Carcass traits and microsatellite distributions in offspring of sires from three geographical regions of Japan[1]

S. B. Smith*[2], M. Zembayashi†, D. K. Lunt*, J. O. Sanders*, and C. D. Gilbert*

*Department of Animal Science, Texas Agricultural Experiment Station, Texas A&M University, College Station 77843-2471 and †Livestock Farm, Graduate School of Agriculture, Kyoto University, 144 Komono Tomita, Tanba-cho, Kyoto, 622-0203, Japan

ABSTRACT: We proposed that cattle sired by bulls from distinct geographical regions of Japan would differ in their ability to accumulate marbling and that they could be distinguished by differences in microsatellite genotypes. Semen was obtained from six, three, and one sire from the Hyogo, Shimane, and Tottori regions, respectively. Cows and heifers (n = 92) were bred by artificial insemination and raised at the Kyoto University Livestock Research Farm over several generations. The calves (n = 145) were 252 ± 0.2 d of age (mean ± SEM) and weighed 164 to 307 kg at the beginning of the finishing phase. Cattle produced from sires from the three regions differed significantly in days on feed (all significant differences $P < 0.05$). Carcass data were collected from 48, 36, and 19 offspring from the Hyogo, Shimane, and Tottori sires, respectively. There was no difference in slaughter weight (550 ± 15 kg). Carcasses from Shimane progeny had more muscle than Hyogo cattle. Hyogo and Shimane cattle contained more kidney and 12th-rib fat than Tottori cattle, whereas Hyogo offspring had more 6th-rib fat than Shimane or Tottori offspring. There were no differences across regions in the monounsaturated:saturated fatty acid ratio. The rate of gain of 6th-rib fat for Hyogo progeny (0.033%/d) was significantly greater than that for Shimane or Tattori progeny (both 0.023%/d). Hyogo 12th-rib fat gain was 0.026%/d, which was significantly greater than that for Tottori progeny (0.010%/d). Shimane 12th-rib fat gain was intermediate (0.016%/d) between the other groups. Blood and muscle samples were used for the collection of DNA from 58, 30, and 18 offspring from the Hyogo, Shimane, and Tottori sires, respectively. Samples of the DNA were analyzed for 11 microsatellites. The BM1824 microsatellites for the Tottori progeny were not in Hardy-Weinberg equilibrium because of successive use of Hyogo sires on previous generations. The TGLA227 microsatellites for the Shimane cattle were not in Hardy-Weinberg equilibrium because of selective removal of progeny for slaughter. There were significant differences in allelic frequencies for the BM1824, ETH10, INRA23, and SPS115 and TGLA53 alleles across regions. These data are consistent with the geographical isolation of the different lines of Japanese Black cattle during their development and indicate the superiority of certain groups of sires in the accumulation of intramuscular lipid.

Key Words: Carcass Composition, Cattle, Geographical Distribution, Microsatellites

 J. Anim. Sci. 2001. 79:3041–3051

Introduction

The various regions and prefectures of Japan are known to produce Japanese Black cattle differing in conformation and carcass quality (Namikawa, 1984). This resulted from the use of a small number of founding cows within each region in the early 1800s. Additionally, there were regional differences in the breeds of cattle used for crossbreeding after the Meiji Restoration in 1868. We proposed that we could provide additional evidence that the offspring of these sires accumulate intramuscular lipid (marbling) at different rates. Additionally, we demonstrated regional differences in microsatellite allele frequencies that would be consistent with the geographical isolation of the lines during their development.

Materials and Methods

Animals and Production. Semen was obtained from six sires, three sires, and one sire from the Hyogo, Shimane, and Tottori regions, respectively. Therefore, the Tottori regional effects must be considered single-sire

[1]Technical article from the Texas Agric. Exp. Sta. This research was funded by the Texas Agric. Exp. Sta. and by grants from the Ito Foundation, Minato-ku, Japan.

[2]Correspondence: 2471 TAMU (phone: 979-845-3939; fax: 979-845-9454; E-mail: sbsmith@tamu.edu).

Received July 25, 2000.
Accepted June 26, 2001.

effects. There were 49 Hyogo-sired, 28 Shimane-sired, and 15 Tottori-sired cows and heifers. Cows and heifers were bred by artificial insemination and were used in subsequent generations as many as six times. Artificial insemination and all rearing were done at the Kyoto University Livestock Research Farm, Tanba-cho, Japan. The production system has been described in detail previously (Zembayashi et al., 1995). All cattle were fed a concentrate ration consisting of 25% flaked corn, 20% steam rolled barley, 10% wheat bran, 15% powder enriched wheat bran, 10% gluten feed, 10% barley bran, 8% rice bran, and 0.02% mineral additives. Although some variation in climate invariably occurred over the several generations represented by this investigation, the offspring of the different sires were distributed equally across the generations. Therefore, production conditions can be considered identical for the offspring.

There were 77, 46, and 22 progeny from the Hyogo, Shimane, and Tottori sires, respectively. The sires from the Hyogo region were Tayasufuku (43 progeny), Takaei (32 progeny), and Okumatsu (2 progeny). Sires from the Shimane region were Itozakura (29 progeny), Itomatsu (15 progeny), and Akizura (2 progeny). The sire from the Tottori region was Sugita. Pedigree analyses of a small number of progeny are indicated for Hyogo and Shimane (Figure 1) and Tattori (Figure 2) progeny. All cows were Japanese Black and either were offspring of the sires used in this investigation (except for Okumatsu and Akizura) or, for the earliest generations, were from various, unknown regions of Japan. As indicated in Figures 1 and 2, offspring from each of the sire/regions frequently were from heifers from other regions.

Carcass data were collected from 48, 36, and 19 offspring from the Hyogo, Shimane, and Tottori sires, respectively. The calves were between 170 and 272 d of age and weighed between 164 and 307 kg at the beginning of the finishing phase. The steer (n = 78) and heifer (n = 25) calves were fed for various periods of time (from 253 to 948 d) to achieve a wide variation in intramuscular lipid content.

Carcass and Sample Composition. Carcasses were dissected after chilling to obtain total muscle, s.c. fat, intermuscular fat, and kidney fat. Samples of longissimus thoracis muscle were obtained from chilled carcasses for the analysis of lipid content. Samples were vacuum packed and stored at –20°C until analyzed. Fatty acid composition of intramuscular neutral lipids of the 6th rib section of the longissimus thoracis muscle was measured as described previously (Zembayashi et al., 1995).

