DEVELOPMENT OF MARBLING PORK WITH MARKER-ASSISTED SELECTION

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ABSTRACT

Intramuscular fat (IMF) contents influence the tenderness, juiciness and flavor in pork as well as beef. High IMF contents are able to be judged by the appearance of sliced meats as "marbling", so it is one of the most important traits affecting consumer acceptability of meat. In Japan, meat animals are generally produced by a three-way cross using Duroc sires and F1 (LW, Landrace and Large White) dams, so genetically improvement of Duroc breed pigs is effective. In 1999, some meat animals showed extremely high IMF contents at a wholesale market in Gifu prefecture at central Japan. We performed an analysis of consanguinity for them and found a common sire (D1). Then, we tried to explore the quantitative trait loci (QTL) for the high IMF contents using a D1-derived pedigree consisting of one Sire (son of D1), six dams and 191 progeny. As a result of linkage analysis with 125 DNA markers, two QTLs were detected on swine chromosomes (SSC) 7 and 14. These two QTL acted independently and QTL effects on SSC7 and SSC14 were 0.7% and 0.4% per one allele, respectively. We then attempted to produce a breeding population, in which QTL types on the both SSC7 and SSC14 were fixed at the IMF-increase types. In 2009, the first population was constructed with nine homozygous sires and 14 homozygous dams on both the QTLs. The meat quality test with 60 individuals, which did not pass the aptitude test for sires and dams, showed that the average IMF contents were 6.3 ± 1.9% (21 barrows) and 5.8 ± 1.3% (39 gilts). These values were approximately two times of those of meat animals produced from a conventional line (3.2% in average) but the back fat thickness was similar to those from a conventional line (≈2 cm in average). This population was named "Bono-Brown", which was constructed from the Italian word of "bono" (delicious in Italian) and "brown" of Duroc hair color. Now, the population of Bono-Brown has been enlarged and a pork brand "Bono Pork" produced with Bono-Brown sires is popular in Gifu prefecture.

Keywords**:** Intramuscular Fat, Marbling, Marker-Assisted Selection

INTRODUCTION

The standards of consumers in the choice of pork are "safe and secure", "low price", and "high quality". In Japan, many people say that domestic pork is safe and they have higher reliability to it than imported one. On the other hand, some consumers think that imported pork (USA, 40.2%; Canada, 22.1%; Denmark, 15.0%; Mexico, 5.9%; Chili, 3.7%; Spain, 3.5%; Others, 9.6%) is low price and has the same quality as domestic pork and that higher price of domestic pork is not worthwhile to purchase. In order to choose the domestic pork for more consumers, improvement of the meat quality is important. Among traits of meat quality, intramuscular fat (IMF) contents influence the tenderness, juiciness and flavor (Wood *et al*. 1996, Brewer *et al*. 1999, Fernandez *et al*. 1999a, Fernandez *et al*. 1999b, van Laack *et al*. 2001,). High IMF contents are able to be judged by the appearance of sliced meats as 'marbling', so it is one of the most important traits affecting consumer acceptability of meat.

Pig breeding programs have focused on mainly growth rate and leanness (Hammond and Leitch 1998). Reproductive traits such as litter size and litter weight have been also targets of genetic improvement (Stewart *et al*. 1991, Roehe and Kennedy 1993). In 2012, finishing feed conversion ratio, average lean meat percentage and pigs weaned per sows per year were 2.91, 58.4% and 25.41, respectively, in EU average (British pig executive 2013). For lean meat percentage, Belgium had the largest value, 63.4% in average. However, such lean meat is not preferred in Japan because they think some marbling contributes tenderness, juiciness and flavor of cooked meat. For pigs weaned per sow per year, Denmark had the largest number, 29.62, and it is a remarkable result of breeding programmes.

