Midiprep. A total of 300 ng of DNA were sequenced by using T7 (5'-TAA TAC GAC TCA CTA TAG GG-3 ') and SP6 (5'-CGA TTT AGG TGA CAC TAT AG-3') primers with the program 96°C for 1 minute, 25 cycles: 96°C for 30 seconds, 50°C for 15 seconds and 60°C for 4 minutes, BigDye Terminator v3.1 Cycle sequencing Kit and ABI 310 PrismTM Sequencer System (Applied Biosystem).

Phylogenetic Analysis of mtDNA Cytochrome b Gene. A phylogenetic tree using mtDNA cytochrome b sequence was constructed by using MEGA 4.

RESULTS AND DISCUSSION

Cattle Phenotype Traits Which was Observed

Indonesia is an archipelagic State which has diverse natural resources, including cattle. We used 28 samples in this study, each breed consisted seven cattle. The Indonesian locals cattle were used in this study, they were PO, Bali, Madura, and local cattle of Pacitan (Table 1). We used these breeds to know the maternal origin of Indonesia local cattle. We hoped that Bali cattle could expect to represent the "*banteng*" (*Bos bibos*), while PO could expect to represent the *Bos indicus* breed.

The PO cattle used as samples were obtained from abattoir in Bantul Regency, Yogyakarta. It has white and slightly dark gray color on the head, hump, and back. Williamson and Payne (1993)

reported that Ongole cattle have white color generally, but the male has dark gray on the head, neck and back, and sometimes has black on the knee. Sometimes, we can find red Ongole cattle or red-white Ongole cattle. We found most of the local cattle in Pacitan also have similar characteristics to PO. Local cattle in Pacitan also have white colour in whole body, so we classified as local cattle of Pacitan like-PO (Table 2).

Table 1. The number of cattle was used in this study

No.	Breed	Number of samples	Location
1.	Ongole-grade (PO)	7	Bantul, Yogyakarta
2.	Bali	7	Bali Island
3.	Madura	7	Pamekasan, Madura Island
4.	Local Pacitan	7	Pacitan

Tabel 2. Phenotype traits which was observed in every cattle breeds

Breed	Sample code	Phenotype traits		
		Whole body	Metacarpus–phalanx	Twist
Ongole-grade (PO)	PO5, PO22	White	White	White
Bali	Bali	Brown	White	White (oval)
Madura	Madura1, Madura2	Brown	Smear	Smear
Local Pacitan (like-PO)	PO1P, PO2P, Pc13	White	White	White
Local Pacitan (like-Madura/Bali)	Pc9, Pc11	Brown	Smear	Smear

Both Bali and Madura cattle used in the study have uniformity in coat color. We also found brown local cattle of Pacitan. Thse cattle have phenotype similarity to either Bali or Madura cattle, brown local cattle of Pacitan, have smear or white color on the legs (*metacarpus–phalanx*) and twist. Wijono and Setiadi (2004) reported that red-brown Madura cattle have black or dark brown back line (*linea spinosum*) and white lower leg (*metacarpus-phalanx*) and white twist. In addition, Williamson and Payne (1993) reported that red, golden or dark brown Bali cattle are usually can be found. Bali cattle have white legs and white oval on twist. Bali bulls are darker than Bali cows. Bali bulls will become dark brown to black when mature. With this fact, we suspected that the local cattle of Pacitan have closer relationship with both PO (*Bos indicus*) and Madura or Bali cattle (*Bos bibos*) in phenotype traits (Fig. 1).

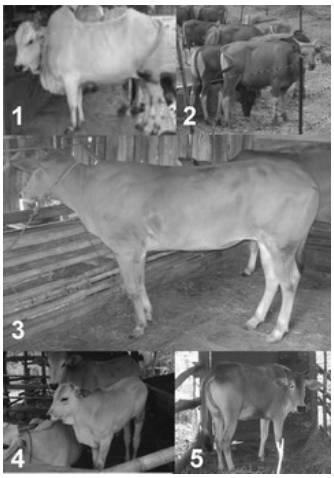

Figure 1. Cattle breed which is used in this study (note: 1 is Ongole-Grade (PO); 2 is Bali cattle; 3 is Madura cattle; 4 is Local cattle of Pacitan (like-PO) and 5 is Local cattle of Pacitan (like-Madura/Bali)

Phylogenetic Tree of Indonesian Local Cattle Based on Partial Sequences of mt-DNA CYT B Gene

To verify the phenotype traits similarity with origins of local cattle of Pacitan, we used mitochondrial DNA (mt-DNA) cytochrome *b* gene as a molecular markers. Cyt *b* gene is a gene that is often used in phylogenetic studies. Research conducted by Wang et al. (2000) and Prusak and Grzybowski (2004) reported that cyt *b* gene of mitochondrial (mt) can be used to study the evolution and phylogenetic relationships of various animals, like birds, mammals, reptiles, amphibians, fishes and some invertebrates. Added by Pfeiffer et al. (2004), mitochondrial cyt *b* gene can be used to distinguish species of mammals such as cattle (*Bos taurus*), sheep (*Ovis aries*) and goats (*Capra hircus*). The reason is the relatively rapid rate of evolution, although the substitution did not cause a change of understanding or amino acid code. Cyt *b* gene is universal, so that it can be easily compared. Cyt *b* gene is vary in populations, and it is suitable for the further study of phylogenetic relationships. In addition, some parts of cyt *b* gene have large conserve areas on each species. Considerable regional variation found in the trans membrane domain encoding regions or amino and carboxyl terminal (Kvist, 2000).