DNA Analysis. Semen (from straws), blood (from 47 cows), and frozen longissimus thoracis muscle (from the remaining animals) provided the source of DNA. Muscle samples had been stored as long as 14 yr at –20°C. The DNA was extracted by standard techniques (Maniatis et al., 1982) from 10 mL of whole blood obtained by venipuncture, from 1 g of frozen muscle, or from one semen straw per sire. Blood samples were collected in 50-mL culture tubes containing 4% EDTA (pH 7.3). Fifteen milliliters of blood was added to two volumes

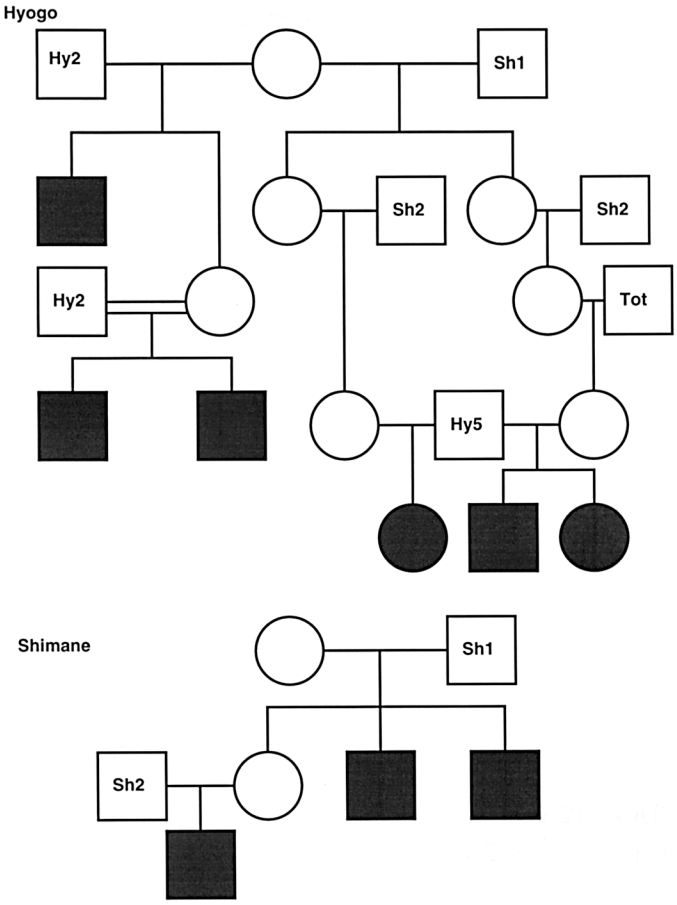

Figure 1. Top. Pedigree analysis of six Hyogo progeny. Open squares, males; open circles, females; closed symbols indicate progeny from which carcass data and(or) microsatellites were determined; double lines between mating pairs indicate cosanguination. Hy2, Hy5, Sh1, Sh2, and Tot indicate the sires Tayasufuku, Takaei, Itozakura, Itomatsu, and Sugita, respectively. Bottom. Pedigree analysis of three Shimane progeny.

of 0.14 *M* NH_4Cl, 0.017 *M* Tris (pH 7.3) and shaken for 15 min at 37°C. The samples were homogenized at 1,500 × *g*, and the pellets were rinsed in the same buffer and centrifuged at 1,500 × *g*. The supernates were discarded, and the pellets were resuspended in 5 mL of STE buffer (100 m*M* NaCl, 10 m*M* Tris, 1 m*M* EDTA, pH 7.3). Proteinase K was added to a final concentration of 0.5 mg/mL, and 1/20th volume of 20% sodium dodecyl sulfate was added. The suspension was mixed gently by hand and incubated overnight at 55°C.

The muscle samples were taken directly from frozen storage, and samples were shaved from the meat block. One gram of muscle was mixed with 2 volumes of 0.14 *M* NH_4Cl, 0.017 *M* Tris (pH 7.3) and shaken for 15 min at 37°C. Afterward, the muscle samples were processed in the same fashion as the blood samples.

Semen samples were thawed and added to 1 mL of 15 m*M* NaCl, 10 m*M* EDTA, which was vortexed and centrifuged at 5,000 × *g* for 3 min. The pellet was resus-

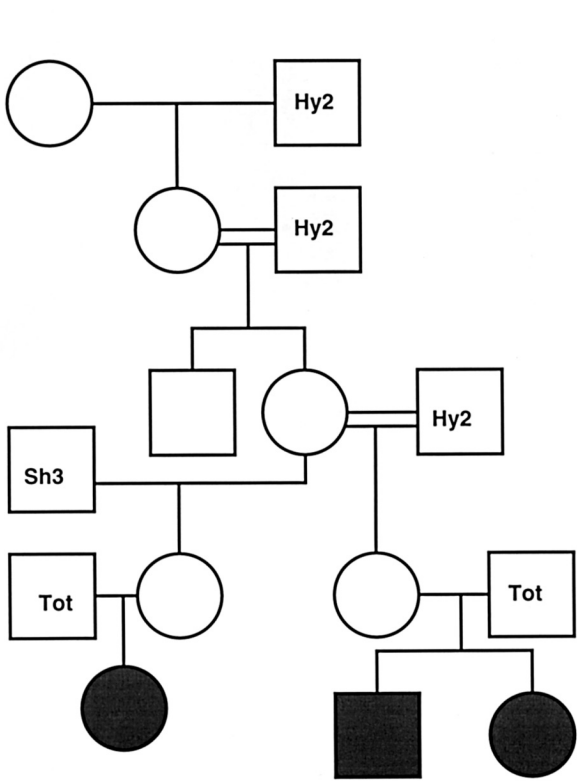

Figure 2. Pedigree analysis of three Tottori progeny. Symbols and sires are defined in Figure 1.

pended in 0.5 mL of the same salt solution, vortexed, and centrifuged again for 3 min. The pellet was resuspended in a sperm lysis solution to a final concentration of 100 m*M* Tris (pH 7.5), 500 m*M* NaCl, 1 m*M* EDTA, 1% SDS, and 2% β-mercaptoethanol. To each sample was added 0.12 mg of proteinase K, and the samples were digested overnight at 37°C.

