Effect of Mitochondrial DNA Variation on Carcass Traits of Japanese Black Cattle[1,2]

H. Mannen*, T. Kojima*, K. Oyama*, F. Mukai*, T. Ishida†, and S. Tsuji*

*Laboratory of Animal Breeding and Genetics, Faculty of Agriculture, Kobe University, Kobe 657, Japan and †Laboratory of Animal Breeding, Faculty of Agriculture, Miyazaki University, Miyazaki 889-21, Japan

ABSTRACT: Japanese Black fattening steers were used to examine relationships between carcass traits and mitochondria displacement loop (**D-loop**) variations. The D-loop region of Japanese Black cattle was sequenced and revealed 26 mitochondrial haplotypes defined by 25 polymorphic sites. The haplotypes were classified into five mitochondrial types (type 1 to 5) using the unweighted pair-group method with arithmetic means. Carcass weight, longissimus muscle area (**LMA**), rib thickness, subcutaneous fat thickness, yield estimate, and beef marbling score (**BMS**) were compared among five mitochondrial types with BLUP procedures. Significant differences between mitochondria types were detected for LMA and BMS. Difference ($P < .05$) was observed between mitochondrial types 2 and 4 for LMA. There was a highly significant difference ($P < .01$) in BMS between types 2 and 4. Difference ($P < .05$) was also found between types 1 and 4 on BMS. These results suggest that cytoplasmic genetic effects are important sources of variation for carcass traits in Japanese Black cattle.

Key Words: Beef Cattle, Mitochondrial DNA, Cytoplasmic Inheritance

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Introduction

Carcass characteristics, especially meat quality, are the main breeding objectives in Japanese Black cattle (*Bos taurus*), the major beef breed in Japan. The extensive use of AI and performance and progeny testing in stations have contributed to the improvement of carcass traits in Japanese Black cattle since the late 1960s. These trials aimed mainly to improve additive genetic values controlled by nuclear genes.

The presence of cytoplasmic genetic effects has been hypothesized because mitochondria contain their own DNA and are maternally inherited (Venge, 1953; Wagner, 1972). Several researchers have detected the cytoplasmic effects on yield traits of dairy cattle (Bell et al., 1985; Kennedy, 1986; Boettcher et al., 1996b). Recently, the relationships between yield traits and mitochondrial DNA (**mtDNA**) sequence variations were shown in dairy cattle (Schutz et al., 1994; Boettcher et al., 1996a). In contrast, previous studies based on maternal lineage showed that the cytoplasmic genetic effects were not important sources of variation for performance traits in beef cattle (Tess and Robison, 1990; Northcutt et al., 1991; Tess and MacNeil, 1994). Cytoplasmic effects on carcass traits in beef cattle have not been evaluated in previous studies. The objective of this study was to evaluate the effects of mtDNA displacement loop (**D-loop**) variations on carcass traits in Japanese Black cattle.

Materials and Methods

Animals and Carcass Traits. Lymphonoduli of the kidney knob were collected for extraction of DNA from carcasses of Japanese Black fattening steers at a carcass market in Osaka, Japan from 1992 to 1995. Numbers of carcasses were 116 in 1992 for a carcass competition and 95 from 1993 to 1995 for field progeny testings. Here we refer to the 1992 carcasses as the **CC group** and the remaining carcasses as the **PT group**. All steers had ad libitum access to concentrates and roughages in feedlots and were killed at an average age of 24.1 mo.

The carcasses were dissected at the sixth and seventh rib section according to the Japanese meat

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grading system by certified graders of the Japan Meat Grading Association to measure carcass traits. Traits examined were carcass weight (**CW**), longissimus muscle area (**LMA**), rib thickness (**RT**), subcutaneous fat thickness (**SFT**), yield estimate (**YE**), and beef marbling score (**BMS**). The RT is the distance between latissimus muscle and pleura membrane measured halfway between the rib ends. The YE is the estimated ratio of wholesale cuts, from which surface fat was trimmed, to carcass weight as a percentage and is calculated by the following equation:

$$YE\ (\%) = 69.419 + .130 \times LMA + .667 \times RT - .025 \times CW' - .896 \times SFT$$

where CW′ is a cold left-side carcass weight. Beef marbling score is a subjective score of the degree of marbling ranging from null (0) to very abundant (5) (Mukai et al., 1995).

