Genetic Diversity and Conservation of South-East Asian Cattle: From Indian Zebu to Indonesian Banteng, and then to the Cambodian Kouprey?

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Most domestic cattle worldwide belongs to the species *Bos taurus* or *Bos indicus* (zebu), which both descend from the wild aurochs (*Bos primigenius*). Other cattle species (Lenstra and Bradley, 1999) are the yak (*Bos grunniens*) in and around Tibet, the gayal (*Bos frontalis*) in Eastern India, derived from the gaur (*Bos gaurus*), and the Indonesian Bali cattle derived from the banteng (*Bos javanicus*).

Since 15 years, DNA analysis has allowed a reconstruction of the origin of most domestic species (Bruford *et al.*, 2003). For instance, analysis of mitochondrial DNA established a taurine maternal origin of all zebu populations outside Asia. Indonesian cattle breeds are supposed to be derived from zebu as well as banteng (Rollingson, 1994), species that in contrast to taurine cattle and zebu are not fully interfertile (Lenstra and Bradley, 1999). However, this mixed species origin is not supported by breeding records, while there are only sporadic molecular data (Namikawa *et al.*, 1981; Kikkawa *et al.*, 1995, 2003; Nijman *et al.*, 2003; Verkaar *et al.*, 2003).

Blood and skin tissue samples were collected from Bali cattle on Bali, Sumatera and Sulawesi. Blood samples from Aceh, Pesisir and Filial Ongole were collected on Sumatera. DNA was isolated by using standard SDS/proteinase K extraction or the Qiagen blood and tissue extraction kit.

Because of the divergence of cattle and zebu, identification of the species origin by PCR-RFLP analysis of mitochondrial DNA (mtDNA) or Y-chromosomal DNA is straightforward (Verkaar *et al.*, 2002; Nijman *et al.*, 2003). Aceh and Pesisir zebu breeds carry zebu mitochondrial DNA, while maternal lineages from both species are represented in Ongole cattle. In two earlier studies, banteng mtDNA has been found in 20 out of 26 (Kikkawa *et al.*, 1995) or 6 out of 7 (Kikkawa *et al.*, 2002) Ongole animals. We found banteng mtDNA also in 56% and 94% of the East-Javanese Madura and Galekan zebu breeds, respectively. However, the maternal origin of Bali cattle from different locations on three isles is almost exclusively banteng with a zebu origin found in only 1 out of 125 animals. This is in contrast to the mixed maternal origin of Bali cattle from Malaysia (Nijman *et al.*, 2003), but agrees with the results recently obtained for a feral population of Bali cattle (Bradshaw, 2006).

Interestingly, Y-chromosomal typing as probe of the paternal lineage does not completely parallel the mtDNA results (Fig. 1). All zebu bulls including those from the East-Javanese breed carry exclusively zebu Y-chromosomes. For the filial Ongole only females were sampled, but Kikkawa *et al.* (2005) found zebu Y-chromosomes in 7 out of 7 bulls. With one

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exception in South Sumatera, all Bali cattle descend from banteng bulls. However, the parental species origin of Madura cattle may depend on the sampling site: bulls on Madura all descend from zebu, but earlier we found banteng Y-chromosomes in two Madura bulls from a breeding station in Malang (Verkaar *et al.* 2003).