Following proteinase K digestion, an equal volume of Tris-saturated phenol was added, and samples were mixed gently and centrifuged at 1,500 × *g* for 10 min. The upper layer was transferred to another tube and extracted with an equal volume of chloroform:isoamyl alcohol (24:1, vol/vol). The samples were mixed and centrifuged at 1,500 × *g* for 10 min. The upper layer was transferred to another tube, to which was added 1/10th volume of 2 *M* NaCl and 2.5 volumes of 100% ethanol. The DNA was precipitated overnight, after which the samples were centrifuged at 10,000 × *g* for 20 min at 0°C. The supernate was decanted and the pellet was rinsed in 85% ethanol and subsequently dried. The DNA was redissolved in 1 mL of 10 m*M* Tris, 1 m*M* EDTA (pH 7.3) and heated to 65°C.

The DNA was extracted at the Kyoto University Livestock Research Farm on different occasions over a 4-yr period and transported by hand on ice on each occasion to Texas A&M University. The DNA was stored at –20°C until analyzed. The DNA was collected from semen from two Hyogo sires (Tayasufuku and Takaei), two Shimane sires (Itozakura and Itomatsu), and from the Tottori sire (Sugita). Thus, DNA was collected from bulls that produced 141 of the 145 progeny. Also collected was DNA from 49 of the cows, and from 58, 30, and 18 offspring from the Hyogo, Shimane, and Tottori sires, respectively. Thus, not all of the progeny providing carcass data were used in the DNA analysis. Furthermore, carcass data were not available for all of the progeny sampled for DNA.

Samples of the DNA were analyzed by PE AgGen, Inc. (Salt Lake City, Utah) for 11 microsatellites. The microsatellites (chromosomal location) were BM1824 (Bta1), BM2113 (Bta2), ETH10 (Bta5), ETH225 (Bta9), ETH3 (Bta 19), TGLA122 (Bta21), TGLA126 (Bta20), TGLA227 (Bta18), TGLA53 (Bta16), and INRA23 and SPS115 (bovine chromosomal assignments unknown as of Nov. 1, 2001). Microsatellite analysis and scoring were essentially as described by Taylor et al. (1998).

Statistics. Data were analyzed by analysis of covariance by the SuperAnova program (Abacus Concepts, Inc., Berkeley, CA). The regressions of 6th- and 12th-rib fat as a function of days on feed were compared across regions by analysis of linear regression (Ott, 1984). For carcass data within region, region was the main effect and days on feed was the covariate. When treatment effects were significant ($P < 0.05$), means were separated by the Fisher's Protected LSD method, which was contained in the same software program. Microsatellite genotypes were tested for Hardy-Weinberg equilibrium across regions and within regions, and frequencies of individual microsatellites were tested by chi-square analysis (Ott, 1984).

Results

Carcass Traits. Cattle produced from sires from the Hyogo, Shimane, and Tottori regions differed significantly in days on feed, due to differences in ADG (Table 1). By design, there was no difference in age at which the calves were placed on feed (252 ± 0.2 d, mean ± SEM) or average slaughter weight (560 ± 15 kg), although cattle were slaughtered over a wide range of weights (335 to 741 kg). Hyogo calves initially were lighter than Shimane calves (203 vs 221 kg). Carcasses of cattle from the Shimane sires had more muscle than Hyogo cattle (all significant differences $P < 0.05$). Hyogo and Shimane cattle contained more kidney fat and 6th- and 12th-rib extractable lipid (i.e., marbling) than Tottori cattle, whereas Hyogo cattle had more 6th rib lipid than Shimane or Tottori cattle. There were no differences across regions in the monounsaturated:saturated fatty acid (**MUFA:SFA**) ratios of the longissimus thoracis muscle (Table 1).

There were significant differences in days on feed across regions. The Hyogo offspring were fed for 553 d, whereas the Shimane and Tottori offspring were fed for 443 and 423 d, respectively ($P = 0.29$, Shimane vs Tottori). This suggested that Hyogo cattle had more 6th- and 12th-rib fat than Tottori progeny because they were on feed longer. However, the rate of gain of 6th-

Table 1. Growth and carcass characteristics of cattle produced from sires from the Hyogo, Shimane, and Tottori production regions[a]

Trait	Sire region			Pooled SEM
	Hyogo (n = 48)	Shimane (n = 36)	Tottori (n = 19)	
Production trait				
Initial age, d	252.1	253.1	252.1	0.2
Initial weight, kg	203.1^{y}	221.4^{x}	216.8^{xy}	4.9
Days on feed	553.3^{x}	443.3^{y}	422.6^{y}	20.5
Average daily gain, g	657^{z}	773^{y}	844^{x}	23
Slaughter weight, kg	553.6	559.6	567.1	14.6
Composition trait				
Total muscle, kg	91.5^{y}	96.2^{x}	94.1^{xy}	2.6
Total subcutaneous fat, kg	17.1	15.5	17.2	1.0
Total intermuscular fat, kg	27.9	25.3	26.8	1.2
Kidney fat, g	429^{x}	436^{x}	416^{y}	13
6th-rib fat, g	17.1^{x}	14.6^{y}	14.1^{y}	0.9
12th-rib fat, g	12.1^{x}	11.3^{x}	9.6^{y}	0.6
5th-loin fat, g	17.8^{x}	16.0^{xy}	12.8^{y}	0.8
MUFA:SFA[b]	1.93	1.47	1.72	0.07

[a]Production traits were analyzed by analysis of variance, whereas composition traits were analyzed by analysis of covariance, with days on feed as the covariate. The 6th- and 12th-rib fat and 5th-loin fat are ether extractable lipids.
[b]MUFA:SFA = monounsaturated:saturated fatty acid ratio.
[x,y,z]Means within rows with common superscripts are not different ($P > 0.05$).

rib fat for Hyogo progeny (0.033%/d) was significantly greater than that for Shimane or Tattori progeny (both 0.023%/d; $P < 0.05$) (Figure 3A). The rate of gain of intramuscular fat was less in the 12th rib than in the 6th rib. Hyogo 12th-rib fat gain was 0.026%/d, which was significantly greater than that for Tottori progeny (0.010%/d) (Figure 3B). Shimane 12th-rib fat gain was intermediate (0.016%/d; $P < 0.05$) between the other groups. For this reason, 6th-rib fat was significantly greater in Hyogo cattle than in Shimane and Tottori cattle even when days on feed was used as a covariate. Similarly, Shimane 12th-rib fat was intermediate between Hyogo and Tottori 12th-rib fat (although not different from Hyogo 12th-rib fat).

