



タイトル Title	Low mitochondrial DNA diversity of Japanese Polled and Kuchinoshima feral cattle
著者 Author(s)	Mannen, Hideyuki / Yonesaka, Riku / Noda, Aoi / Shimogiri, Takeshi / Oshima, Ichiro / Katahira, Kiyomi / Kanemaki, Misao / Kunieda, Tetsuo / Inayoshi, Yousuke / Mukai, Fumio / Sasazaki, Shinji
掲載誌・巻号・ページ Citation	Animal Science Journal,88(5):739-744
刊行日 Issue date	2017-05
資源タイプ Resource Type	Journal Article / 学術雑誌論文
版区分 Resource Version	author
権利 Rights	© 2016 Japanese Society of Animal Science. This is the peer reviewed version of the following article: [Animal Science Journal, 88(5):739-744, 2017], which has been published in final form at https://doi.org/10.1111/asj.12716 . This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.
DOI	10.1111/asj.12716
JaLDOI	
URL	http://www.lib.kobe-u.ac.jp/handle_kernel/90005793

1 **Low mitochondrial DNA diversity of Japanese Polled and Kuchinoshima**
2 **feral cattle**

3

4 Hideyuki MANNEN,^{1,*} Riku YONESAKA,¹ Aoi NODA,¹ Takeshi SHIMOGIRI,²
5 Ichiro OSHIMA,² Kiyomi KATAHIRA,² Misao KANEMAKI,³ Tetsuo KUNIEDA,⁴
6 Yousuke INAYOSHI,⁵ Fumio MUKAI⁶ and Shinji SASAZAKI¹

7

8 *¹Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural*
9 *Science, Kobe University, Nada, Kobe 657-8501, Japan, ²Faculty of Agriculture,*
10 *Kagoshima University, Korimoto, Kagoshima 890-8580, Japan, ³Institute for*
11 *Animal Science, Shitara, Aichi 441-2423, Japan, ⁴Graduate School of*
12 *Environmental and Life Science, Okayama University, Tsushima-naka, Kita-ku,*
13 *Okayama 700-8530, Japan, ⁵Yamaguchi Prefectural Agriculture & Forestry*
14 *General Technology Center, Yamaguchi 753-0214, Japan, ⁶Wagyu Registry*
15 *Association, Kyoto 604-0845, Japan*

16

17 *Correspondence: Hideyuki Mannen, Graduate School of Agricultural Science,
18 Kobe University, Nada, Kobe 657-8501, Japan. (E-mail:
19 mannen@kobe-u.ac.jp)

20 TEL: +81-78-803-5803, FAX: +81-78-803-5803

21

22 Running title: mtDNA diversity of Japanese cattle

23

1 **ABSTRACT**

2 This study aims to estimate the mitochondrial genetic diversity and structure of
3 Japanese Polled and Kuchinoshima feral cattle, which are maintained in small
4 populations. We determined the mtDNA D-loop sequences for both cattle
5 populations and analyzed these in conjunction with previously published data
6 from Northeast Asian cattle populations. Our findings showed that Japanese
7 native cattle have a predominant, Asian-specific mtDNA haplogroup T4 with
8 high frequencies (0.43–0.81). This excluded Kuchinoshima cattle (32 animals),
9 which had only one mtDNA haplotype belonging to the haplogroup T3.
10 Japanese Polled showed relatively lower mtDNA diversity in the average
11 sequence divergence (0.0020) than other Wagyu breeds (0.0036–0.0047).
12 Japanese Polled have been maintained in a limited area of Yamaguchi, and the
13 population size is now less than 200. Therefore, low mtDNA diversity in the
14 Japanese Polled could be explained by the decreasing population size in the
15 last three decades. We found low mtDNA diversity in both Japanese Polled and
16 Kuchinoshima cattle. The genetic information obtained in this study will be
17 useful for maintaining these populations and for understanding the origin of
18 Japanese native cattle.