Recently, meat quality has become an important issue in genetic selection, but it takes a lot of labor and time to measure phenotypic traits associating meat quality. So, marker-assisted selection might be a useful tool to improve selection efficiency for meat quality traits (Spelman and Bovenhuis 1998). In pigs, many studies for quantitative traits loci (QTL) have reported genetic regions affecting meat quality, but most of them have detected between an improved breed and a local breed or wild boar (Ovilo *et al.* 2000, Nii *et al.* 2005, Nii *et al.* 2006), or between improved breeds (Grindflek *et al.* 2001, Sanchez *et al.* 2007). In Japan, meat animals are generally produced by an LWD three-way cross using Duroc sires and F1 (Landrace and Large White) dams, so genetically improvement of Duroc breed pigs is effective. Recently, it was shown that QTL for growth rate can be detected in a European breed (Nagamine *et al.* 2003). Therefore, we aimed to explore genetic regions affecting IMF content in the Duroc breed and to produce a population showing high IMF content by marker-assisted selection.

MATERIALS AND METHOD

Parentage test

DNA samples of pork meat were extracted by phenol extraction method and those of live animals in a pig farm were extracted from hair roots by the same method. For parentage test, a panel reported by Yamaguchi *et al.* (2008) containing 10 markers (*ACR*, *SW24*, *SW2429*, *SW1550*, *SW1027*, *SW1328*, *SW443*, *S0316*, *SWR1921* and *SW1263*) was used. These microsatellite markers have many alleles, which can be distinguished with their PCR product sizes. All animals have two copies (alleles) of each marker; one copy from its mother and one from its father. Therefore, if a particular allele is present in the piglet, but absent in the nominated sire, then the nominated sire must be excluded from the true father. Although all the markers do not deny the parentage, it is possible that nominated sire is not the true father of the piglet. So, for each microsatellite marker, the probability of paternity exclusion (PE) was calculated with allele frequencies. Combined probability of paternity exclusion (CPE) was also calculated as $CPE=1 - [(1 - PE_1) \times (1 - PE_2) \times \cdots\times (1 - PE_{10})]$. The PE and CPE were calculated from allele frequencies among Landrace, Large White and Duroc population in Japan. The PE of the 10 markers ranged from 0.512 to 0.688, and the CPE were 0.9999 with sires', dams' and offspring's genotypes and 0.9973 with sires' and offspring's genotypes, in commercial three-way crossed (LWD) pigs in Japan.

Genotyping of microsatellite markers

For genotyping of microsatellite markers, one primer of each primer-pair was labeled with a fluorescence tag as recommended by Applied Biosystems. Microsatellite markers were amplified by PCR using 25 ng of genomic DNA as a template, 0.5 pmol of PCR primer-pair and AmpliTaqGold DNA polymerase, and were analyzed using ABI 3130xl genetic analyzer / GeneMapper system.

Construction of experimental family and traits measurement

The Duroc sire (D1), of which progeny had shown high intramuscular fat (IMF) content, was mated to a dam from a conventional Duroc line (named Sakura) in Japan. One progeny male pig (D2) was selected and mated to six dams from a Large White line (named Nagara York) constructed by Gifu prefecture in Japan. For measurement of phenotypic traits, 191 F1 progeny were fattened to 115 kg of body weight (BW) and slaughtered. Traits scored in this study were body weights (at birth, 7 days, 20 days, 28 days and 60days), age of 115 kg of BW, back fat thick ness at center of body length at slaughter, IMF content of *longissimus* muscle (LM) in the three parts corresponding to the positions from the first to 9th thoracic vertebra, from 10th to the last thoracic vertebra and lumber vertebrae, meat color (L*, a*, b*) and drip loss of LM. The percentage of IMF in the LM (fresh-tissue basis) was determined using the Soxhlet apparatus (Ellis *et al.* 1996). Methods for measurement of other traits were listed in the report by Nii *et al.* (2005). All procedures involving live animals were approved by the Gifu prefecture animal care and use committee.

QTL scanning for IMF

A QTL scan was performed for IMF content in a population, which was consisting of 191 progenies derived from one Duroc sire (D2) and six Large White dams. For linkage analysis, 125 microsatellite markers were used and the summary of a linkage map is shown in Table 1. An interval mapping based on the least-squares method developed for outbred population (Haley *et al*. 1994) was used, whereby back fat thickness was used as a covariate. The male pig (D2) of the parental generation was a son of D1 sire, which had produced meat animals showing very high IMF content, so we assumed that the D1 sire was homozygous of IMF-increase type (Q) allele and the D2 sire was heterozygous of Q allele and IMF-not increase type (q) allele at QTLs. Alleles in Large White pigs were assumed to be another IMF-not increase type (w) (Fig. 1)