Microsatellite Alleles. Those bulls that were analyzed from each region were characterized by distinct microsatellite alleles (Tables 2 and 3). For example, the 201- and 209-bp INRA23 alleles were unique to the Hyogo sires, the 213-bp INRA23 allele was unique to the Shimane sires, and the 215- and 217-bp INRA23 alleles were unique to the Tottori sire. The cows and heifers that were progeny of the sires used in this investigation contained several common alleles with their sires. The cows from unknown regions of Japan (used in the earliest generations) possessed microsatellite alleles that were not observed in the bulls. There were no cows produced from the two bulls (Okumatsu and Akizura) for which there were only two progeny each, so the "other cow alleles" in Tables 2 and 3 must have arisen from the older-generation cows. These alleles were in very low frequency in the offspring and were not used in further analyses.

Allelic frequencies and Hardy-Weinberg analyses of the 11 microsatellites of the sires, dams, and progeny are provided (Table 4). Genotypes based on the frequencies of microsatellite alleles were calculated, and all were in Hardy-Weinberg equilibrium when data were pooled across regions (genotypes not shown). Microsatellite allelic frequencies within each production region are indicated in Tables 5 and 6. Chi-square analysis indicated that there were significant differences among the BM1824, ETH10, INRA23, SPS115, and TGLA53 allelic frequencies across sire regions, as predicted from the differences in alleles for these microsatellites among sires (Tables 2 and 3).

The BM1824 genotypes for the Tottori cattle (Table 7) and TGLA227 genotypes for the Shimane cattle (Table 8) were not in Hardy-Weinberg equilibrium. The AA (180, 180-bp) BM1824 genotype frequency was less than predicted, whereas the AB (180, 182-bp) genotype frequency was higher than predicted. Similarly, frequencies of the AA (84, 84-bp), DE (96, 100-bp), DF (96, 106-bp), and EF (100, 106-bp) TGLA227 genotypes were lower than predicted, whereas the frequency of the AD (84, 96-bp) genotype was higher than predicted (Table 8). All other genotypes for the remaining microsatellites were in Hardy-Weinberg equilibrium (not shown).

Discussion

One of the earliest reports describing the ability of Japanese cattle to accumulate large amounts of marbling was that of Yamazaki (1981), which indicated that Japanese beef marbling scores (**BMS**) increased to 24 mo of age and then attained a plateau. However, Zembayashi et al. (1999) demonstrated a linear increase in extractable lipid of the longissimus thoracis muscle (hence, BMS) in Japanese Black cattle over 1,100 d

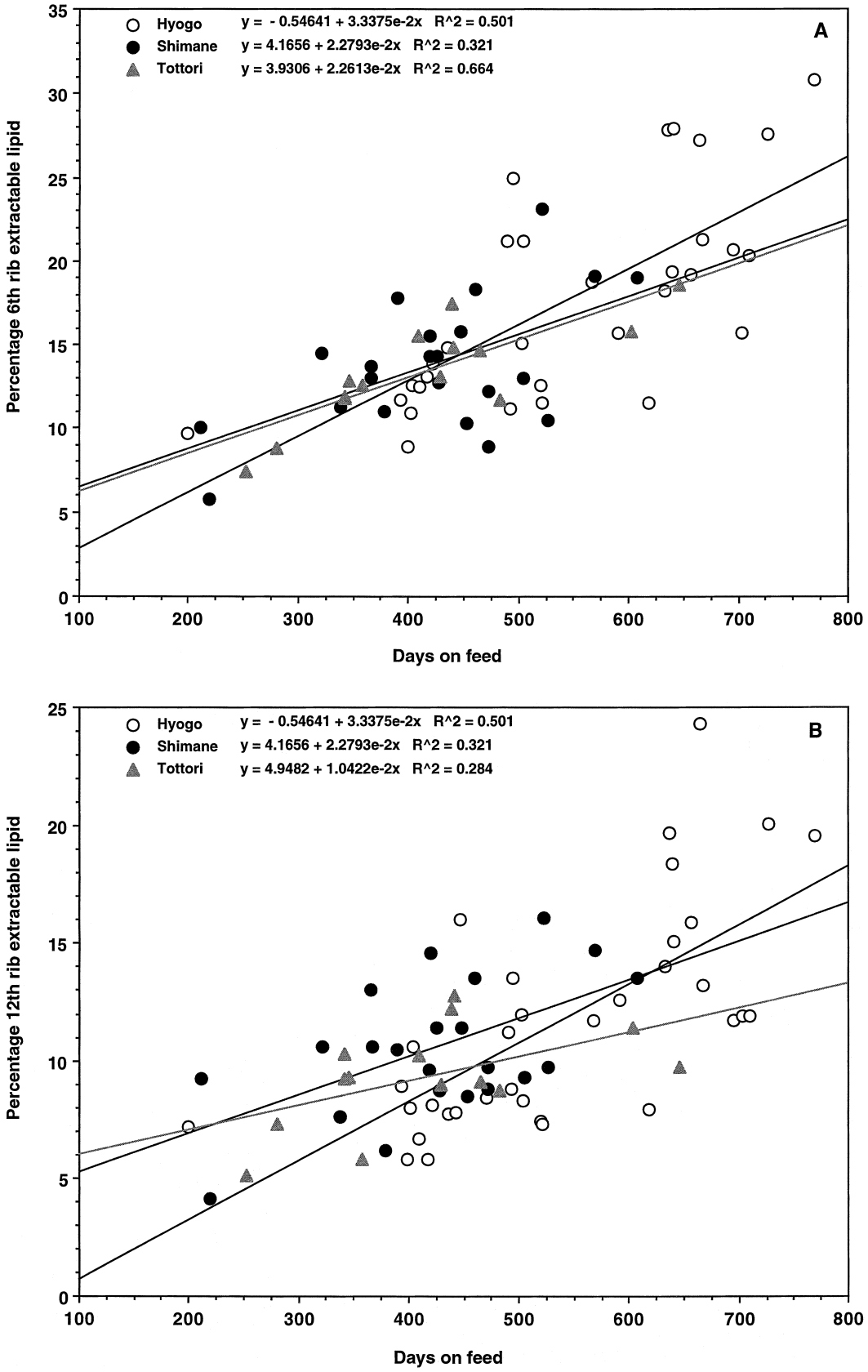

Figure 3. A) Regression of the percentage of 6th-rib extractable intramuscular lipid on days on feed. Symbol legends and regression coefficients are indicated in the figure. The rate of lipid accumulation was greater ($P < 0.05$) in Hyogo cattle than in Shimane and Tottori cattle. B) Regression of percentage 12th-rib extractable intramuscular lipid on days on feed. Rates of lipid accumulation were different for each region ($P < 0.05$).