19

20 **Key words:** *genetic diversity, Japanese Polled, Kuchinoshima feral cattle,*
21 *mtDNA, small population size*

22

1 INTRODUCTION

2 Four breeds of native beef cattle (Wagyu) have been established in Japan:
3 Japanese Black, Japanese Brown, Japanese Shorthorn, and Japanese Polled.
4 Japanese Black cattle are the dominant beef breed in Japan and are famous for
5 its high-quality meat. Japanese Brown are categorized into two different
6 substrains: Kochi (Tosa-strain) and Kumamoto (Higo-strain). The Japanese
7 Shorthorn have been improved by crossbreeding with imported Shorthorn bulls
8 to the indigenous native cattle in the northern region of Japan. Japanese Polled
9 have also been improved by crossbreeding with imported Aberdeen Angus
10 bulls and have been maintained in a limited region of Yamaguchi in Japan.
11 These breeds were established by crossing Japanese native cattle with several
12 breeds of European cattle during the mid-19th century to improve the native
13 stock.

14 In addition to the Wagyu breeds, there are two unique native cattle
15 populations in Japan: Mishima and Kuchinoshima cattle. Mishima cattle have
16 been isolated on Mishima Island for at least 200–300 years and are conserved
17 as a closed colony. Mishima cattle retain the characteristics of native Japanese
18 cattle and were declared a “national natural treasure” in 1928 (Tsuda *et al.*
19 2013). Kuchinoshima cattle are unique feral cattle and originated from grazing
20 Japanese native cattle on Kuchinoshima Island in the Tokara Island chain of
21 Kagoshima during the Meiji and Taisho periods (Kawahara-Miki *et al.* 2011;

1 Siqintuya *et al.* 2014). So far, these native cattle have been maintained without
2 any genetic influences from European breeds.

3 Mitochondrial DNA (mtDNA) is a powerful genetic marker for
4 investigating the origins of livestock. The mtDNA variations in the displacement
5 loop (D-loop) region have been widely used for studying the origin, genetic
6 diversity, and relationships in cattle (Chen *et al.* 2010; Loftus *et al.* 1994;
7 Mannen *et al.* 1998; Sasazaki *et al.* 2006; Troy *et al.* 2001). Previous studies
8 demonstrated the genetic information and relationships in Japanese Black
9 (Mannen *et al.* 1998, 2004), Japanese Brown (Sasazaki *et al.* 2006) and
10 Mishima cattle (Shi *et al.* 2002) using mtDNA variations.

11 Japanese Polled and Kuchinoshima cattle are maintained as small
12 populations (< 200) compared with other Japanese breeds and have been
13 subject to significant inbreeding. Knowledge of the basic genetic information,
14 such as mtDNA sequences, is fundamental to the conservation of native cattle
15 populations. The objective of this study is to determine mtDNA variations and
16 genetic diversity in Japanese Polled and Kuchinoshima cattle and to analyze
17 these sequences in conjunction with previously published mtDNA data from
18 Northeast Asian cattle populations to better understand the genetic diversity
19 and structure of Japanese Polled and Kuchinoshima cattle.

20

1 MATERIALS AND METHODS

2 ***Ethical Conditions***

3 All procedures in the present study were performed according to the Research
4 Guidelines for Kobe University.

5

6 ***Animals***

7 Genomic DNA was extracted from blood samples of 57 Japanese Polled and 32
8 Kuchinoshima cattle. Kuchinoshima cattle have been maintained in Kagoshima
9 University (13 animals used in this study) and Nagoya University (19 animals).
10 The Kagoshima and Nagoya populations have originated from captured 7
11 males and 5 females, and 4 males and 6 females, respectively (personal record
12 in Kagoshima and Nagoya Universities). Since the relationships were unclear,
13 we used maximum available number of the cattle (32 Kuchinoshima) in this
14 study. Representative published mtDNA sequences and genetic information
15 were included to construct the phylogenetic tree; 32 Japanese Black (Mannen
16 *et al.* 1998; accession nos. U87633-U87650), 60 Japanese Brown (30
17 Kumamoto and 30 Kochi strains) (Sasazaki *et al.* 2006; AB244486-AB244514)
18 and 2 Mishima cattle (Shi *et al.* 2002; AB177788, AB177789). In addition, the
19 sequences and genetic information of 30 Korean native cattle (Hanwoo)
20 (Mannen *et al.* 2004; AB117037-AB117059) and 48 Mongolian native cattle
21 (Mannen *et al.* 2004; AB117060-AB117092) were used to construct reduced

1 median networks.