Table 1. Summary of microsatellite markers for linkage analysis

Chromosome	Number of marker	Map length (cM)	Average of interval (cM)
SSC1	11	144.0	13.1
SSC2	8	132.1	16.5
SSC3	7	129.3	18.5
SSC4	9	130.1	14.5
SSC5	6	118.7	19.8
SSC6	9	165.7	18.4
SSC7	11	156.6	14.2
SSC8	4	127.7	31.9
SSC9	10	138.5	13.9
SSC10	7	124.1	17.7
SSC11	5	84.9	17.0
SSC12	4	113.1	28.3
SSC13	7	126.2	18.0
SSC14	6	111.5	18.6
SSC15	9	111.8	12.4
SSC16	4	93.2	23.3
SSC17	5	97.0	19.4
SSC18	3	57.6	19.2
Total	125	2162.1	17.3

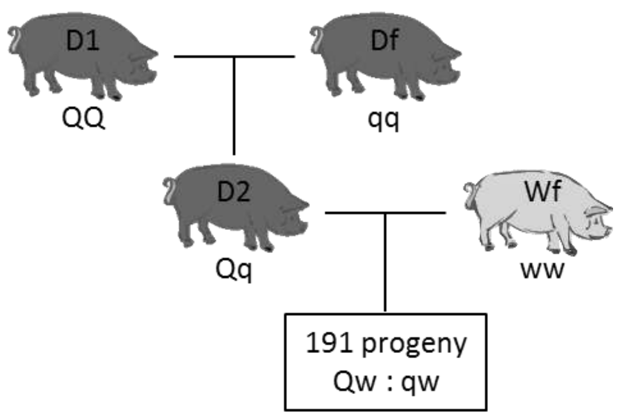

Fig. 1. Construction of an experimental family and the model of QTL analysis. The meat animals produced from the D1 sire showed very high IMF contents in a wholesale market. The D1 sire was mated to a Duroc dam (Df) in a population showing low IMF content, and D2 sire was produced. We assumed the QTL types of the D1 and D2 sires as Q/Q and Q/q, respectively. QTL analysis was performed with 191 progenies derived from the D2 sire and six Large White dams. In this model, Q allele was IMF-increase type and q and w alleles were IMF-not increase type; w allele was that of Large White pigs.

Marker-assisted selection

The Duroc D2 sire, which was heterozygous at the both QTLs on *Sus scrofa* chromosome (SSC) 7 and SSC14, was mated to six dams from a Duroc line (named Iris Nagara) constructed by Gifu prefecture. For produced progeny, seven and six microsatellite markers at the QTL on SSC7 and SSC14, respectively, were genotyped. Haplotypes of microsatellite markers of progeny were constructed by using genotypes of the sire, dam and progeny itself. When progeny had the same haplotype as that at the IMF-increase type allele of the D2 sire, the progeny was judged to be transmitted the IMF-increase type allele. In this generation, animals having IMF-increase type alleles at either QTL were selected. Then mating between selected animals was repeated for more two generations in order to produce a population of homozygotes at the both QTLs. An aptitude test for sires and dams was also used to select animals for production stock. Animals, which did not pass the test, were fattened and slaughtered, and then were scored for phenotypic traits.

RESULTS AND DISCCUSION

Identify a sire having genetic ability to increase IMF

In 1999, some meat animals, which were three-way crossed (LWD) pigs, showed extremely high intramuscular fat (IMF) contents (≈10%) at a wholesale market in Gifu prefecture at central Japan. They were shipped in a short time and we expected genetic factors responsible for those high IMF. We tracked shipping records and found a pig farm, which produced the meat animals. Then in order to identify their parents, we performed a parentage test with 10 microsatellite markers for Duroc sires and F1 (Landrace × Large White) dams bred in the pig farm. The result showed that a sire (D1) was found as the father of the meat animals showing high IMF content. Unfortunately dams were not identified, maybe because they were already culled.

Construction of experimental family for linkage analysis of IMF

We planned to construct an experimental family for the genetic analysis to detect quantitative trait loci (QTLs) of the high IMF content by using the D1 sire. We thought that the D1 sire had genetic ability to increase the IMF content and that progenies produced by a cross of the D1 sire to Duroc dams in another population were heterozygous (Q/q) at the loci for the IMF content; Q and q are IMF-increase type and wild type alleles, respectively. In production of meat animals by using such heterozygous sires, segregation of IMF content would occur in the progeny population due to the allele types (Q or q) derived from the heterozygous sires and enables us to identify the genetic region affecting the IMF content.