Sequencing. The D-loop region of mtDNA was amplified using PCR with primers constructed from the published proline tRNA (5′-CTGCAGTCTCAC-CATCAACC-3′) and 12S rRNA (5′-CTCCTCGGACAAGATATTAG-3′) gene sequences (Loftus et al., 1994). Amplification and purification of product were carried out according to the method of Loftus et al. (1994). Standard double-strand DNA sequencing was performed using approximately 200 to 500 ng of amplified product. Mutations in the D-loop region of Japanese Black cattle were defined by comparison with the standard bovine mtDNA sequence (accession number V00654) published by Anderson et al. (1982).

Statistical Analysis. We constructed a phylogenetic tree to classify carcasses into several maternal lineages depending on D-loop sequences by the unweighted pair-group method with arithmetic means (**UPGMA**) incorporated in the MEGA package (Kumar et al., 1993) using Tamura-Nei distance (Tamura and Nei, 1993). The UPGMA method was originally developed for constructing a phenogram (Sokal and Michener, 1958), but it can be used for constructing a phylogenetic tree (Nei, 1987).

Means of carcass traits were 410 ± 2.7 kg (CW), 48.1 ± .45 cm^2 (LMA), 6.7 ± .048 cm (RT), 2.7 ± .055 cm (SFT), 72.5 ± .083% (YE), and 1.59 ± .059 (BMS). Differences among mitochondrial types classified by mtDNA D-loop variation on the six carcass traits were estimated by the following mixed linear model including additive nuclear genetic effects and nuclear maternal genetic effects as random effects:

$$y_{ijkl} = \mu + MT_i + HY_j + b_1(x_{ijkl} - \bar{x}) + b_2 (x_{ijkl} - \bar{x})^2 + b_3(F_{ijkl} - \bar{F}) + a_k + m_l + e_{ijkl}$$

where

y_{ijkl} = observation for the carcass trait,
μ = overall mean,
MT_i = fixed effect of i^{th} mitochondrial type (i =1...5),
HY_j = fixed effect of j^{th} herd-year (j = 1...55),
b_1, b_2 = partial linear and quadratic regression coefficients for slaughter age, respectively,
x_{ijkl} = slaughter age,
$\bar{x}$ = average of slaughter age (24.1 mo),
b_3 = partial linear regression coefficient for inbreeding coefficient,
F_{ijkl} = inbreeding coefficient of k^{th} animal (k = 1...211),
a_k = random effect of additive nuclear genetic effect of k^{th} animal (k = 1...211),
m_l = random nuclear maternal genetic effect of l^{th} dam (l = 1...211), and
e_{ijkl} = random environmental effect.

Although the influence of nuclear maternal effect is assumed to be small on carcass traits, such an effect should be taken account to avoid confusion with the cytoplasmic genetic effect. Pedigrees of steers were traced to ancestors born in 1975 as the base, and then the additive relationship matrix included 1,793 animals. The variance components assumed are shown in Table 3 (Mukai, 1994) and four maternal heritabilities, .00, .01, .03, and .10, were examined because we do not have a reliable variance component of the nuclear maternal genetic effect. Individual comparisons between the mitochondrial type effects were conducted with linear contrasts using elements of inverse of coefficient matrix. Analyses were conducted on 211 carcasses combining the CC and the PT groups because no difference was found between averages of the traits and variance components of the two groups.

Results

The complete D-loop region of mtDNA was sequenced for 116 steers of the CC group (GenBank accession numbers: U87633 to U87650, U87893 to U87905). Table 1 shows the location and the frequency of D-loop variants of the CC group. Most of the variants (18/25) showed very low frequencies (less than 5%). Table 2 shows the 26 mitochondria haplotypes defined by variations at 25 sites and their frequencies. Most of the haplotypes (21/26) were rare. Two sequence variants, at bp 16,093 (notation I) and 16,302 (notation U), were observed simultaneously, except one individual of haplotype MUY.

In addition to these variations, deletion and insertion events in the D-loop were also observed (data not shown). These variations were in a poly C tract at bp 103 to 105 (deletion) and at bp 211 to 216 (insertion). Variation at bp 103 to 105 was detected in all animals examined in this study; however, this variation has not been reported in the European and African *Bos taurus* (Bradley et al., 1996). This could be due to the gel artifact of band compression.