2

3 **Sequencing**

4 We amplified the complete D-loop region of mtDNA using primers constructed
5 from cytochrome b (5'- ACAACTAACCTCCCTAAGACTC-3') and 12S rRNA (5'-
6 GATTATAGAACAGGCTCCTC-3') gene sequences. The mtDNA amplification
7 and sequencing were performed according to previous studies (Mannen *et al.*
8 2004). Variations in the D-loop region of Japanese Black were defined by
9 comparison with the reference bovine mtDNA sequence (accession nos.
10 V00654) published by Anderson *et al.* (1982).

11

12 **Sequence Analysis**

13 Sequence alignment of the D-loop region was performed using CLUSTAL W
14 (Thompson *et al.* 1994). To investigate the genetic relationship among
15 mitochondrial sequences, an un-rooted neighbor-joining phylogenetic tree
16 (Saitou & Nei 1987) was constructed using the Tamura-Nei distance (Tamura &
17 Nei 1993). The distance computation and phylogenetic tree construction were
18 incorporated into the MEGA package Ver. 5.03 (Tamura *et al.* 2011). All sites
19 containing alignment gaps were excluded from the analysis. Reduced median
20 networks were constructed using NETWORK 4.5 (Bandelt *et al.* 1995).

21

1 RESULTS

2 We analyzed the complete D-loop sequences of 57 Japanese Polled and 32
3 Kuchinoshima cattle, and deposited these sequences in the DDBJ database
4 (accession nos. LC013966 - LC013978). Figure 1 indicates the alignment of the
5 D-loop sequence with the representative sequences of Japanese Black,
6 Japanese Brown, Mishima, and Friesian cattle (reference sequence: V00654).
7 Comparison of these sequences revealed 40 variants, including 1 indel, 2
8 transversions and 37 transitions. On the basis of these variants, Japanese
9 Polled had 12 mitochondrial haplotypes and Kuchinoshima cattle had one
10 mitochondrial haplotype.

11 The average sequence divergence values between populations and within
12 populations and the genetic distances among populations are presented in
13 Table 1. Japanese Black, Japanese Brown and Mishima cattle populations
14 displayed similar levels of divergence (0.36–0.55%), while Kuchinoshima and
15 Japanese Polled cattle showed low divergence (0.00–0.20%).

16 Figure 2 shows a phylogenetic reconstruction of Japanese native cattle
17 using mtDNA sequences. All Japanese native cattle belonged to the *Bos taurus*
18 mtDNA haplogroups T1, T2, T3, and T4 defined by Troy *et al.* (2001). All
19 Kuchinoshima cattle had only one mtDNA haplotype belonging to haplogroup
20 T3.

21 The genetic frequencies of mtDNA haplogroups in Japanese cattle

1 populations are presented in Table 2. Reduced median networks of the
2 mitochondrial haplotypes in the North East Asian cattle are illustrated in Figure
3 3. All Japanese cattle populations had the Asian-specific mtDNA haplogroup T4
4 with high frequencies (0.43–0.81), except for Kuchinoshima cattle. The
5 haplogroup T4 consists in a common, phylogenetically central haplotype with
6 derivative sequences that differed by only a few substitutions. Three Japanese
7 Wagyu breeds have a similar mtDNA topology composed by the major
8 haplogroup T4 and the other haplogroups T1-T3. The Mongolian and Korean
9 networks are composed in several haplogroups T and show high mtDNA
10 diversity (Mannen *et al.* 2004).

11

12 **DISCUSSION**

13 In this study, we have determined the complete mtDNA D-loop sequences of
14 Japanese Polled and Kuchinoshima cattle, and analyzed these sequences in
15 conjunction with previously published data from Northeast Asian cattle
16 populations. With the exception of Kuchinoshima cattle, Japanese native cattle
17 have a predominant, Asian-specific mtDNA haplogroup T4 with high
18 frequencies. By contrast, lower frequencies of the haplogroup T4 were
19 observed in Korean (0.07) and Mongolian (0.12) cattle (Table 2 and Fig. 3).

