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Mitochondrial DNA Polymorphism in Native Philippine Cattle Based on Restriction Endonuclease Cleavage Patterns

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An analysis of patterns of cleavage of mtDNA by restriction endonucleases was performed for nine individuals from the Philippine population of native cattle. MtDNA polymorphisms were detected in the restriction patterns generated by the following six enzymes, BamHI, BglII, EcoRV, HindIII, PstI, *and* ScaI. *The restriction patterns showing polymorphisms were distributed nonrandomly among the nine individuals examined from the Philippine population of native cattle, indicating the existence of two separate types of mtDNA. These two types of mtDNA are very different from each other, at the level of subspecies. Since the native Philippine cattle are considered to represent an admixture of European and Indian cattle, the two types of mtDNA must be derived from the mtDNAs of both varieties. The polymorphic sites in mtDNA have been located on a restriction map, and the nucleotide substitutions at some of the sites have also been estimated.*

KEY WORDS: cattle; mitochondrial DNA; restriction endonuclease; Philippines.

INTRODUCTION

Mitochondrial DNA (mtDNA) polymorphisms based on an analysis of restriction endonuclease digests have allowed us to clarify the genetic relation-

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ships between various species, since mtDNA is maternally inherited and the evolutionary rate of nucleotide substitution is very rapid (Brown *et al.*, 1979, 1982). We are investigating polymorphism in mtDNA of domestic animals, such as the pig, cow, and chicken (Watanabe *et al.*, 1985a, b, c, 1986; Wakana *et al.*, 1986). In the case of the cow, there are two major groups in the world, namely, European cattle (humpless cattle) and Indian cattle (humped or Zebu cattle). In a previous report (Watanabe *et al.*, 1985c), the restriction patterns of mtDNA in cattle were examined in the three breeds maintained in Japan: Japanese Black, Japanese Shorthorn, and Holstein. However, few polymorphisms were detectable. The three breeds in Japan are all European-type cattle. Therefore, in the present study we examined the mtDNA from native cattle in the Philippines which are thought to represent an admixture of European and Indian cattle.

MATERIALS AND METHODS

Chemicals. Fifteen restriction endonucleases, *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Pst*I, *Pvu*II, *Sal*I, *Sac*I, *Sca*I, *Stu*I, *Xba*I, and *Xho*I, were purchased from Toyobo Biochemicals. DNaseI was purchased from Worthington Biochemical Co. Agarose was obtained from Nakarai Chemical Ltd., and ethidium bromide from Katayama Chemical Ltd. Other reagents were commercial preparations of the highest purity available.

Preparation of mtDNA, Restriction Endonuclease Digestion, and Gel Electrophoresis. Mitochondrial DNA was prepared from kidneys of Philippine native cattle which were obtained from a slaughterhouse in the suburbs of Manila. The cattle were randomly chosen from the population so that their maternal relatedness is not known. The procedures for the preparation of mtDNA, restriction endonuclease digestion, and agarose gel electrophoresis have been described previously (Watanabe *et al.*, 1985a, c).

Mapping of Cleavage Sites on mtDNA and Analysis of Nucleotide Substitutions. Molecular weights of restriction fragments of mtDNA were determined by comparison of their mobilities relative to those of fragments of λ-DNA digested with *Hin*dIII and used as standards. Double-digestion methods were used for the mapping of cleavage sites on the mtDNA molecule. Determinations of the sites of cleavage by the restriction enzymes and of nucleotide substitutions were made using a personal computer (NEC, PC-8201), comparing them to the complete sequence of Anderson *et al.* (1982). The A restriction pattern for all enzymes except one in this study matches that inferred from the published sequence; for *Msp*I pattern B is derived from the known sequence.

RESULTS

Analysis of patterns of cleavage of mtDNA by restriction endonucleases was performed for nine individual Philippine native cows. Fifteen restriction enzymes which each recognize six base pairs were used in this study. MtDNA polymorphisms were detected in the restriction patterns generated by the six enzymes, *Bam*HI, *Bgl*II, *Eco*RV, *Hin*dIII, *Pst*I, and *Sca*I, but no difference was observed in the patterns generated by the other nine enzymes. In the restriction patterns of enzymes in which no differences were detected, the numbers of cleavage sites and the sizes of fragments were the same as previously reported (Watanabe *et al.*, 1985c); there were seven fragments detected for *Dra*I, which is the only enzyme not used in the earlier report. In total, 36 restriction sites by nine enzymes revealing no variation were recognized. Figure 1 shows some restriction patterns that reveal polymorphisms, and Table I shows the number of cleavage sites of restriction enzymes