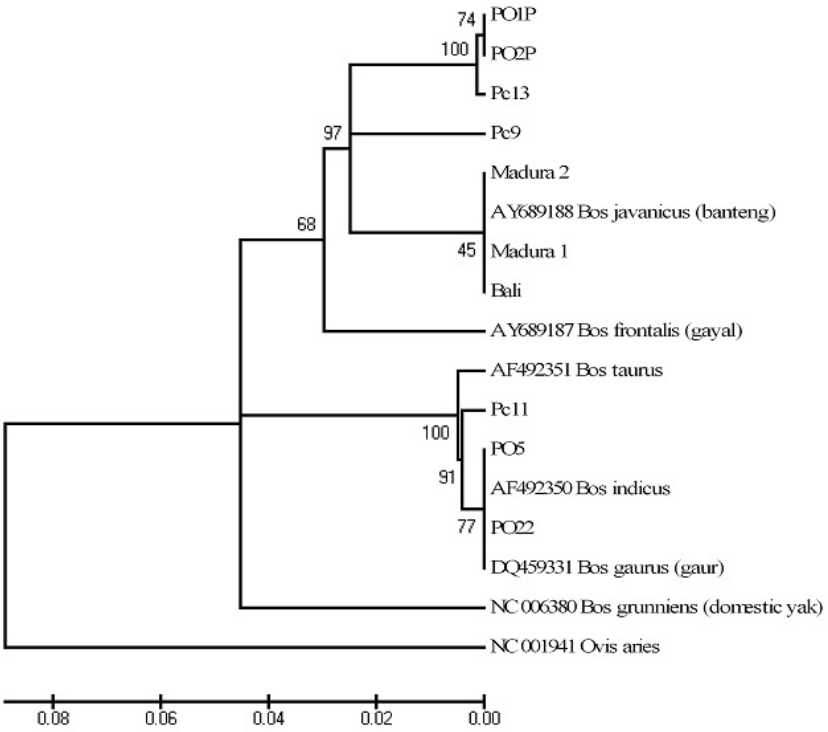

Figure 2. Phylogenetic tree of Indonesian local cattle based on partial sequences of mi-DNA cyt *b* gene (373 bp)

We also used partial sequences of mt-DNA cyt *b* gene (373 bp) from NCBI in constructing the phylogenetic tree. They were AY689188 (Bull), AY689187 (gayal), AF492351 (*Bos taurus*), AF492350 (*Bos indicus*), DQ459331 (Gaur), NC006380 (domestic yak), and NC001941 (*Ovis aries*). These sequences were used to determine the local cattle of Pacitan relationship based on phylogenetic tree. *Ovis aries* were used as an out group to produce a tree that is easier to be understood.

Analysis of partial cyt *b* gene sequences shows that both Bali and Madura cattle were related to AY689188 *Banteng* (*Bos bibos*) (Fig. 2). Local cattle of Pacitan (PO1P, PO2P and Pc13 [white colour, like-PO breed], Pc9 [brown colour, like-Madura/Bali breed]) are closer to Bali cattle, Madura cattle and *Banteng* AY689188 than *Bos indicus* (AF492350), *Bos taurus* (AF49235) or PO cattle from Yogyakarta. These results indicated that the like-PO cattle of Pacitan has maternal inheritance to Madura cattle, Bali and *Banteng*. In addition, these results showed that Pacitan District had Bali or

Madura cattle population in the past. Cattle crossbred program (*Ongolisasi*) made local cattle of Pacitan population decrease (Hardjosubroto and Astuti, 1993). Sustainable cross-breeding towards bulls breed (Ongole) will cause the offspring exterior appearance getting closer to the bulls breed. As a result, Pacitan local cattle like-PO (PO1P, PO2P and Pc13) has similar appearance with PO cattle, while it has maternal relationship with Bali cattle, Madura and *Banteng*. This opinion was strengthened with the local cattle of Pacitan like-Madura/Bali (Pc9) which has brown colour and white color on legs and twist (Williamson and Payne, 1993); Wijono and Setiadi, 2004).