20 The haplogroup T4 in Japanese native cattle had relatively low genetic
21 diversity, because one of the haplotypes T4 showed predominance and

1 topological centrality within haplogroup T4 (Fig. 3). These results may reflect
2 the geographical and historical background of Japanese native cattle. The
3 primary ancestral cattle were presumably introduced to the Japanese Islands
4 around the second century A.D. (Mukai *et al.* 1989). It is believed that a limited
5 number of animals were introduced to the Japanese Islands at that time
6 because of the Japanese sea barrier. This would have caused a pronounced
7 founder effect in maternal mtDNA. The predominance of the haplogroup T4
8 may originate from the primary mtDNA haplotype in Japanese cattle.

9 Thirty-two Kuchinoshima cattle had only one mtDNA haplotype belonged
10 to haplogroup T3 (Fig. 2 and Table 2). Kuchinoshima cattle are feral cattle that
11 originate from the grazing Japanese native cattle from Kuchinoshima Island
12 (Siqintuya *et al.* 2014). It was reported that a small number of Japanese native
13 cattle were introduced to Kuchinoshima Island in 1918 or 1919 (Hayashida &
14 Nozawa, 1964). Some of these cattle then escaped to the mountains and
15 became the founder of the Kuchinoshima feral cattle. So far, this population has
16 proliferated naturally in the mountains of Kuchinoshima Island.

17 The genetic diversity of Kuchinoshima cattle has been estimated using
18 autosomal polymorphisms. Siqintuya *et al.* (2014) reported that four out of six
19 functional gene variants (*NCAPG*, *FASN*, *SCD*, *SREBP-1*, *F11* and *MC1R*)
20 were monomorphic in 32 Kuchinoshima cattle. Saito *et al.* (2016) genotyped
21 54K SNPs in Kuchinoshima, Japanese Black, Japanese Brown and Japanese

1 Holstein cattle, and found that the average minor allele frequency in
2 Kuchinoshima cattle was lower (0.089) than in other populations (0.181–0.251).
3 These results also indicate the extremely low genetic diversity of Kuchinoshima
4 cattle, which may be explained by a founder effect and/or genetic drift in the
5 small population (< 100 animals) that has survived on Kuchinoshima Island for
6 many generations.

7 Japanese Polled cattle have relatively lower mtDNA diversity (0.0020)
8 than other Wagyu breeds (0.0036 – 0.0047) (Table 1). Japanese Polled cattle
9 have been maintained in a limited area of Yamaguchi and the population is now
10 less than 200. This breed has experienced serious inbreeding, which has
11 reduced the population size and increased the risk of extinction. The low
12 mtDNA diversity in Japanese Polled cattle may be explained by the decreasing
13 population size in the last three decades.

14 In conclusion, we have defined the structure and diversity of mtDNA in
15 Japanese Polled and Kuchinoshima feral cattle. Our findings demonstrated low
16 mtDNA diversity in Japanese Polled and Kuchinoshima cattle. Kuchinoshima
17 cattle had only one mtDNA haplotype belonging to haplogroup T3, indicating a
18 strong founder effect. The genetic information obtained in this study will be
19 useful for maintaining these populations and for understanding the origin of
20 Japanese native cattle.

21

1 **ACKNOWLEDGEMENTS**

2 We thank Wagyu Registry Association for sampling and data collection. This
3 work was supported in part by JSPS KAKENHI Grant Numbers 23380165 and
4 16K15025.

