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1 Low mitochondrial DNA diversity of Japanese Polled and Kuchinoshima

2 feral cattle

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- 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 $\mathbf{5}$ 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). 12Japanese Polled have been maintained in a limited area of Yamaguchi, and the population size is now less than 200. Therefore, low mtDNA diversity in the 1314Japanese Polled could be explained by the decreasing population size in the last three decades. We found low mtDNA diversity in both Japanese Polled and 1516 Kuchinoshima cattle. The genetic information obtained in this study will be 17useful for maintaining these populations and for understanding the origin of 18 Japanese native cattle.

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

1 INTRODUCTION

Four breeds of native beef cattle (Wagyu) have been established in Japan: $\mathbf{2}$ 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 $\mathbf{5}$ 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 12breeds of European cattle during the mid-19th century to improve the native stock. 13

14In addition to the Wagyu breeds, there are two unique native cattle populations in Japan: Mishima and Kuchinoshima cattle. Mishima cattle have 1516 been isolated on Mishima Island for at least 200–300 years and are conserved 17as 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 Japanese native cattle on Kuchinoshima Island in the Tokara Island chain of 2021Kagoshima during the Meiji and Taisho periods (Kawahara-Miki et al. 2011;

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

Mitochondrial DNA (mtDNA) is a powerful genetic marker for 3 investigating the origins of livestock. The mtDNA variations in the displacement 4 $\mathbf{5}$ loop (D-loop) region have been widely used for studying the origin, genetic diversity, and relationships in cattle (Chen et al. 2010; Loftus et al. 1994; 6 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.

Japanese Polled and Kuchinoshima cattle are maintained as small 11 12populations (< 200) compared with other Japanese breeds and have been subject to significant inbreeding. Knowledge of the basic genetic information, 1314such as mtDNA sequences, is fundamental to the conservation of native cattle populations. The objective of this study is to determine mtDNA variations and 1516 genetic diversity in Japanese Polled and Kuchinoshima cattle and to analyze 17these 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.

1 MATERIALS AND METHODS

2 **Ethical Conditions**

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

 $\mathbf{5}$

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 12in Kagoshima and Nagoya Universities). Since the relationships were unclear, we used maximum available number of the cattle (32 Kuchinoshima) in this 1314study. Representative published mtDNA sequences and genetic information were included to construct the phylogenetic tree; 32 Japanese Black (Mannen 1516 et al. 1998; accession nos. U87633-U87650), 60 Japanese Brown (30 17Kumamoto 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.

 $\mathbf{2}$

3 Sequencing

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

11

12 Sequence Analysis

Sequence alignment of the D-loop region was performed using CLUSTAL W 1314(Thompson et al. 1994). To investigate the genetic relationship among 15mitochondrial sequences, an un-rooted neighbor-joining phylogenetic tree 16 (Saitou & Nei 1987) was constructed using the Tamura-Nei distance (Tamura & 17Nei 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 20networks were constructed using NETWORK 4.5 (Bandelt et al. 1995).

1 **RESULTS**

 $\mathbf{2}$ 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 $\mathbf{5}$ 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.

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

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

21 The genetic frequencies of mtDNA haplogroups in Japanese cattle

1 populations are presented in Table 2. Reduced median networks of the $\mathbf{2}$ 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 with high frequencies (0.43-0.81), except for Kuchinoshima cattle. The 4 $\mathbf{5}$ 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 haplogroup T4 and the other haplogroups T1-T3. The Mongolian and Korean 8 9 networks are composed in several haplogroups T and show high mtDNA 10 diversity (Mannen et al. 2004).

11

12 **DISCUSSION**

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

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

1 topological centrality within haplogroup T4 (Fig. 3). These results may reflect the geographical and historical background of Japanese native cattle. The $\mathbf{2}$ 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 $\mathbf{5}$ 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(Sigintuya et al. 2014). It was reported that a small number of Japanese native cattle were introduced to Kuchinoshima Island in 1918 or 1919 (Hayashida & 1314Nozawa, 1964). Some of these cattle then escaped to the mountains and became the founder of the Kuchinoshima feral cattle. So far, this population has 1516 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 Holstein cattle, and found that the average minor allele frequency in Kuchinoshima cattle was lower (0.089) than in other populations (0.181–0.251). These results also indicate the extremely low genetic diversity of Kuchinoshima cattle, which may be explained by a founder effect and/or genetic drift in the small population (< 100 animals) that has survived on Kuchinoshima Island for many generations.

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

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

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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. Mitchondrial 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. Mitchondrial 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, <i>et al</i> . 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.

1 FIGURE LEGENDS

Figure 1 Variations of D-loop sequences in Japanese native cattle. Bovine $\mathbf{2}$ 3 D-loop sequence codes and numbers are given in the first column. The initials represent the breed and population code: JP, Japanese Polled; KU, 4 $\mathbf{5}$ Kuchinoshima cattle; BrH, Japanese Brown Kumamoto (Higo) strain; BrT, Japanese Brown Kochi (Tosa) strain; JB, Japanese Black; MI, Mishima cattle. 6 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 12deletion by a hyphen.

13

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

17

Figure 3 Reduced median networks of *Bos taurus* mitochondrial haplotypes in Japan, Korea, and Mongol. The lower left figure panel shows the relationships of the five primary *Bos taurus* haplogroups T–T4 based on a previous study (Troy *et al.* 2001; Mannen *et al.* 1998, 2004; Sasazaki *et al.* 2006). The numbers on lines denote the positions of substitutions in bovine mtDNA sequence (V00654).
 a: Japanese Black, b: Japanese polled, c: Japanese Brown Kochi strain, d:
 Kuchinoshima feral cattle, e: Japanese Brown Kumamoto strain, f: Korean native
 cattle (Hanwoo), g: Mongolian native cattle.

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	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	0.0036	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>	

Table 1 Sequence divergence and genetic distance of Japanese native cattle

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).

	Mitochondrial Haplogroup [†]											
population -	T1	T2	Т3	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)								

Table 2 Distribution of mitochondrial haplogroups in Japanese native cattle populations

†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).

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JP4 (4)		. C					Α														А	. C		G.			
JP5 (2)		. C					Α														А			GG	. (с.	
JP8 Ú		. C					Α														А	Α.		G.			
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JB2		. C					А					С									А			G.			
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