Table 1. Sites and types of variants detected in the D-loop region of Japanese Black cattle (CC group)

Location of variant and polymorphic event[a]	Notation of polymorphism	Frequency, %	Location of variant and polymorphic event	Notation of polymorphism	Frequency, %
T16019C	A	.86	T16122C	N	12.07
T16042C	B	62.93	G16185A	O	.86
C16050T	C	3.45	G16200A	P	.86
T16055C	D	2.59	A16250G	Q	.86
G16057C	E	.86	T16255C	R	5.17
C16058T	F	.86	T16294C	S	.86
C16088G	H	.86	G16302A	U	66.38
G16093A	I	65.52	T16308C	V	.86
C16104T	J	1.72	G8A	W	3.45
T16112C	K	1.72	A166G	X	.86
T16116C	L	.86	A169G	Y	93.97
T16119C	M	12.93	—	—	—

[a]Sites are numbered according to Anderson et al. (1982). T16019C indicates T → C transition mutation on bp 16,019. A = adenine; C = cytosine; G = guanine; T = thymine.

Variation at bp 211 to 216 was readily detected and the haplotype frequency was 25.9% in the CC group. However, incorporation of the information of insertion/deletion into the estimation of genetic distance had no influence on the structure of the phylogenetic tree, so this variation was excluded in the following analysis.

Figure 1 shows the phylogenetic tree constructed using the D-loop DNA sequences using UPGMA. The CC group were classified into five clusters (mitochondria type 1 to 5) by the haplotypes of the D-loop region. The most highly variable region of the D-loop was between bp 16,042 and 16,122 in Japanese Black. The effective sequences for classification into five clusters were from bp 16,042 to bp 16,119. To increase the number of carcasses in the following carcass trait analysis, this region was sequenced for an additional

Table 2. Definition of mitochondria displacement loop haplotypes and their respective frequencies

Haplotype[a]	Frequency, %	Haplotype	Frequency, %
BIUY	49.14	IJUY	.86
BISUY	.86	DKNY	1.72
BIUX	.86	DFHY	.86
BINUY	2.59	MY	9.48
BIQUY	.86	MTY	.86
BIUVY	.86	MUY	.86
BILUY	.86	GMY	.86
BIUWY	3.45	JMY	.86
ABIUY	.86	CRY	3.45
BEIUY	.86	RY	1.72
BIPUY	.86	NY	6.03
IOUY	.86	BNY	6.90
IUY	1.72	None	6.03

[a]Haplotypes are shown by notations defined in Table 1.

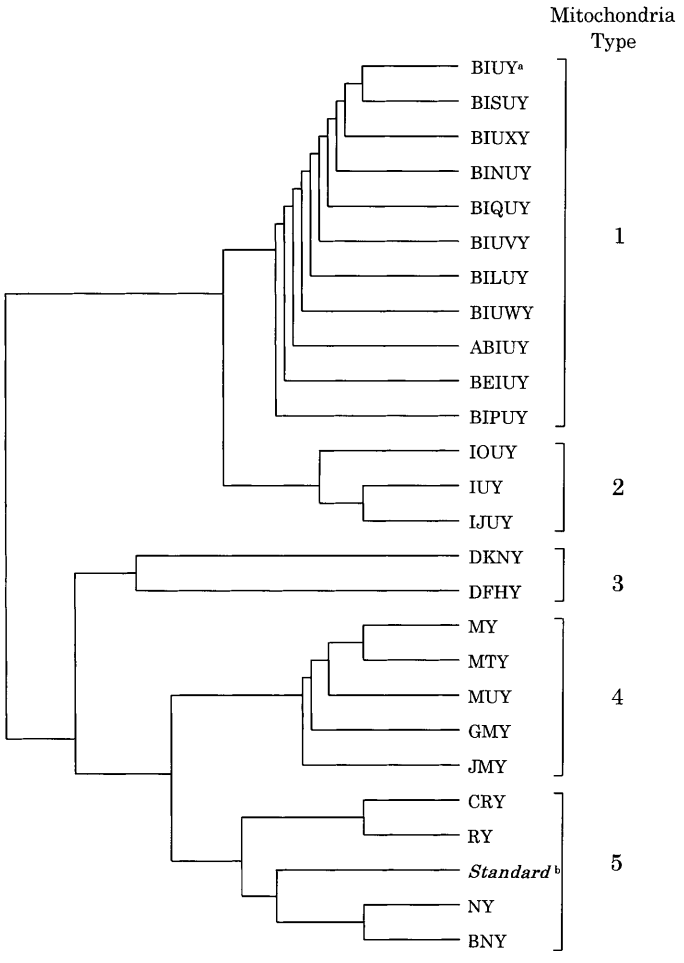

Figure 1. Phylogenetic tree showing the relationship between the nucleotide sequences of the mitochondrial displacement loop of Japanese Black cattle. [a]Haplotypes are shown by notations defined in Table 1. [b]Standard means sequence published by Anderson et al. (1982).