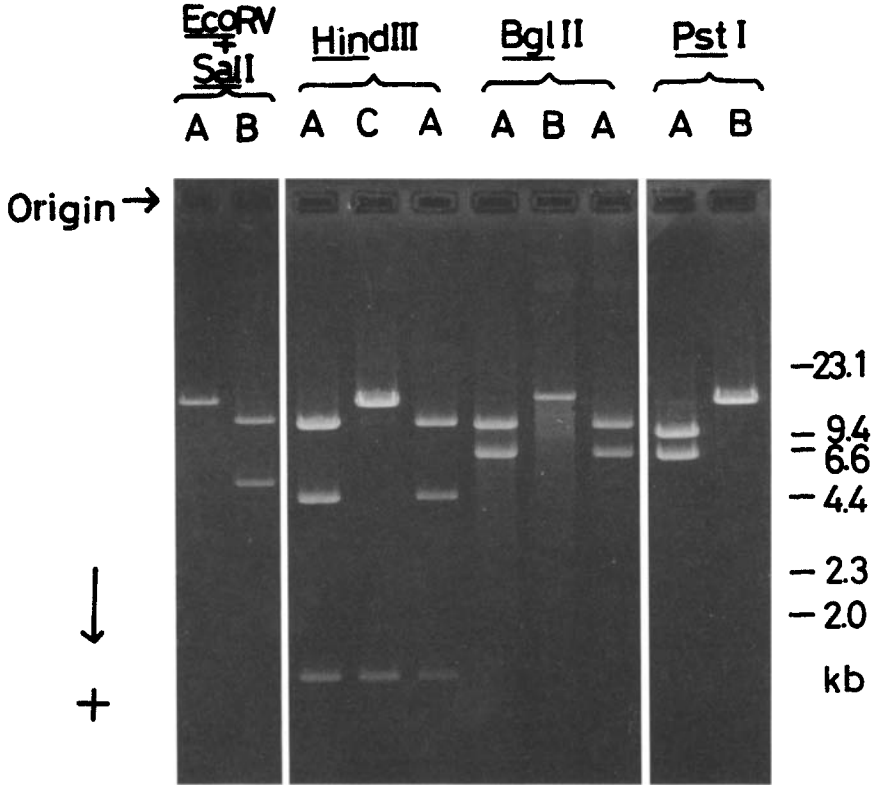

Fig. 1. Comparison of patterns of cleavage by restriction endonucleases of mtDNA from Philippine native cattle after electrophoresis on 0.9% agarose gels.

Table I. Number of Sites of Cleavage by Various Restriction Endonucleases, Demonstrating Polymorphism in Bovine mtDNA, and the Size of Fragments

Restriction endonuclease	Type	Number of cleavage sites	Length of fragments (kb)
*Bam*HI	A	3	11.2 3.2 1.9
	B	4	9.8 3.2 1.9 1.4
*Bgl*II	A	2	9.7 6.6
	B	1	16.3
*Eco*RV + *Sal*I[a]	A	1	16.3
	B	2	11.1 5.2
*Hind*III	A	3	10.2 4.5 1.7
	B	4	10.2 2.4 2.0 1.7
	C	2	14.6 1.7
*Pst*I	A	2	9.4 7.0
	B	1	16.3
*Sca*I	A	9	4.2 2.6 2.6 1.9 1.8 1.3 1.2 0.5 0.2
	B	9	6.0 2.6 2.6 1.7 1.3 1.2 0.5 0.2 0.2

[a]There is a *Sal*I restriction site common to A and B types of mtDNA.

that allow polymorphisms to be recognized and the length of relevant fragments. No *Eco*RV cleavage site was detected in the A type of mtDNA, but in the B type there was a single *Eco*RV restriction site. The B type of *Hind*III restriction pattern was not found in this study, even though it was found previously in Japanese Shorthorn and Holstein cows. The A and C types of *Hind*III restriction pattern were observed in the Philippine native cattle. The C type pattern contains only two bands of 14.6 and 1.7 kb, while the A type pattern contains three bands, because of the presence of one more restriction site in the fragment of 14.6 kb than is present in the C type. This additional site is responsible for generation of the fragments of 10.2 and 4.5 kb. The B type mtDNA generates four bands, since fragments of 2.4 and 2.0 kb result from cleavage at yet one more site in the 4.5 kb fragment of the A type mtDNA. On the basis of the differences in cleavage sites, the A type of *Hind*III restriction pattern can be considered to be intermediate between the B and the C types. In the *Sca*I restriction patterns, the A and B types each generate nine fragments, but two of the cleavage sites are different from each other. In the restriction patterns generated by *Bam*HI, *Bgl*II, and *Pst*I, a single cleavage site differs between each of the A and B types.