5

1 REFERENCES

- 2 Anderson S, Debruijn MHL, Coulson AR, Eperon IC, Sanger F. 1982. Complete
3 sequence of bovine mitochondrial DNA. *Journal of Molecular Biology* **156**,
4 683-717.
- 5 Bandelt, HJ, Forster P, Sykes BC, Richards MB. 1995. Mitochondrial portraits of
6 human populations using median networks. *Genetics* **141**, 743-753.
- 7 Chen S, Lin BZ, Baig M, Mitra B, Lopes RJ, Santos AM, *et al.* 2010. Zebu cattle
8 are an exclusive legacy of the South Asia Neolithic. *Molecular Biology and*
9 *Evolution* **27**, 1-6.
- 10 Hayashida S, Nozawa K. 1964. Cattle in Tokara Islands. Report of the
11 Research Group on the Native Farm Animals in Japan and its Adjacent
12 Localities 1, 24-29.
- 13 Kawahara-Miki R, Tsuda K, Shiwa Y, Arai-Kichise Y, Matsumoto T, Kanesaki Y,
14 *et al.* 2011. Whole-genome resequencing shows numerous genes with
15 nonsynonymous SNPs in the Japanese native cattle Kuchinoshima-Ushi.
16 *BMC Genomics* **12**, 103.
- 17 Loftus RT, MacHugh DE, Bradley DG, Sharp PM, Cunningham P. 1994.
18 Evidence for two independent domestications of cattle. *Proceedings of the*
19 *National Academy of Sciences USA* **91**, 277-2761.
- 20 Mannen H, Tsuji S, Loftus RT, Bradley DG. 1998. Mitochondrial DNA variation
21 and Evolution of Japanese Black cattle (*Bos taurus*). *Genetics* **150**,

- 1 1169-1174.
- 2 Mannen H, Kohno M, Nagata Y, Tsuji S, Bradley DG, Yeo JS, *et al.* 2004.
3 Independent Mitochondrial Origin and Historical Genetic Differentiation of
4 North Eastern Asian cattle. *Molecular Phylogenetics and Evolution* **32**,
5 539-544.
- 6 Mukai F, Tsuji S, Fukazawa K, Ohtagaki S, Nambu Y. 1989. History and
7 population structure of a closed strain of Japanese Black cattle. *Journal of*
8 *Animal Breeding and Genetics* **106**, 254-264.
- 9 Saito Y, Sasazaki S, Shimogiri T, Ohima I, Katahira K, Kanemaki M, *et al.* 2016.
10 Estimating chromosomal genetic diversity of Kuchinoshima feral cattle using
11 high density SNP chip. *Nihon Chikusan Gakkaiho* (in press).
- 12 Saitou N, Nei M. 1987. The neighbor-joining method: a new method for
13 reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**,
14 406–425.
- 15 Sasazaki S, Odahara S, Hirua C, Mannen H. 2006. Mitochondrial DNA variation
16 and genetic relationship in Japanese and Korean cattle. *Asia-Australasian*
17 *Journal of Animal Sciences* **19**, 1394-1398.
- 18 Shi J, Hosoi E, Harada Y, Miyake S, Sakata S, Shinoda T, *et al.* 2002. Genetic
19 variants of Mishima cattle based on analysis of mitochondrial DNA D-loop
20 region. *Nihon Chikusan Gakkaiho* **73**, 261-264.
- 21 Siqintuya, Nishimaki T, Ibi T, Tsuji T, Yoneda K, Oshima I, *et al.* 2014. Allelic

- 1 distributions of genes involved in economical traits, hereditary disorder, and
2 coat color in a population of Kuchinoshima cattle. *Journal of Animal*
3 *Genetics* **42**, 11-19.
- 4 Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in
5 the control region of mitochondrial DNA in humans and chimpanzees.
6 *Molecular Biology and Evolution* **10**, 512-526.
- 7 Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5:
8 Molecular Evolutionary Genetic Analysis using Maximum Likelihood,
9 Evolutionary Distance, and Maximum Parsimony Methods. *Molecular*
10 *Biology and Evolution* **28**, 2731-2739.
- 11 Thompson J, Higgins DDG, Gibson TJ. 1994 CLUSTAL W: improving the
12 sensitivity of progressive multiple sequence alignment through sequence
13 weighting, position-specific gap penalties and weight matrix choice. *Nucleic*
14 *Acids Research* **22**, 4673-4680.
- 15 Troy CS, Machugh D, Balley JF, Magee DA, Loftus RT, Cunningham P, *et al.*
16 2001. Genetic evidence for Near-Eastern origins of European cattle. *Nature*
17 **410**, 1088-1091.
- 18 Tsuda K, Kawahara-Miki R, Sano S, Imai M, Noguchi T, Inayoshi Y, *et al.* 2013.
19 Abundant sequence divergence in the native Japanese cattle Mishima-Ushi
20 (*Bos taurus*) detected using whole-genome sequencing. *Genomics* **102**,
21 372-378.
22