Table 3. Frequencies and effects (BLUE) of mitochondria types on six carcass traits of Japanese Black cattle

Mitochondrial type[a]	Frequency, %	BLUE[b] CW, kg[c]	LMA, cm^{2c}	RT, cm[c]	SFT, cm[c]	YE, %[c]	BMS, unit[c]
Type 1	61.6	1.75	1.06	–.02	–.09	.19	.13
Type 2	3.8	–5.39	–2.06	–.16	.11	–.41	–.43
Type 3	1.9	–1.32	–2.54	–.12	–.03	–.36	–.32
Type 4	12.8	9.60	3.45	.27	.07	.44	.54
Type 5	19.9	–4.65	.09	.02	–.05	.14	.07
Additive genetic variance[d]		723.98	18.30	.23	.22	.58	.31
Environmental variance[d]		874.86	21.05	.36	.38	.86	.33

[a]Types are defined by the phylogenetic tree in Figure 1.
[b]BLUE = best linear unbiased estimates.
[c]CW = carcass weight; LMA = longissimus muscle area; RT = rib thickness; SFT = subcutaneous fat thickness; YE = yield estimate; BMS = beef marbling score.
[d]Data from Mukai (1994).

95 animals of the PT group and then the group was categorized into five types (type 1 to 5), as was the CC group. All carcass data from both groups were combined and analyzed.

Table 3 shows the frequencies of mitochondrial types and the effects expressed by best linear unbiased estimates (**BLUE**) on six carcass traits. The BLUE were obtained when maternal heritability was assumed to be .03. However, no obvious difference was observed for other maternal heritabilities examined. Type 1 was the predominant mitochondrial type (61.6%) and type 5 was next frequent (19.9%). The effects of both types showed the average for all traits. Types 2 and 3 represented a minor proportion of the population (3.8 and 1.9%, respectively). The frequency of type 4 was 12.8%.

Differences between BLUE for the effects of five mitochondrial types are shown in Table 4. Significant differences between the mitochondrial types were detected for LMA and BMS. Difference ($P < .05$) was indicated between types 2 and 4 on LMA; the type 4 carcasses had on average 5.52 cm^2 larger LMA than the type 2 carcasses. There was a highly significant difference of .97 units ($P < .01$) in BMS between types 2 and 4. Difference ($P < .05$) was also found between types 1 and 4 and between types 4 and 5 with respect to BMS. Given that BMS ranges from null (0) to very abundant (5), with an average of 1.59, a difference of .97 units is indeed a large effect. The type 4 animals tended to have better meat quality than the other types. These results are important because the type 4 marker can be used for cows that produce offspring of higher meat quality than the average. Although no significant differences were observed for CW, RT, SFT, and YE between mitochondrial types, the type 4 animals tended to be superior in all comparisons to the other types, and the minority groups of types 2 and 3 generally tended to be inferior.

Table 4. Differences between the effects of mitochondria types on six carcass traits of Japanese Black cattle

Comparison of mitochondrial type	BLUE[a] CW, kg[b]	LMA, cm^{2b}	RT, cm[b]	SFT, cm[b]	YE, %[b]	BMS, unit[b]
1–2	7.14	3.12	.14	–.20	.59	.55†
1–3	3.06	3.59	.09	–.06	.55	.44
1–4	–7.86	–2.40	–.29	–.16	–.26	–.42*
1–5	6.39	.97	.05	–.05	.05	.06
2–3	–4.07	.47	–.04	.14	–.04	–.11
2–4	–14.99	–5.52*	–.43	.04	–.85	–.97**
2–5	–.74	–2.15	–.18	.15	–.55	–.50
3–4	–10.92	–5.99†	–.39	–.10	–.81	–.86†
3–5	3.33	–2.62	–.14	.01	–.50	–.38
4–5	14.25	3.37†	.25	.11	.31	.47*

[a]BLUE = best linear unbiased estimates.
[b]CW = carcass weight; LMA = longissimus muscle area; RT = rib thickness; SFT = subcutaneous fat thickness; YE = yield estimate; BMS = beef marbling score.
†$P < .10$.
*$P < .05$.
**$P < .01$.