The D1 sire was mated to a dam from conventional Duroc line and a sire (D2) was randomly selected from the progenies, which were assumed to be heterozygous, to produce an experimental family for a linkage analysis. The D2 sire mated to six dams from a Large White breed line and 191 F1 animals were produced and scored for phenotypic traits from 2004 to 2006 (Fig. 1). Data from 191 progenies showed that the average IMF contents of barrows and gilts were 4.6 ± 1.5% and 3.5 ± 1.0%, respectively; 4.1% in average of all the 191 individuals (Table 2). These values were significantly higher than those of meat animals produced by a conventional line; 3.3 ± 0.9% (73 barrows), 3.0 ± 0.9 % (78 gilts), and 3.2% in average. In this experimental family, IMF content varied from 1.0% to 10.5% and some individuals presented higher than 10% (Fig. 2). The IMF contents were therefore thought to be segregated in this population.

Other phenotypic data in this population were listed in Table 2. A positive correlation was observed between IMF content and back fat thickness (barrows; 023, gilts; 0.23). A negative correlation was observed between IMF content and average daily gain (barrows, -0.33; gilts, -0.22), similar to the report by Da Costa *et al*. (2004).

Table 2. Traits measurement of experimental family for QTL analysis

Traits	Barrows (n=94)	Gilts (n=97)
Body weight (kg)		
at birth	1.2 ± 0.28	1.2 ± 0.28
7 days	2.1 ± 0.53	2.1 ± 0.58
20 days	4.8 ± 1.25	4.8 ± 1.39
28 days	6.5 ± 1.69	6.6 ± 1.93
60 days	22.9 ± 5.13	24.2 ± 5.46
Age at 115 kg of BW (day)	158.8 ± 14.0	160.4 ± 13.3
Average daily gain (kg)	0.73 ± 0.06	0.72 ± 0.06
Back fat thickness (mm)	25.0 ± 4.23	21.4 ± 4.35
IMF content (%)	4.6 ± 1.50	3.5 ± 1.03
Drip loss (%)	3.9 ± 2.28	4.1 ± 2.27
Meat color		
L*	47.3 ± 2.28	46.8 ± 2.89
a*	8.5 ± 0.96	8.7 ± 2.89
b*	8.6 ± 0.97	8.7 ± 0.97

BW, body weight
IMF, intramuscular fat

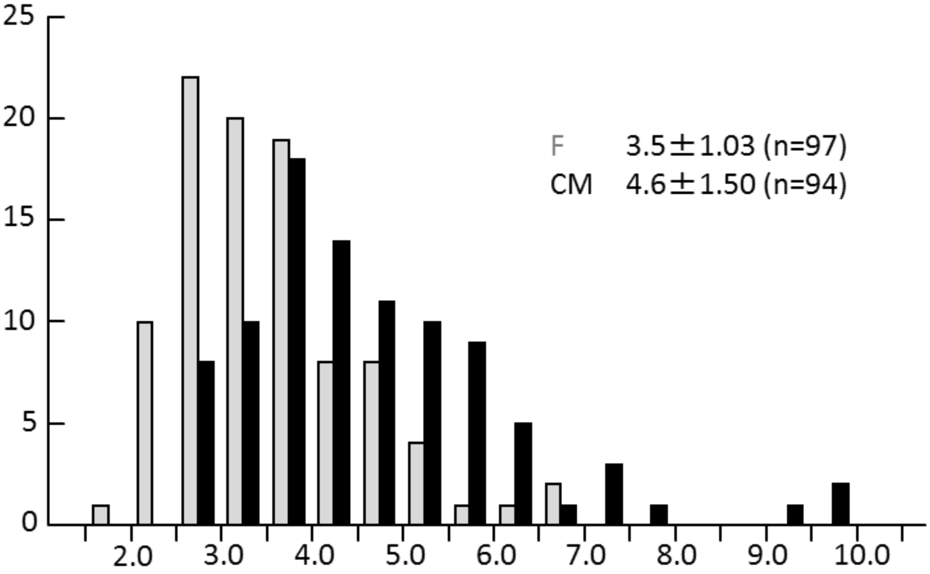

Fig. 2. Distribution of IMF content in the experimental family.
F and CM mean females and castrated males, respectively.