1 **FIGURE LEGENDS**

2 **Figure 1** Variations of D-loop sequences in Japanese native cattle. Bovine
3 D-loop sequence codes and numbers are given in the first column. The initials
4 represent the breed and population code: JP, Japanese Polled; KU,
5 Kuchinoshima cattle; BrH, Japanese Brown Kumamoto (Higo) strain; BrT,
6 Japanese Brown Kochi (Tosa) strain; JB, Japanese Black; MI, Mishima cattle.
7 V000543 is a reference mtDNA sequence belonging to haplogroup T3
8 published by Anderson *et al.* (1982). Numbers in parentheses indicate the
9 number of animals observed within Japanese native cattle. Only variable sites,
10 with the sequence positions given above, are shown. Identity with the first
11 sequence is denoted by a dot, substitution by a different base letter, and a
12 deletion by a hyphen.

13

14 **Figure 2** Unrooted neighbor-joining tree constructed from mtDNA sequences of
15 Japanese native cattle. Bar indicates the genetic distance by Tamura & Nei
16 (1993).

17

18 **Figure 3** Reduced median networks of *Bos taurus* mitochondrial haplotypes in
19 Japan, Korea, and Mongol. The lower left figure panel shows the relationships of
20 the five primary *Bos taurus* haplogroups T–T4 based on a previous study (Troy
21 *et al.* 2001; Mannen *et al.* 1998, 2004; Sasazaki *et al.* 2006). The numbers on

- 1 lines denote the positions of substitutions in bovine mtDNA sequence (V00654).
- 2 a: Japanese Black, b: Japanese polled, c: Japanese Brown Kochi strain, d:
- 3 Kuchinoshima feral cattle, e: Japanese Brown Kumamoto strain, f: Korean native
- 4 cattle (Hanwoo), g: Mongolian native cattle.
- 5

Table 1 Sequence divergence and genetic distance of Japanese native cattle

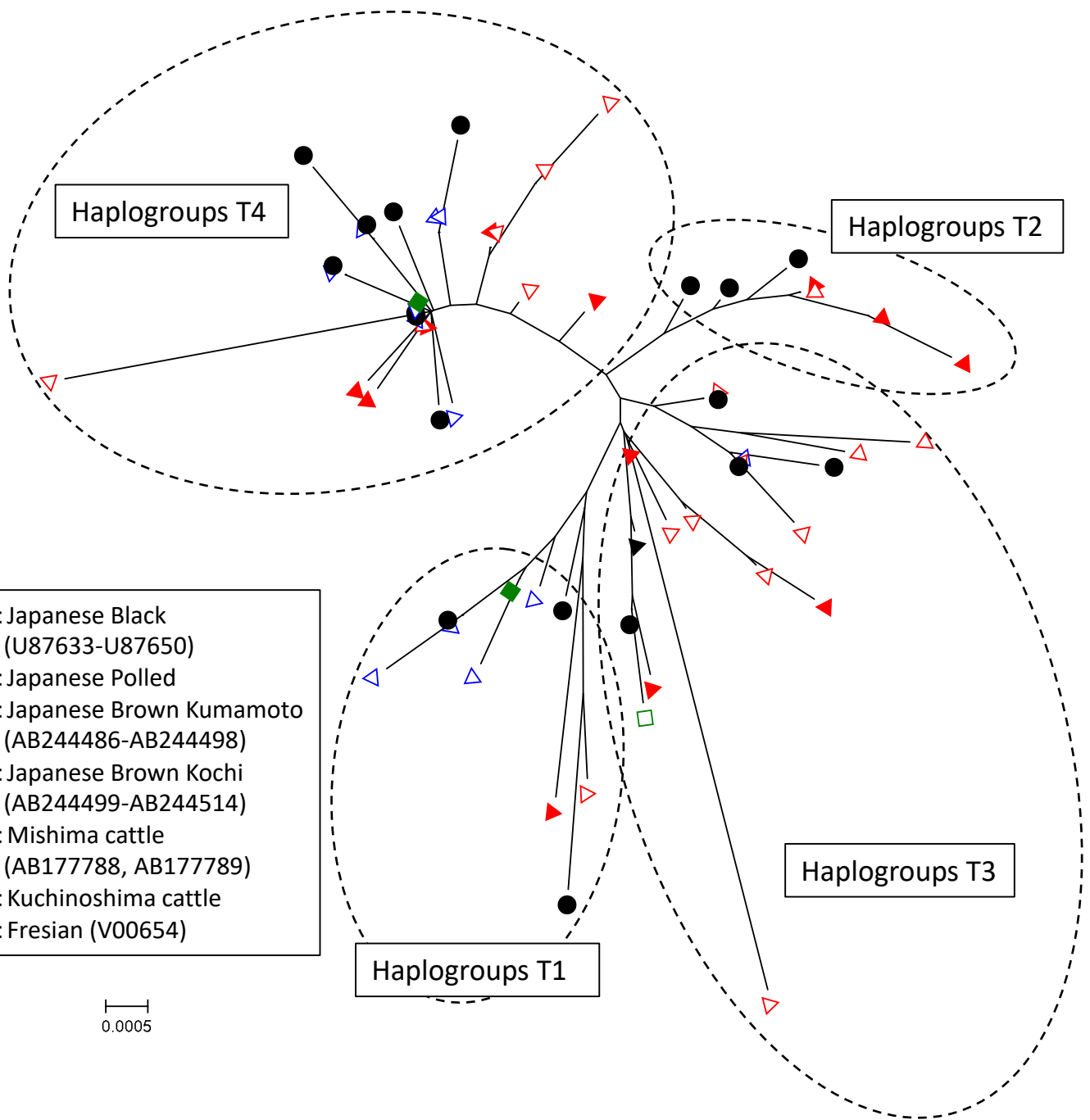
	J. Polled	Kuchinoshima	J. Black	J. Brown-Kumamoto	J. Brown-Kochi	Mishima
J. Polled	<u>0.0020</u>	0.0056	0.0035	0.0033	0.0041	0.0030
Kuchinoshima	0.0061	<u>0.0000</u>	0.0058	0.0061	0.0059	0.0060
J. Black	0.0033	0.0057	<u>0.0047</u>	0.0044	0.0047	0.0041
J. Brown-Kumamoto	0.0032	0.0062	0.0038	<u>0.0036</u>	0.0042	0.0038
J. Brown-Kochi	0.0040	0.0062	0.0043	0.0042	<u>0.0044</u>	0.0040
Mishima	0.0031	0.0061	0.0037	0.0039	0.0042	<u>0.0055</u>