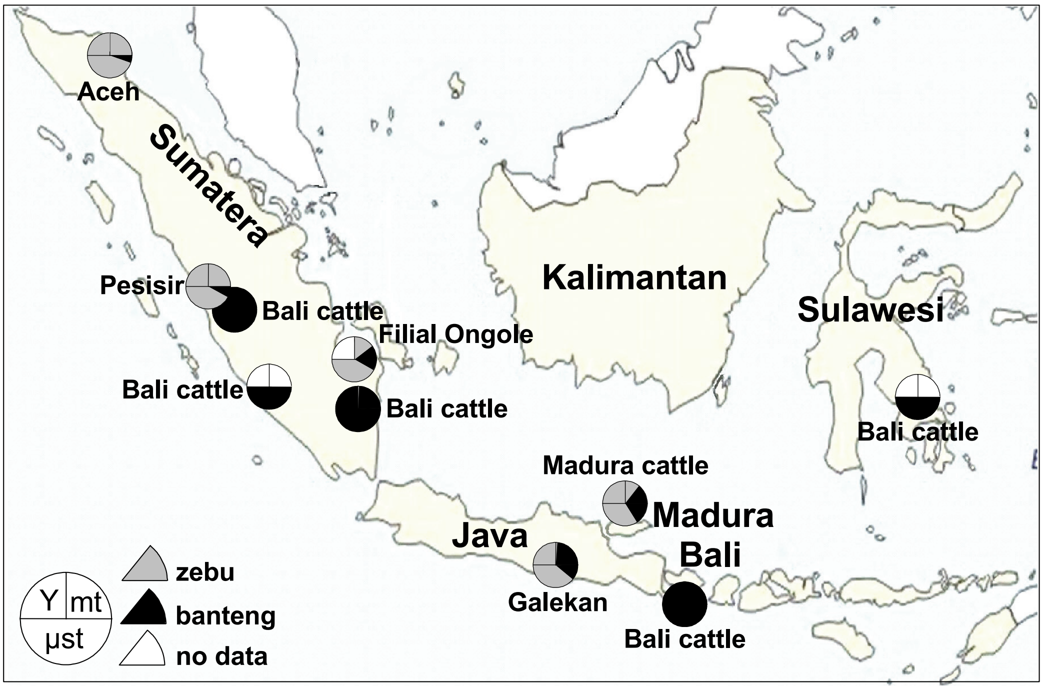

Figure 1. Locations of sampling and genetic constitution of Indonesian cattle populations. The species origin of the paternally transmitted Y-chromosomes (Y), maternally transmitted mitochondrial DNA (mt) and autosomal microsatellite alleles (µst) is represented by black and gray shading of the indicated circle segments.

For a quantitative estimation of admixture, we carried out a microsatellite analysis with 16 markers (*INRA63*, *INRA5*, *ETH225*, *ILSTS5*, *HEL1*, *INRA35*, *ETH152*, *ETH10*, *CSSM66*, *ETH3*, *BM2113*, *BM1824*, *HEL13*, *BM1818*, *ILSTS6* and *CSRM60*) from the panel of 30 microsatellites recommended by the FAO for diversity studies and compared with genotypes for the same markers from seven Indian zebu populations (Kumar *et al.*, 2003). Allele distributions of Indonesian and Indian breeds matched well. However, for several markers additional alleles were observed that are also present in Bali cattle. Quantification of the autosomal species composition was achieved by calculation of genetic distances and model based clustering (Pritchard *et al.*, 2000). For Aceh, Pesisir and Filial Ongole banteng introgression is in the range of 11-16 %, but is clearly higher for Galekan (22%) and Madura cattle (31%).

Domestication of banteng probably took place around 3500 BC (Rollinson, 1994; Felius, 1995). Bali cattle is currently the main representative of the domestic banteng, and is kept in several Indonesian Isles. There is no reliable dating of the first entry of IndoChinese zebu cattle. Singalese immigrants may have brought Indian cattle already 1500 years ago. At the end of the 19th century Ongole zebu bulls were imported and became predominant on Java and other Indonesian isles, but not on Madura or Bali (Payne and Rollinson, 1976; Felius, 1995).

The predominance of zebu mitochondria (Fig. 1) shows that not only zebu bulls but also cows have been imported. This is in contrast to the zebu populations in Africa and America, which emerged by crossing imported zebu bulls with taurine cattle (Bradley, 1996). Banteng mitochondria in Indonesian Ongole populations as well as microsatellite analysis indicate crossbreeding of zebu and local cattle with a banteng maternal origin. Galekan cattle descends from original Java cattle and has a completely banteng maternal origin. Madura cattle predates the import of Ongole bulls, but DNA analysis shows a combination of a zebu paternal lineage with a mixed zebu-banteng maternal origin. Our data also indicate that Bali cattle on different locations in Indonesia has been kept separate from zebu, this in contrast to the situation in Malaysia (Nijman *et al.*, 2003).

We propose that DNA analysis would also be most informative for continental South-East Asian cattle. First, Siamese cattle is supposed to have a similar mixed zebu-banteng origin as Javanese cattle (Felius, 1995). However, there is no information on the preservation of maternal and/or paternal banteng lineage or on quantitative estimates of the species composition. Second, DNA analysis of species hybridization may shed light on the mysterious and contentious origin of the kouprey from Cambodia, which now is probably extinct. Galbreath *et al.* (2006) observed a similarity of a published kouprey mitochondrial DNA sequence (Hassanin and Roupiquet, 2004) and the sequence of continental banteng. It was proposed that kouprey was a zebu-banteng hybrid. On the other hand, Hassanin *et al.* (2006) found a kouprey sequence in a museum specimen that may have been domestic. This raises the intriguing possibility that the Cambodian kouprey has been domesticated and that genetic traces of this extinct species are still present in local breeds. This would be revealed by an analysis of mitochondrial and Y-chromosomal DNA of museum specimen as well as Cambodian local cattle. We also emphasize that information about the species composition and history of cattle breeds are most essential for strategic choices regarding breed management and conservation.

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