Altogether, polymorphisms were detected at seven restriction sites, and the different patterns of restriction were distributed characteristically in the nine individual Philippine native cattle examined. Five cows were A type for

*Bam*HI, *Bgl*II, *Eco*RV, *Hin*dIII, *Pst*I, and *Sca*I and are designated Philippines-1, and this combination corresponds to that observed in the case of Japanese-1. Four of the native, Philippine cows gave patterns that were B, B, B, C, B, and B type, respectively, and these cows are designated Philippines-2. Since the Philippine native cattle are considered to represent an admixture of European and Indian cattle, the mtDNA in Philippines-1 must be derived from the European cattle's type of mtDNA, and the Philippines-2 mtDNA must be derived from the Indian cattle's type of mtDNA. Furthermore, combining the data in this study with those from a previous report, we can summarize the restriction types, as shown in Table II. In the mtDNA of the three Japanese breeds, three combinations were seen, A, A, and A (Japanese-1A), A, A, and B (Japanese-1B), and B, B, and B (Japanese-2), with respect to digestions with *Hin*dIII, *Taq*I, and *Msp*I, respectively. *Taq*I and *Msp*I are enzymes that recognize 4 base pairs. On the basis of these results and the restriction map (Fig. 2), the genetic distance between types of mtDNA detected in the three breeds in Japan and in the Philippine native cattle can be estimated from the numbers of restriction sites observed. First, the distance was calculated by the methods of Nei and Li (1979) for the cases in which enzymes that recognize only 6 base pairs were used. These were 0.0015 ± 0.0015 between Philippines-1 and Japanese-2, 0.0095 ± 0.0040 between Philippines-1 and Philippines-2, and 0.0111 ± 0.0043 between Japanese-2 and Philippines-2. Second, the distance was calculated by the methods of Nei and Tajima (1983) for the cases in which enzymes that recognize both 4 and 6 base-pairs were used. These were 0.0011 ± 0.0011 between Japanese-1A and Japanese-1B, 0.0032 ± 0.0019 between Japanese-1A and Japanese-2, and 0.0021 ± 0.0015 between Japanese-1B and Japanese-2. Based on these genetic distances, phylogenetic trees of mtDNA types were constructed by UPGMA (Fig. 3). The genetic distances estimated by the two procedures are consistent. Philippines-2 is very distant from the other groups, to the extent that it can be considered to be a subspecies.

Table II. Types of Restriction Polymorphisms in mtDNA Among Three Japanese Breeds and Philippine Native Cattle

Line	*Bam*HI	*Bgl*II	*Eco*RV	*Hin*dIII	*Pst*I	*Sca*I	*Taq*I[a]	*Msp*I[a]
Japanese-1A	A	A	A	A	A	A	A	A
Japanese-1B	A	A	A	A	A	A	A	B
Japanese-2	A	A	A	B	A	A	B	B
Philippines-1	A	A	A	A	A	A	—[b]	—
Philippines-2	B	B	B	C	B	B	—	—

[a] *Taq*I and *Msp*I, which were used in the previous study (Watanabe *et al.*, 1985c), are enzymes that recognize 4 base pairs.
[b] Not determined.

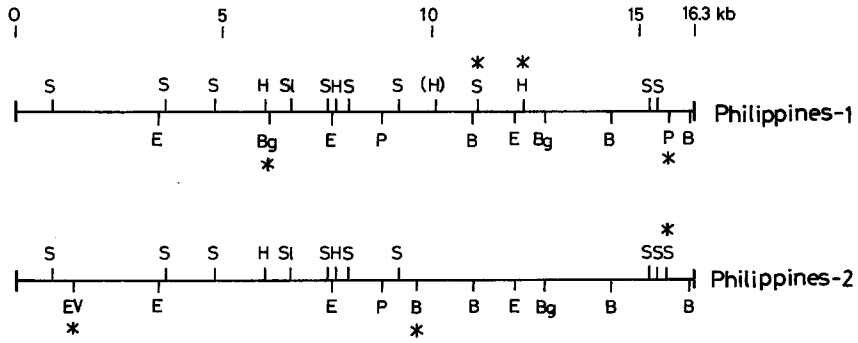

Fig. 2. Maps of cleavage by restriction endonucleases of Philippines-1- and Philippines-2-type mtDNA. Asterisks indicate the polymorphic sites detected in Philippine native cattle, and the polymorphic site detected previously in two Japanese breeds is in parentheses. B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; Ev, *Eco*RV; H, *Hin*dIII; S, *Sca*I; Sl, *Sal*I; P, *Pst*I.