QTL analysis of IMF content

We performed a linkage analysis for the IMF content with the phenotype data and the genotype data of 125 microsatellite markers covering the whole genome except for the chromosome X from the 191 individuals produced with the D2 sire. As a result, two QTLs were detected on *Sus scrofa* chromosomes (SSC) 7 and 14 (Fig. 3). In SSC7, the peak position was detected at microsatellite marker *SW1418* (82.8 cM) with 21.7 of *F*-value, which was above the chromosome-wide 1% significance level. In SSC14, the peak position was detected at microsatellite marker *SW1081* (72.1 cM) with 9.4 of *F*-value corresponding to the chromosome-wide 5% significance level. These two

QTLs acted independently and QTL effects on SSC7 and SSC14 were 0.7% and 0.4% per one allele, respectively (Fig. 4). The progeny not having any IMF-increase type alleles showed 3.3% of IMF content in average and those heterozygous at the both QTL showed 4.4% of IMF content in average; the difference of IMF contents was 1.1%. At the time, we could not evaluate the effects of homozygotes at these two QTL.

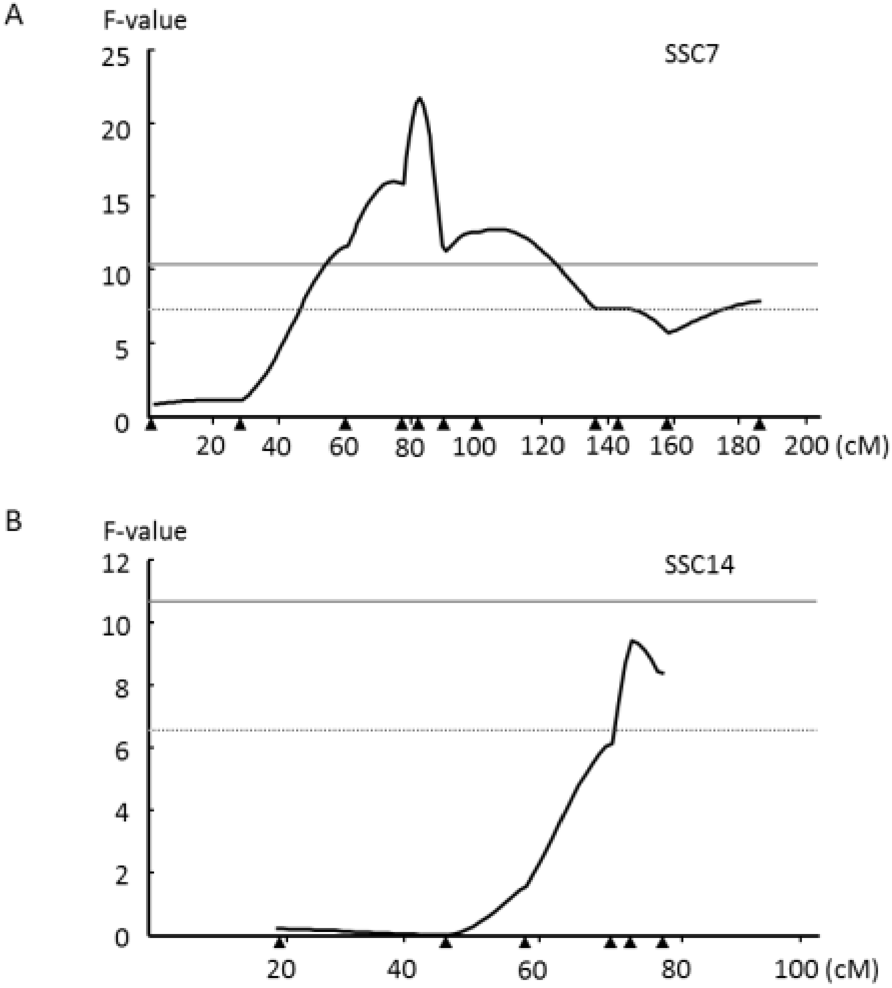

Fig. 3 QTLs for intramuscular fat (IMF) content detected on SSC7 (A) and SSC14 (B) in the D2-derived experimental family. Solid and dotted lines indicates chromosome-wide significance level of 1% and 5%, respectively. Triangles indicate the positions of microsatellite markers used for an interval mapping analysis.