Table 2. Microsatellite alleles for sires and dams for BM1824, BM2113, ETH10, ETH225, ETH3, and INRA23[a]

	Allele designation						
Microsatellite/region	p	q	r	s	t	u	Other cow alleles
BM1824/Hyogo		182		190			
BM1824/Shimane	180	182	184				
BM1824/Tottori	180			190			
BM1824/Cows	180	182	184	190			186, 192
BM2113/Hyogo			138	140			
BM2113/Shimane		134	138	140			
BM2113/Tottori	128			140			
BM2113/Cows	128	134	138	140			126, 132, 136
ETH10/Hyogo	217			225			
ETH10/Shimane		221		225			
ETH10/Tottori			223	225			
ETH10/Cows	217	221	223	225			214, 215, 219
ETH225/Hyogo		149	151				
ETH225/Shimane		149	151				
ETH225/Tottori	144		151				
ETH225/Cows	144	149	151				146
ETH3/Hyogo	119						
ETH3/Shimane	119	121	127				
ETH3/Tottori		121	127				
ETH3/Cows	119	121	127				117, 125, 129
INRA23/Hyogo	201	207	209				
INRA23/Shimane		207		213			
INRA23/Tottori					215	217	
INRA23/Cows	201	207	209	213	215	217	203, 211

[a]Alleles were determined for two Hyogo sires, two Shimane sires, and one Tottori sire, and for 49 cows and heifers.

Table 3. Microsatellite alleles for sires and dams for SPS115, TGLA122, TGLA126, TGLA227, and TGLA53[a]

	Allele designation						
Microsatellite/region	p	q	r	s	t	u	Other cow alleles
SPS115/Hyogo		255	259				
SPS115/Shimane		255	259				
SPS115/Tottori	253		259				
SPS115/Cows	253	255	259				247, 249, 251
TGLA122/Hyogo		153					
TGLA122/Shimane	145	153					
TGLA122/Tottori	145	153					
TGLA122/Cows	145	153					143, 147, 151, 155, 165, 167, 182
TGLA126/Hyogo	118						
TGLA126/Shimane	118	120					
TGLA126/Tottori	118		124				
TGLA126/Cows	118	120	124				122, 126
TGLA227/Hyogo	84	94					
TGLA227/Shimane	84			100			
TGLA227/Tottori			96		106		
TGLA227/Cows	84	94	96	100	106		77, 79, 86, 90, 92
TGLA53/Hyogo	159	167	169	179			
TGLA53/Shimane	159	167					
TGLA53/Tottori	159				181		
TGLA53/Cows	159	167	169	179	181		153, 161, 163, 165, 171, 173, 175

[a]Alleles were determined for two Hyogo sires, two Shimane sires, and one Tottori sire, and for 49 cows and heifers.

Table 4. Overall microsatellite allele frequencies for cattle produced from sires from the Hyogo, Shimane, and Tottori production regions

Microsatellite	n	Allele p	q	r	s	t	u	χ^2
BM1824	145	0.148	0.338	0.217	0.286	0.010		13.9[x]
BM2113	137	0.149	0.080	0.014	0.361	0.394		13.9
ETH10	133	0.037	0.127	0.067	0.086	0.071	0.609	9.8
ETH225	136	0.132	0.330	0.529	0.007			7.9
ETH3	140	0.010	0.560	0.160	0.257	0.003	0.007	13.7
INRA23	119	0.226	0.420	0.142	0.084	0.063	0.063	15.1
SPS115	133	0.086	0.015	0.097	0.387	0.375	0.037	9.1
TGAL122	133	0.015	0.357	0.015	0.026	0.530	0.056	6.8
TGLA126	134	0.794	0.070	0.115	0.014	0.003		8.6
TGLA227	122	0.532	0.024	0.118	0.077	0.151	0.094	12.3
TGLA53	125	0.032	0.416	0.268	0.172	0.084	0.028	19.3

[x]Chi-square values are indicated for genotypes. All genotypes were in Hardy-Weinberg equilibrium.

of age, with no indication of a plateau. In contrast, Charolais × Japanese Black/Holstein crossbred cattle contained less extractable lipid in the longissimus thoracis muscle than purebred Japanese Black cattle, and there was no further increase in intramuscular lipid after 800 d of age in the Charolais crossbred cattle (Zembayashi et al., 1999). We have established that the BMS of American Wagyu steers is significantly higher (7.3) than that of Angus steers (4.5) raised under identical conditions for the Japanese market (Lunt et al., 1993). Reports to date support the hypothesis that Japanese Black cattle are genetically predisposed to deposit marbling over longer periods of time than British or Continental European beef cattle.

Fat firmness is an important characteristic of the Japanese beef grading system (JMGA, 1988) and reflects the fatty acid composition of the adipose tissue. Adipose tissues of cattle fed grain-based finishing diets typically display a general decrease in saturated fatty acids and an increase in monounsaturated fatty acids (Mitsuhashi et al., 1988a,b; Huerta-Leidenz et al., 1996). Japanese Black cattle are characterized by unusually high concentrations of monounsaturated fatty acids relative to Japanese Shorthorn and Holstein cat-

Table 5. Microsatellite allele frequencies for BM1824, BM2113, ETH10, ETH225, and ETH3 for sires, dams, and offspring of sires from the Hyogo, Shimane, and Tottori production regions[a]