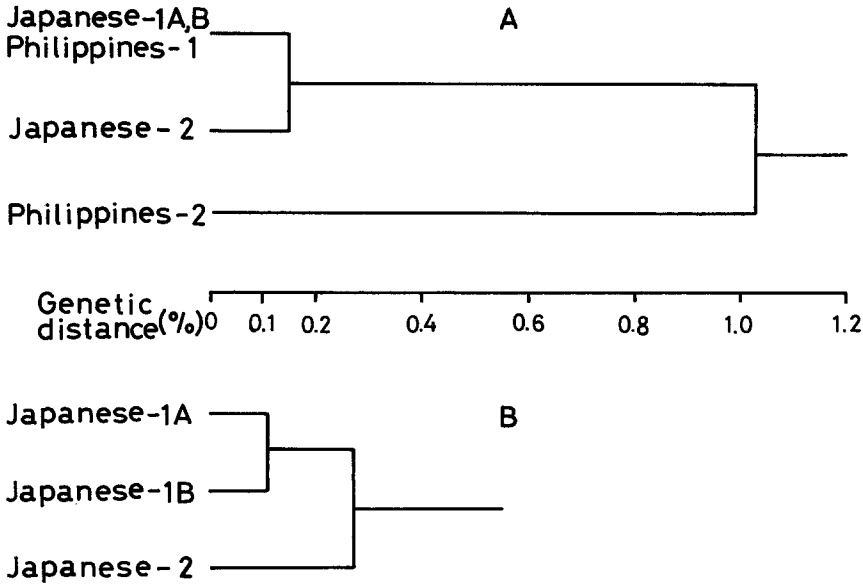

Fig. 3. Dendrogram of three Japanese breeds and Philippine native cattle, based on genetic distances. The distance in A is calculated by the methods of Nei and Li (1979) for the cases in which only 6 base pairs recognizing enzymes were used, and the distance in B is estimated by the methods of Nei and Tajima (1983) for the cases that both 4- and 6-base-pair enzymes were used. The scale is the same in A and B.

The complete nucleotide sequence of bovine mtDNA has already been reported by Anderson *et al.* (1982). Therefore, the restriction map, including the polymorphic sites, is easily drawn. Figure 2 shows the restriction map of both the Philippines-1 and the Philippines-2 types of mtDNA, and if a single nucleotide substitution has occurred between the two types of mtDNA, the positions of seven polymorphic sites can be estimated on the nucleotide sequence of mtDNA, by computer analyses, to be those shown. The seven variable sites in Philippines-2 relative to the completely reported sequence are as follows, listed according to enzyme, gene, location of site start, and, for the three site gains, the inferred base change and its location: (1) *Eco*RV, $tRNA^{Val}$, 1389, A → C, 1395; (2) *Bgl*II, COI, 6115; (3) *Bam*HI COIII, 9600, G → A, 9602; (4) *Sca*I, ND4, 11067; (5) *Hind*III, ND5, 12174; (6) *Sca*I Cytb, 15605, G → A, 15608; and (7) *Pst*I, 15738, $tRNA^{Pro}$. The mutations at 9602 and 15605 are third-position silent changes.

DISCUSSION

In Philippine native cattle, the presence of two types of mtDNA has clearly been demonstrated in this study. These two types of mtDNA are very different from each other. In comparison with the mouse mtDNA sequence divergence among subspecies (Yonekawa *et al.*, 1982; Ferris *et al.*, 1983), it has been considered that the sequence divergence, somewhat over 1%, between Philippines-1 and Philippines-2 is at the level of subspecies. No shifting type of mtDNA, showing intermediate characteristics, was observed. This result means that repeated nucleotide substitutions have not occurred in the Philippine native cattle and that two groups of cattle of different maternal origin were admixed in that country. From investigations of polymorphisms in the beta chain of hemoglobin, Namikawa (1981) suggested that the Philippine native cattle developed from an admixture between European and Indian types of cattle. One set of mtDNA restriction patterns examined in this study must, thus, be derived from European cattle and another from Indian cattle. In future, it will be necessary to examine the restriction patterns of mtDNA from authentic Indian cattle.

In domestic animals, the mtDNA polymorphisms offer important information for the identification of their origins. The situation with respect to polymorphism of cow mtDNA is very similar to that for pig mtDNA. Watanabe *et al.* (1985a, 1986) have indicated from studies of mtDNA polymorphism that domestic pigs must be derived from two different maternal origins, namely, European and Asian wild boars, and that one breed, the Large White, must have arisen as an admixture of European and Asian pigs. Mitochondrial DNA polymorphisms of the chicken, *Gallus gallus domesticus* are quite different from those of the pig and cow (Wakana *et al.*, 1986). The

cleavage patterns generated by 11 restriction enzymes, each of which recognizes 6 base pairs, were identical in all the 16 lines of domestic fowl examined, including the red jungle fowl, *Gallus gallus gallus*. Only one variant was found, in a line of White Leghorns, in the pattern of digestion by *Msp*I, which recognizes 4 base pairs. The domestic fowl seems to have a less divergent population of mtDNAs than the pig and the cow.

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