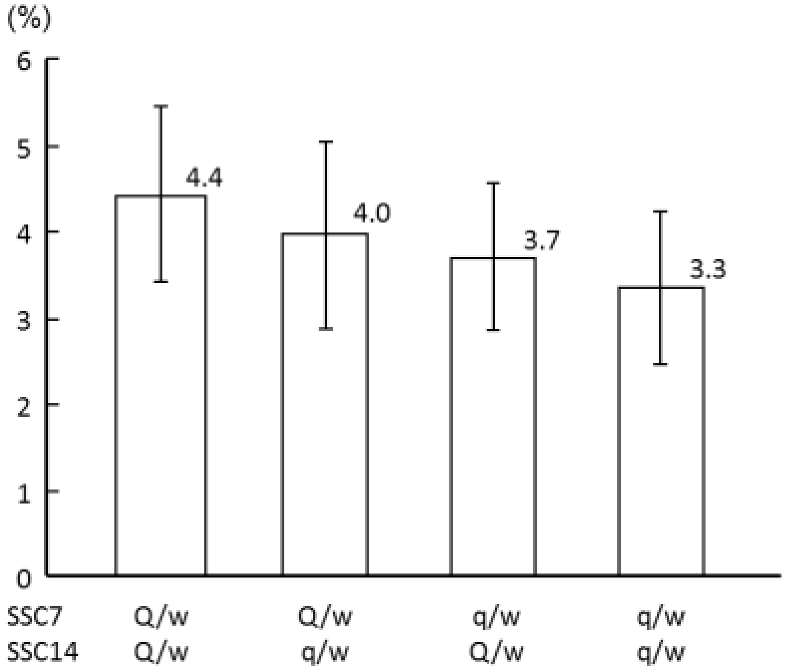

Fig. 4 Effects of two QTLs for IMF content in the experimental family derived from the D2 sire and Large White dams. Q means the IMF-increase type allele at the QTLs on SSC7 and SSC14, q means the not-increase type in Duroc pigs, and w means alleles of Large White, which was assumed as not-increase type.

At the beginning of this study, we found some meat animals showing very high IMF content (≒10%). However, the QTLs on SSC7 and SSC14 did not have such strong effects. We think that some of QTLs could not be detected because the number of animals showing remarkable high IMF content was small; only three animals showed more than 9% of IMF content. It is possible that multiple QTLs may be responsible for the very high IMF content (≒10%). In this study, we used a half-sib family for a linkage analysis. Because homozygotes were not present in this model, it is difficult to detect recessive QTLs.

In fact, 10% of IMF content was too high and such pork meat was not desired even in Japan. Furthermore, Duroc breed pigs are used as terminal sires for production of three-way crossed meat animals, so recessive QTLs may not be useful. For these reasons, we aimed to construct a pig population by using a marker-assisted selection of the QTLs on SSC7 and SSC14. Desired value of IMF content was approximately 6%, which was two times of conventional pork meat.

Marker-assisted selection of two QTLs responsible for high IMF content

We then attempted to produce a Duroc breeding population, in which QTL types on the both SSC7 and SSC14 were fixed at the IMF-increase type (Q) alleles. The D2 sire, which was heterozygous (Q/q), was mated to independent six Duroc dams (q/q). From the progeny (male; 18, female; 16), five males and nine females were selected by using haplotype of seven and six microsatellite markers on SSC7 and SSC14, respectively. In this generation, animals having IMF-increase type alleles at either QTL were selected. For the selection of the homozygous individuals (Q/Q) on the both of two QTLs on SSC7 and SSC14, 411 progeny (male; 176, female; 235) were produced with the total of 54 mating between heterozygous animals through several generations. As the 1st parent stock population, nine homozygous sires and 14 homozygous dams on both the QTLs were selected (Fig. 5).