Sequences analysis showed that the Ongole-grade cattle from DIY (PO5 and PO22) and local cattle of Pacitan (Pc11) have relationship with *Bos indicus* (AF492350) and *Bos gaurus* / Gaur (DQ459331) (Fig. 2). The white PO cattle from DIY have hump and jowls, while red-brown local cattle of Pacitan (Pc11) has white colour on legs and twist. Although different in exterior performance, PO5, PO22 and Pc11 cattle were related to *Bos indicus* (AF492350). Blakely and Bade (1991) and Williamson and Payne (1993) reported that PO cattle was derived from *Bos indicus*. Exterior performance differences were due to cross-breeding beetwen local cattle with *Bos Taurus*, such as Simmental and Limousin, which is red-brown. Sustainable crossbreeding toward bulls breed will cause the offspring performance getting closer to the bulls. Crossbreeding is often occurred in Indonesia. Crossbreeding have been introduced almost in every region by artificial insemination (AI). According to the Syrstrad (1985) report, that most local cattle crossed with import breed from Europe which aims to improve the productivity of local livestock (Ayalew et al., 2003).

CONCLUSION

This results indicated that similarity phenotype trait did not always has similar maternal origin. The majority Pacitan local cattle were closed to *Bos javanicus* "*Banteng*" in maternal origin and it has different phenotype trait.

LITERATURE CITED

Ayalew, W., B. Rischowsky, J. M. King and E. Bruns. 2003. Crossbreed did not generate more net benefits than Indigenous goat in Ethiopian smallholdings. Journal Agricultural System 76 (2003), 1137 – 1156.Birstein, V. J., J. Betts and R. DeSalle. 1998. Molecular identification of Acipenser sturio specimens: A warning note for recovery plans. Biol. Conserv., 84, 97-101.

Birstein, V. J. and R. DeSalle. 1998. Molecular phylogeny of Acipenserinae. Mol. Phylogenet. Evol., 9, 141-155.

Blakely, J. dan D. H. Bade. 1991. Ilmu Peternakan. Edisi keempat. Gadjah Mada University Press, Yogyakarta.

Brown, J. R., K.Beckenbach, A. T. Beckenbach and M. J. Smith. 1996. Length variation, heteroplasmy and sequence divergence in the mitochondrial DNA of four species of sturgeon (Acipenser). Genetics, 142, 525-35.

Esposti, M. D., S. De Vries, M. Crimi, A. Ghelli, T. Patarnello and A. Meyer. 1993. Mitochondrial cytochrome b: evolution and structure of the protein. Biochim. Biophys. Acta, 1143, 243-71.

Hardjosubroto, W. dan J. M. Astuti. 1993. Buku Pintar Peternakan. PT Gramedia, Jakarta.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Paabo, F. X. Villablanca and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. U.S.A. (86), 6196-6200.

Kvist, L. 2000. Phylogeny and phylogeography of European Parids. Available at:http://herkules.oulu.-fi/isbn9514255364/html/x128.html

Nijman, I. J., M. Otsen, Edward, LC. Verkaar, Christa de Ruijter, E Hanekamp, J.Y. Ochieng, SBM Shamshad, Edward O Rege, O. Hanotte, MW. Barwegen, T. Sulawati dan JA. Lenstra, 2003, Hybridization of Banteng (Bos Javanicus) and Zebu (Bos Indicus) Revealed by Mitochondrial DNA, Satelite DNA, AFLP, and microsatelite. HEREDITY, 90(1), 10 – 16.

Payne, W.J.A and J. Hodges. 1997. Tropical cattle: origin, breeds and breeding. Oxford (etc.): Blackwell Science, United Kingdom.

Pfeiffer, I., J. Burger and B. Brenig. 2004. Diagnostic polymorphisms in the mitochondrial cytochrome b gene allow discrimination between cattle, sheep, goat, roe buck and deer by PCR-RFLP. BMC Genetics 5 (30), 1-5.

Prusak, B. and T. Grzybowski. 2004. Non-random base composition in codons of mitochondrial cytochrome b gene in vertebrates. Acta Biochemica Polonica, 51 (4), 897-905.

Sambrook J., E. F. Fritsch and T. Maniatis. 1989. Molecular Cloning, A Laboratoty Manual. 2nd edition, Vol. 3. Cold Spring Harbour Laboratory Press: Cold Spring Harbour, USA.

Sosroamidjojo, M. 1980. Ternak Potong dan Kerja. CV Yasaguna, Jakarta.

Susilawati, T., I. Subagiyo, A. Budiarto, G. Ciptadi dan Kuswati. 2002. Katalog Performan Sapi Lokal Jawa Timur. Fakultas Peternakan Universitas Brawijaya dan Dinas Pternakan Propinsi Jawa Timur.

Wang, L., K. Yokoyama, M. Miyaji, and K. Nishimura. 2000. Mitochondrial Cytochrome b Gene Analysis of Aspergillus fumigatus and Related Species. Journal of Clinical Microbiology, 38 (4), 1352–1358.

Wijono, D. B. dan B. Setiadi. 2004. Potensi dan Keragaman Sumberdaya Genetik. Lokakarya Nasional Sapi Potong, Hal. 43-52

Williamson, G. Dan W. J. A. Payne. 1993. Pengantar Peternakan di Daerah Tropis. Cetakan Pertama. Gadjah Mada University Press, Yogyakarta.

Wolf, C., J. Rentsch and P. Hubner. 1999. PCR-RFLP Analysis of Mitochondrial DNA: A Reliable Method for Species Identification. J. Agric. Food Chem., 47 (4), 1350-1355.