Discussion

Cytoplasmic variation in a closed herd could be diminished by founder effects arising from the strict maternal inheritance of mtDNA. The use of limited maternal lineages might have reduced the power of the test to detect the cytoplasmic effects because of scanty variation. In this study, the Japanese Black steers examined were collected from all over Japan to avoid this problem. Recent studies have shown that Japanese Black cattle are highly variable in mtDNA D-loop sequences (our unpublished observations), which may reflect influences by British and Continental breeds dating back 100 yr (Mukai et al., 1989; Ogawa et al., 1989). Crossbreeding was prominent for several years but has not been conducted since the early 1900s. We detected 26 mitochondrial haplotypes defined by variations at 25 sites. Hence, data examined were suitable for studying the cytoplasmic genetic effects on economic traits because of their high variability.

We constructed the phylogenetic tree to classify the mitochondria types. There are some reasons for adopting this strategy. First, the same substitutions sometimes occur among entirely different maternal lines (e.g., at bp 16,042, 16,104, 16,122, and 16,302, in Table 2). Moreover, Boettcher et al. (1996a) described that the degree of sequence variation was surprisingly high between animals with the same maternal lineage. These shared or unexpected substitutions reduce the power of tests when variations at one polymorphic site were used as criteria of classification as a fixed level. Second, the D-loop region does not code any gene products. Variations of this region can be used as genetic markers for the remaining regions of mtDNA because no recombination would occur between the D-loop region and the remaining parts of mtDNA; the former evolves faster than the latter regions. This fact suggests that haplotypes having mutation(s) in the coding regions are expected to have mutation(s) in D-loop regions, although it does not suggest that haplotypes having mutation(s) in the D-loop region have distinct mutation(s) in the coding regions. Therefore, animals having the same mitochondrial types classified by D-loop sequence variations would have the same or similar gene products in the coding regions influencing some traits. We adopted the above assumption to detect the cytoplasmic effects for carcass traits; however, it might be necessary to identify the coding region influencing the traits in the future.

Some researchers who have examined the relationships between yield traits and mtDNA mutations in cows indicated some pitfalls when interpreting the results (Schutz et al., 1994; Boettcher et al., 1996a). A number of significant results occur by chance alone. The total number of statistical comparisons in this study was 60 (10 pair-wise comparisons × six traits) in Table 4. The expected number of significant results by chance would be 6.0, 3.0, and .6 for $P < .10$, $P < .05$, and $P < .01$, respectively. The number of significant differences detected in this study was 8, 4, and 1, respectively. It was almost equal to the values, which were expected by chance. However, the significant differences were only restricted to LMA and BMS. The extent of the differences are considerable because the additive genetic standard deviations were .557 for BMS and 4.23 for LMA. These results suggest that the haplotype of mitochondria affects only LMA and BMS.

Meat quality, especially BMS, is the most important economic attribute in Japanese beef markets. Nowadays, the genetic evaluation of carcass traits is practiced based on field records with BLUP procedures under animal models. If cytoplasmic genetic effects are important sources of variation in particular traits, this effect must be taken into consideration to evaluate the additive genetic values precisely. This information can be easily used by introducing the cytoplasmic or maternal lineage effects in the mixed linear model under an animal model. Combination of BLUP of the additive effects and BLUE of the cytoplasmic effects may be very useful criteria for selection of cows as breeding stock and good fattening calf producers in future herds. Because cytoplasmic effects are passed from dam to dam without recombination, it is easy to classify maternal lines according to D-loop variations, and then it may be possible to screen the maternal lineage with preferable genetic potential in nuclear and cytoplasmic effects. Further research on the relationship between the additive and cytoplasmic effects will be necessary.

Confirmation of these results with more carcass data will be necessary and may be possible, for example with mixed model methodologies for variance component estimation and prediction of cytoplasmic effects using a large carcass data set from the field. Relationships of specific mtDNA variations in a region encoding a transcribed product and D-loop type should be studied using Japanese Black cattle to reveal the causal region(s), that affect carcass traits.

Implications

Significant cytoplasmic genetic effects were observed with two carcass traits, longissimus muscle area and beef marbling score, in Japanese Black cattle. Therefore, mitochondrial DNA sequence variations in the displacement-loop region may be used as genetic markers for selection in the breed. This result recommends that if the genetic evaluation of carcass traits is carried out with animal model BLUP, the cytoplasmic effects should be taken into consideration in Japanese Black cattle. The approach in this study might be used to detect the cytoplasmic effects on the other breeds.

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