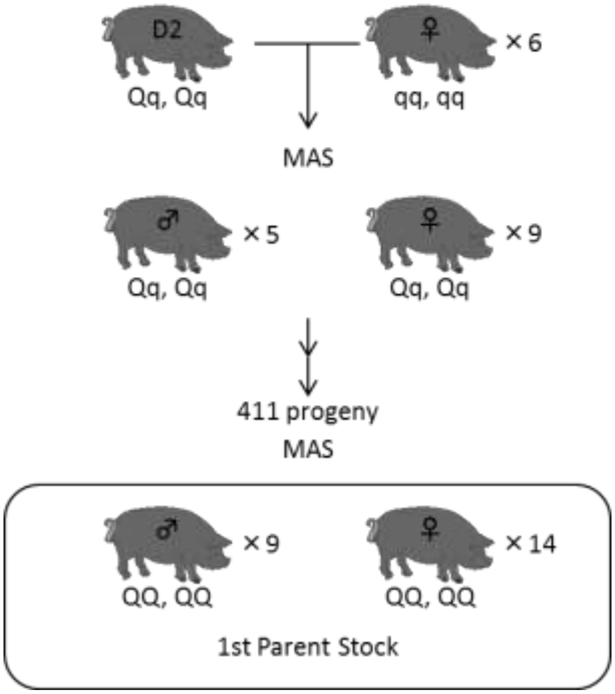

Fig. 5 Marker-assisted selection (MAS) of the QTLs on SSC7 and SSC14. The D2 sire was mated to six Duroc dams. With haplotype of microsatellite markers in the QTL regions, heterozygotes (5 males and 9 females) at the QTLs were selected. Through some generations, the 1st parent stock population consisting 9 males and 14 females, which were homozygous at the both QTLs, were established.

For the analysis of meat quality of this population, 60 individuals, which did not pass the aptitude test for sires and dams, were fattened and scored for phenotypic traits. As a result of analysis of the meat quality, the average IMF contents were 6.3 ± 1.9% (21 barrows) and 5.8 ± 1.3% (39 gilts), which were approximately two times of those of meat animals produced from a conventional line; 3.2% in average (Table 3). This population was named "Bono-Brown", which was constructed from the Italian word of "bono" (delicious in Italian) and "brown" of Duroc hair color (Fig. 6).

Table 3. Summary for IMF contents in Bono-Brown population

	Barrows (n=21)	Gilt (n=39)
Days of slaughter (115 kg of live weight)	174.6 ± 9.2	184.2 ± 15.7
Back fat thickness (mm)	2.1 ± 0.6	1.8 ± 0.4
IMF content (%)	6.3 ± 1.9 (3.1 ± 1.7)	5.8 ± 1.3 (3.0 ± 0.8)

The values in parentheses indicates those in a conventional line

Fig. 6. A sire of Bono-Brown population and pork meat of Bono-Brown

After that, the parent stock population has been enlarged. Homozygotes were used for mating to another line of Duroc pigs in order to re-produce heterozygotes, which were then crossed each other to produce homozygotes. Bono-Brown has been used as terminal sires in producing meat animals by three-way crossing, and approximately 30,000 meat animals were produced in 2013. Among them, 10,000 animals were fattened with a special diet (data not shown) and sold as a pork brand 'Bono-Pork'. Bono-Pork is produced from LWD three-way crossbred pigs but showing 6% of IMF content in average, which was 2% higher than pork meat of conventional LWD pigs with the special diet (4% in average). We think that there is some interaction between the genetic effects and dietary regulation.

CONCLUSION

We identified two quantitative trait loci (QTL) for intramuscular fat (IMF) contents segregated in Duroc breed pigs. QTL effects on SSC7 and SSC14 were 0.7% and 0.4% per one allele, respectively, and these two QTL acted independently. Then we undertook to produce a breeding population with a marker-assisted selection. Through some generations, a breeding population, in which QTL types on the both SSC7 and SSC14 were fixed at the IMF-increase types, was constructed. The average IMF content was approximately 6% in average, which was two times of those of meat animals produced from a conventional line. We named this population 'Bono-Brown', which has been used as terminal sires to produce three-way crossed meat animals. Pork meats showing high IMF content (6% in average) are sold with a brand name 'Bono Pork', which has become popular in Gifu prefecture. This is a successful example of marker-assisted selection in pigs.

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