Microsatellite	n	Allele p	q	r	s	t	u
BM1824/Hyogo	61	0.073	0.360	0.139	0.426	0	
BM1824/Shimane	30	0.142	0.428	0.428	0	0	
BM1824/Tottori	18	0.863	0.045	0	0.090	0	
(size of alleles, bp)		(180)[x]	(182)	(184)[x]	(190)[x]	(192)	
BM2113/Hyogo	59	0.129	0.051	0.017	0.344	0.456	
BM2113/Shimane	26	0.153	0.192	0.019	0.326	0.307	
BM2113/Tottori	23	0.239	0.021	0.021	0.152	0.565	
(size of alleles, bp)		(128)	(134)	(136)	(138)	(140)	
ETH10/Hyogo	61	0.032	0.131	0.032	0.024	0.032	0.745
ETH10/Shimane	29	0.017	0.068	0.034	0.275	0.086	0.517
ETH10/Tottori	18	0	0.111	0.027	0.111	0.250	0.500
(size of alleles, bp)		(215)	(217)	(219)	(221)[x]	(223)	(225)
ETH225/Hyogo	61	0.107	0.410	0.482			
ETH225/Shimane	27	0.148	0.314	0.537			
ETH225/Tottori	18	0.250	0.083	0.667			
(size of alleles, bp)		(144)	(149)	(151)			
ETH3/Hyogo	61	0.008	0.713	0.065	0.008	0.196	0.008
ETH3/Shimane	27	0	0.425	0.240	0	0.333	0
ETH3/Tottori	18	0	0.222	0.277	0	0.500	0
(size of alleles, bp)		(117)	(119)	(121)	(125)	(127)	(129)

[a]Only alleles with frequencies > 0.01 for overall frequency (Table 4) are shown.
[x]Allele frequencies within sire regions differ from overall frequencies (Table 4).

Table 6. Microsatellite allele frequencies for INRA23, SPS115, TGLA122, TGLA126, TGLA227, and TGLA53 for sires, dams, and offspring of sires from the Hyogo, Shimane, and Tottori production regions[a]

		Allele					
Microsatellite	n	p	q	r	s	t	u
INRA23/Hyogo	58	0.301	0.405	0.198	0.060	0.017	0.017
INRA23/Shimane	24	0.083	0.687	0.062	0.145	0.020	0
INRA23/Tottori	17	0.058	0.294	0	0.147	0.176	0.323
(size of alleles, bp)		(201)	(207)	(209)	(213)	(215)	(217)[x]
SPS115/Hyogo	61	0.098	0.040	0	0.049	0.303	0.508
SPS115/Shimane	27	0	0	0.018	0.037	0.740	0.203
SPS115/Tottori	18	0.055	0	0.027	0.027	0.250	0.388
(size of alleles, bp)		(247)	(249)	(251)	(253)	(255)[x]	(259)
TGLA122/Hyogo	61	0.008	0.245	0.016	0.016	0.672	0.040
TGLA122/Shimane	25	0.040	0.340	0	0.040	0.580	0
TGLA122/Tottori	18	0	0.638	0	0	0.333	0.027
(size of alleles, bp)		(141)	(145)	(147)	(151)	(153)	(155)
TGLA126/Hyogo	60	0.858	0.041	0.091	0.008		
TGLA126/Shimane	27	0.722	0.203	0.055	0.018		
TGLA126/Tottori	18	0.750	0.027	0.222	0		
(size of alleles, bp)		(118)	(120)	(124)	(126)		
TGLA227/Hyogo	59	0.601	0.008	0.186	0.033	0.118	0.050
TGLA227/Shimane	25	0.580	0.060	0.040	0.060	0.200	0.060
TGLA227/Tottori	17	0.294	0.029	0	0.323	0.117	0.235
(size of alleles, bp)		(84)	(92)	(94)	(96)	(100)	(106)
TGLA53/Hyogo	56	0.294	0.330	0.241	0.133	0	
TGLA53/Shimane	26	0.615	0.326	0.057	0	0	
TGLA53/Tottori	18	0.666	0.138	0.027	0	0.166	
(size of alleles, bp)		(159)	(167)	(169)	(179)	(181)[x]	

[a]Only alleles with frequencies > 0.01 for overall frequency (Table 4) are shown.
[x]Allele frequencies within sire regions differ from overall frequencies (Table 4).

tle (Tanaka, 1985). The MUFA:SFA ratios in s.c. adipose tissue from Angus and American Wagyu steers raised for the Japanese market were 1.2 and 1.5, respectively (May et al., 1993), supporting a genetic basis for the higher MUFA:SFA ratios in tissues from Japanese Black cattle. The MUFA:SFA ratio of s.c. adipose tissue from Japanese Black cattle raised in Japan usually is around 2.0, but it can exceed 2.5 (Sturdivant et al., 1992; Smith et al., 1998). The highest MUFA:SFA ratios typically are observed in adipose tissues and muscle from those Japanese Black cattle that achieve the highest BMS scores, suggesting a genetic relationship between fatty acid composition and marbling.

We had reported previously that Japanese Black cattle from the Kagoshima, Miyazaki, and Gunma regions of Japan differed significantly in the MUFA:SFA ratios

Table 7. Expected and observed genotypes for BM1824 for offspring of sires from the Hyogo, Shimane, and Tottori production regions[a]

	Hyogo		Shimane		Tattori	
Genotypes	Expected	Observed	Expected	Observed	Expected	Observed
AA	0.005	0	0.020	0	0.745	0.167
AB	0.053	0.016	0.122	0.0067	0.078	0.389
AC	0.021	0	0.122	0.200	0	0.222
AD	0.062	0.131	0	0.067	0.157	0.111
BB	0.130	0.147	0.183	0.133	0.002	0
BC	0.100	0.131	0.367	0.467	0	0
BD	0.307	0.278	0	0	0.008	0.055
CC	0.019	0.016	0.183	0.067	0	0
CD	0.118	0.114	0	0	0	0
DD	0.181	0.164	0	0	0.008	0.055
χ^2		8.7		6.6		40.2[x]

[a]A, B, C, and D alleles were 180, 182, 184, and 190 bp, respectively.
[x]Not in Hardy-Weinberg equilibrium.