Above the diagonal and on the diagonal are the average sequence divergences between populations and within populations, respectively. Below the diagonal are genetic distances between populations calculated by the Tamura-Nei distance (Tamura & Nei 1993). We used the published mtDNA sequences and the genetic information obtained from previous studies (Mannen *et al.* 1998, 2004; Sasazaki *et al.* 2006; Shi *et al.* 2002).

Table 2 Distribution of mitochondrial haplogroups in Japanese native cattle populations

population	Mitochondrial Haplogroup [†]			
	T1	T2	T3	T4
J.Polled	0.16 (9)	-	0.03 (2)	0.81 (46)
Kuchinoshima	-	-	1.00 (32)	-
J.Black	0.06 (2)	0.03 (1)	0.38 (12)	0.53 (17)
J.Brown-Kumamoto	-	0.03 (1)	0.33 (10)	0.63 (19)
J.Brown-Kochi	0.03 (1)	0.03 (1)	0.50 (15)	0.43 (13)
Mishima	0.50 (1)	-	-	0.50 (1)

[†]Mitochondrial haplogroups T1, T2, T3 and T4 defined by Troy *et al.* (2001) and Mannen *et al.* (1998, 2004). Numbers in parenthesis indicate number of animals. We added published genetic information from previous studies (Mannen *et al.* 1998, 2004; Sasazaki *et al.* 2006; Shi *et al.* 2002).



Haplogroups T4

Haplogroups T2

- : Japanese Black (U87633-U87650)
- △ : Japanese Polled
- ▲ : Japanese Brown Kumamoto (AB244486-AB244498)
- △ : Japanese Brown Kochi (AB244499-AB244514)
- : Mishima cattle (AB177788, AB177789)
- : Kuchinoshima cattle
- ▲ : Fresian (V00654)

0.0005

Haplogroups T1

Haplogroups T3