Table 8. Expected and observed genotypes for TGLA227 for offspring of sires from the Hyogo, Shimane, and Tottori production regions[a]

	Hyogo		Shimane		Tottori	
Genotypes	Expected	Observed	Expected	Observed	Expected	Observed
AA	0.362	0.339	0.336	0.200	0.086	0
AB	0.010	0	0.069	0.080	0.017	0
AC	0.224	0.228	0.046	0.080	0	0
AD	0.040	0.051	0.069	0.120	0.190	0.333
AE	0.142	0.118	0.232	0.360	0.069	0
AF	0.061	0.067	0.069	0.120	0.138	0.222
BB	0.001	0	0.003	0	0.001	0
BC	0.003	0.017	0.005	0	0	0
BD	0.001	0	0.007	0	0.019	0.055
BE	0.002	0	0.024	0.04	0.007	0
BF	0.001	0	0.007	0	0.014	0
CC	0.034	0.017	0.001	0	0	0
CD	0.126	0.017	0.004	0	0	0
CE	0.044	0	0.016	0	0	0
CF	0.019	0.017	0.005	0	0	0
DD	0.001	0	0.004	0	0.104	0
DE	0.008	0	0.024	0	0.076	0.111
DF	0.003	0	0.007	0	0.152	0.111
EE	0.014	0.051	0.040	0	0.014	0
EF	0.012	0.017	0.024	0	0.055	0.111
FF	0.002	0	0.004	0	0.055	0.055
χ^2		15.6		82.5[x]		11.2

[a]A, B, C, D, E, and F alleles were 84, 92, 94, 96, 100, and 106 bp, respectively.
[x]Not in Hardy-Weinberg equilibrium.

of their muscle and adipose tissues (1.65 to 2.10, pooled across tissue types; Sturdivant et al., 1992). This indicated either production or genetic differences across these geographical regions. The values reported for the current investigation were somewhat lower but were taken from animals slaughtered over a wide range of weights and days on feed. Unlike 6th- and 12th-rib fat, there were no differences in the MUFA:SFA ratios among progeny groups when days on feed was used as a covariate. This suggests that there is at best a weak relationship between intramuscular lipid and the MUFA:SFA ratio in Japanese Black cattle, which we had concluded earlier (Sturdivant et al., 1992).

We earlier demonstrated differing allele frequencies of *Taq*I restriction fragment length polymorphisms for the stearoyl coenzyme-A desaturase gene (*SCD1*) for the Hyogo, Shimane, and Tottori regions using a subset of the cattle from the current investigation (Wilson et al., 1993). Tottori offspring exhibited only the smaller, 9.4-kb *SCD1* allele (reflecting the single sire). The Shimane offspring either were heterozygous or were homozygous for the 23-kb allele, whereas the Hyogo offspring were heterozygous or exhibited only the 9.4-kb allele. Thus, our earlier results suggested genetic differences among these populations of Japanese Black cattle; further support for this supposition is provided by our microsatellite analyses.

As for other breed types, sire can significantly affect marbling scores in Japanese Black cattle (Mitsumoto et al., 1989). However, for Japanese Black cattle, differences among sires may reflect the geographical regions in which they were produced. There are four old inbred lines of Japanese Black cattle, the Takenotani-zuru, Bokura-zuru, Iwakura-zuru, and Shusuke-zuru. The Bokura-zuru is a branch line of the Takenotani-zuru. Each line developed in separate-but-adjacent regions in the southwest corner of Honshu (the main Japanese island) just before the Meiji Restoration in 1868. Three of these *tsuru-ushi* (also spelled *zuru-ushi*, literally "inbred lines of cattle") were sampled in the present investigation. Tottori, Shimane, and Hyogo cattle were established in the Takenotani-zuru, Bokura-zuru, and Shusuke-zuru regions, respectively. The origin of the Takenotani-zuru (Tottori) line is well documented (Namikawa, 1984) and illustrates how the separate lines were established. Cattle of the Takenotani-zuru region originated from one cow, which produced 19 calves. Two of her best quality daughters were backcrossed to one of their sons to fix the traits of body size and dairy character, and these formed two sub-lines. Cows of the two lines were bred to two selected offspring bulls reciprocally in successive generations. Cattle in the different prefectures were selected for varying carcass and body conformation traits. Hyogo cattle were selected for carcass quality, which is reflected in their greater amounts of 6th- and 12th-rib intramuscular lipid we observed in this study. Tottori cattle were selected for large size and a strong back line, which apparently selected against carcass quality. Shimane cattle were selected for traits similar to those of the Tottori cattle (being a sub-line of the Takenotani-zuru line) but apparently retained greater carcass quality.

The geographical isolation of each of the inbred lines is suggested by the distribution frequency of alleles for each of the microsatellites. The two Hyogo sires we sampled contained a single allele for EHT3, TGLA122, and TGLA126 and only two alleles for six of the remaining eight microsatellite markers. The two Shimane sires we sampled were more genetically diverse, in that there were two to three alleles at all loci. Whereas the Hyogo and Shimane sires shared several common alleles, the Tottori bull was more apt to contain unique alleles. This could have been caused by a more rigorous isolation of the Tottori line. Alternatively, it may reflect differences in the breed types used in establishing each of the lines. Subsequent to the Meiji Restoration, several imported European breeds were crossed with native cattle to improve specific economic traits (Namikawa, 1984). In the Hyogo region, the foreign breeds were Shorthorn, Devon, and Brown Swiss, whereas, in the Shimane region, the foreign breeds were Devon, Brown Swiss, Simmental, and Ayrshire. The original line in the Tottori region was crossed only with Brown Swiss and Shorthorn imported cattle. The degree of inbreeding varied within region, so that the contribution of each breed type to overall production characteristics would be impossible to document. However, the extent of inbreeding clearly would influence the number of alleles present in cattle from each region.

The cattle produced at the Kyoto University Livestock Research Farm experienced some inbreeding (Figures 1 and 2). Therefore, we expected a greater degree of homozygosity, which would have caused Hardy-Weinberg disequilibrium. Instead, for the two microsatellites not in Hardy-Weinberg equilibrium (BM1824 and TGLA227), we observed lesser homozygosity. For the Tattori BM1824 allele, successive use of the Hyogo bull Tayasafuku (Hy2 in Figure 2) in earlier generations of Tottori cattle led to an overabundance of the 182-bp allele and the 180, 184-bp heterozygotic genotype. Conversely, there were not sufficient generations of the Shimane-sired cattle to establish Hardy-Weinberg equilibrium for TGLA227 (Figure 1).

The original experimental design for the production of these animals was to kill animals at several intervals of days on feed to allow regression analysis of the accumulation rate of the various fat and muscle depots (Zembayashi, 1994). We extend the findings of Zembayashi (1994) in demonstrating that differences exist within populations of Japanese Black cattle (based on region of origin of sire) in their rates of accumulation of intramuscular lipid. The differences in rates of lipid accumulation between Hyogo and Tattori progeny are remarkable (43 and 160% greater in Hyogo progeny for 6th- and 12th-rib fat, respectively). This was in spite of the fact that Shimane- and Tottori-sired cows and heifers were used in earlier generations of the Hyogo progeny. The reverse was true for the Shimane and Tottori progeny. Thus, the terminal sire had a strong impact on the ability of the progeny to accumulate intramuscular lipid.

Recently, Nomura et al. (2001) documented a reduction in the effective population size in Japanese Black cattle subsequent to the liberalization of beef import restrictions in 1991. This was caused primarily by the intensive use of a few popular sires during this time. As a direct consequence of using a small number of sires, genetic differences among prefectures have "essentially disappeared" (Nomura et al., 2001). Thus, the diversity in carcass composition we documented in the current study across production regions may soon be lost.

In summary, we have described a small set of microsatellites in cattle from three geographical regions of Japan. Although there was a statistical relationship between occurrence of alleles and important carcass traits, we have not interpreted this to mean that the microsatellites we have characterized are linked to regulatory genes. Rather, these data indicate that the microsatellites segregated into the different lines due to geographical isolation and(or) selected cross- and inbreeding. Finally, although offspring of the Tottori sire exhibited carcass characteristics typical of cattle from that region, it must be emphasized that these animals were derived from a single sire. Thus, any extension of these data to cattle from the Tottori region must be conservative.

Implications

Our microsatellite analysis of Japanese Black cattle has provided genetic evidence for the geographic isolation of sires from three separate production regions of Japan. The data suggest that it may be possible to determine whether cattle were produced in the Hyogo, Shimane, or Tottori regions based on allelic frequencies. Recent data indicate that this genetic diversity may soon be lost for Japanese Black cattle if the use of a small number of sires continues.

Literature Cited

Huerta-Leidenz, N. O., H. R. Cross, J. W. Savell, D. K. Lunt, J. F. Baker, and S. B. Smith. 1996. Fatty acid composition of subcutaneous adipose tissue from male calves at different stages of growth. J. Anim. Sci. 74:1256–1264.

JMGA. 1988. Japan Meat Grading Association, Chiyoda-du, Tokyo, Japan.

Lunt, D. K., R. R. Riley, and S. B. Smith. 1993. Growth and carcass characteristics of Angus and American Wagyu steers. Meat Sci. 34:327–334.

Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, Cold Spring Harbor, New York.

May, S. G., C. A. Sturdivant, D. K. Lunt, R. K. Miller, and S. B. Smith. 1993. Comparison of sensory characteristics and fatty acid composition between Wagyu crossbred and Angus steers. Meat Sci. 35:289–298.

Mitsuhashi, T., Y. Kitamura, M. Mitsumoto, Y. Yamashita, and S. Ozawa. 1988a. Effect of barley and corn feeding on fatty acid composition and color values of adipose tissue from Japanese Black Steers. Bull. Chugoku Natl. Agric. Exp. Sta., Fukuyama, Hiroshima, Japan. pp 71–79.

Mitsuhashi, T., M. Mitsumoto, Y. Kitamura, Y. Yamashita, and S. Ozawa. 1988b. Age-associated changes in melting points and fatty acid composition in certain adipose tissues from Japanese Black Steers. Bull. Chugoku Natl. Agric. Exp. Sta., Fukuyama, Hiroshima, Japan. pp 43–51.

Mitsumoto, M., S. Ozawa, T. Mitsuhashi, and Y. Yamashita. 1989. The influence of finish weight, sires and kinds of concentrate during the latter fattening period on the carcass characteristics of Japanese Black steers. Jap. J. Zootech. Sci. 60:351–358.

Namikawa, K. 1984. Historical breeding processes of Japanese beef cattle and preservation of genetic resources as economic farm animals. In: Wagyu, Japanese Beef Cattle. Wagyu Cattle Registry Association, Kyoto, Japan.

Nomura, T., T. Honda, and F. Mukai. 2001. Inbreeding and effective population size of Japanese Black Cattle. J. Anim. Sci. 79:366–370.

Ott, L. 1984. An Introduction to Statistical Analysis. 2nd ed. Duxbury Press, Boston, MA.

Smith, S. B., A. Yang, T. W. Larsen, and R. K. Tume. 1998. Positional analysis of triacylglyerols from bovine adipose tissue lipids varying in degree of unsaturation. Lipids 33:197–207.

Sturdivant, C. A., D. K. Lunt, G.C. Smith, and S. B. Smith. 1992. Fatty acid composition of subcutaneous and intramuscular adipose tissues and *M. longissimus dorsi* of Wagyu cattle. Meat Sci. 32:449–458.

Tanaka, S. 1985. Chemical studies on the adipose tissues and muscle lipids of fattening cattle IV. Fatty acid composition and their interrelationships of subcutaneous, intermuscular and abdominal adipose tissues from three beef breeds. Japan. J. Dairy Food Sci. 34:92–102.

Taylor, J. F., L. L. Coutinho, K. L. Herring, D. S. Gallagher Jr., R. A. Brennenman, J. Burney, J. O. Sanders, J. W. Turner, S. B. Smith, R. K. Miller, J. W. Savell, and S. K. Davis. 1998. Candidate gene analysis of *GH1* for effects on growth and carcass composition of cattle. Anim. Genet. 29:194–201.

Wilson, J. J., M. Zembayashi, D. K. Lunt, and S. B. Smith. 1993. Rapid communication: A *Taq*I restriction fragment length polymorphism in the stearoyl-CoA desaturase gene in DNA from purebred Japanese Black cattle. J. Anim. Sci. 71:2575.

Yamazaki, T. 1981. The effect of age and fatness on the meat quality and quantity of beef cattle. IV. The changes of marbling score of the cut surface of loin, and inner muscular fat contents of various cut with the increase of age. Bull. Natl. Grassl. Res. Inst. 58:69–77.

Zembayashi, M. 1994. Beef Production. pp 66–82. Yokendo Ltd., Tokyo, Japan.

Zembayashi, M., D. K. Lunt, and S. B. Smith. 1999. Dietary tea reduces the iron content of beef. Meat Sci. 53:221–226.

Zembayashi, M., K. Nishimura, D. K. Lunt, and S. B. Smith. 1995. Effect of breed type and sex on the fatty acid composition of subcutaneous and intramuscular lipids of finishing steers and heifers. J. Anim. Sci. 73:3325–3332.