Mapping Quantitative Trait Loci for Meat Quality on Pig Chromosome 3, 4 and 7**

Bo Zuo, YuanZhu Xiong *, YuHong Su[1], ChangYan Deng, Rong Zheng and Siwen Jiang
Agricultural Ministry Key Laboratory of Swine Genetics and Breeding. College of Animal Science and Veterinary Medicine, Huazhong Agriculture University, Wuhan 430070. P. R. China

ABSTRACT : The objective of this study was to localize QTL affecting meat quality in a pig family of three generations. All animals were genotyped for twenty-four microsatellites on SSC3 (*Sus scrofa* chromosome 3), SSC4 and SSC7. One hundred and forty F_2 offsprings were scored for eleven meat quality traits. Least square regression interval mapping revealed quantitative trait loci (QTL) effect for meat pH (*m. Semipinalis Capitis*, SC) on SSC4 and SSC7; for moisture (*m. Longissimus Dorsi*, LD) on SSC3. Furthermore, there was suggestive evidence for a QTL on SSC4 affecting intramuscular fat (IMF) content that nearly approached the chromosome-wise (p=0.05) significance threshold. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 3 : 320-324)***

Key Words : Genome Scan, Quantitative Trait Loci (QTL), Meat Quality, Pigs

INTRODUCTION

For the last years, meat quality traits have increasingly attached more attention in pig breeding because selection for high growth rate and lean meat deposition resulted in reduction of meat quality. Marker assisted selection has been suggested as a promising strategy for genetic improvement of such recording intensive traits (Meuwissen and Goddard, 1996), and much focus is now on mapping individual loci controlling these traits. Genome scans using anonymous molecular markers, especially, microsatellites, serve as an important tool for mapping QTL. In swine, a number of studies have been conducted to detect QTL for meat quality (Andersson-Eklund et al., 1998; Milan et al., 1998; Wang et al., 1998; Moser et al., 1998; Olivo et al., 2000; De Koning et al., 1999, 2000a, 2000b; Malek et al., 2001).

In this study, a search for QTL affecting meat quality traits on SSC3, SSC4 and SSC7 was conducted to identify and map QTL in our resource family.

MATERIALS AND METHODS

Animals and traits

The pig reference family used in this study was established by mating three Large White boars to seven Meishan sows. Five males and twenty-three females in the F_1 generation were selected for intercrossing randomly. One hundred and forty F_2 individuals were slaughtered for testing. Eleven meat quality traits were recorded according to the method of Xiong and Deng (1999) (Table 1). For each trait, One hundred and forty phenotypic records were available. All animals in the experiment were halothane tested. Of all animals, thirteen animals including one LargeWhite boar, two F_1 boars and ten F_2 individuals were carrier of halothane negative.

Microsatellite amplication and genotyping

Twenty-four microsatellites on SSC3, SSC4, and SSC7 were selected from the USDA-MARC Genome Database based on position, ease of scoring, and number of alleles. PCR of microsatellites were carried out on PE intergrated thermalcycler. The reaction volume of each PCR was 20 μl containing 200 ng genomic DNA, 1×PCR buffer, 1.5 mM $MgCL_2$, 200 μmol/l dNTP, 5 pm of each primer, and 1 *U Taq* DNA polymerase (Biostar International, Canada). The thermocycling conditions were: pre-denaturation for 10 min at 95°C, followed by 35 cycles of reaction at a fixed annealing temperature, and ending at 72°C for extension. Each pooled sample representing 8 μl of PCR product and 2 μl of internal size standard-formamide mixture were loaded upon electrophoresis on 8% polyacrylamide gels and fragment markers were determined after sliver-staining.

Linkage analysis

Linkage analysis was performed by using the CRIMAP version 2.4 (Green et al., 1990).

Statistical methods

QTL analysis at chromosome-wise level was carried out on the Internet (http://qtl.cap.ed.ac.uk). The QTL analysis was based on the line-cross concept (Haley et al., 1994). Least square regression interval mapping was used for QTL

** Supported by the national "973" project of P. R. China (G2000016105).
* Corresponding Author: Xiong Yuanzhu. Tel: +86-27-87287390, Fax: +86-27-87394184, E-mail: xiongyzh@public.wh.hb.cn
[1] Basic School, JinZhou Medical College, JinZhou, 121001, P. R. China.
Received July 2, 2002; Accepted November 4, 2002

Table 1. Phenotypic mean, standard deviation (SD), maximum and minimum for F_2 individuals

Trait	Abbr.	Unit	Max.	Min.	Mean	SD
Meat pH (*m. Longissimus Dorsi*, LD)	pH (LD)	pH	6.69	5.55	6.33	0.22
Meat pH (*m. Biceps Femoris*, BF)	pH (BF)	pH	6.8	5.84	6.42	0.17
Meat pH (*m. Semipinalis Capitis*, SC)	pH (SC)	pH	6.74	6.02	6.45	0.16
Drip loss rate	DLR	%	35.6	4.09	6.83	5.12
Water holding capacity	WHC	%	94.53	51.06	90.65	7.10
Meat color, Elrepho(*m. Longissimus Dorsi*, LD)	MC (LD)	%	44	18	22.10	4.02
Meat color, Elrepho(*m. Biceps Femoris*, BF)	MC (BF)	%	26	18	20.6	1.49
Marbling (*m. Longissimus Dorsi*, LD)	Marbling (LD)		3.8	3	3.25	0.20
Marbling (*m. Biceps Femoris*, BF)	Marbling (BF)		4.8	4	4.15	0.18
Intramuscular fat (*m. Longissimus Dorsi*, LD)	IMF	%	4.47	1.26	2.4	0.64
Moisture (*m. Longissimus Dorsi*, LD)	Moisture	%	75.27	72.04	73.9	0.79

detection, and significance thresholds were determined by permutation tests. At every centimorgan (cM) across the genome, the following model was fitted:

$$Y = \mu + sex + family + Hal + age\ at\ slaughter + C_a a + C_d d + e$$

Where Y was the observations; μ was the mean; *Sex, family, Hal* was the fixed effect of traits; *age at slaughter* was the covariance of traits; C_a, C_d were the coefficients for the additive and dominance component for individual i at the given location; a, d was the additive and dominance effect of a putative locus at the given position; the additive QTL effect was defined as half the phenotypic difference between homozygous pigs for the QTL alleles originating from the Meishan and the LargeWhite lines, the dominant effects were estimated as the deviation of heterozygous pigs from the mean of the homozygous pigs. In this study, the additive effects were estimated for LargeWhite QTL allele, thus, positive values of the additive effects denote an increase of the trait due to the LargeWhite QTL allele; e was the residual error. Additionally, the additive fraction of F_2 phenotypic variation (σ_y^2) explained by a QTL was computed as $h^2_Q = a^2/2\sigma_y^2$ (Olivo et al., 2000).

RESULTS AND DISCUSSION

Linkage analysis

Marker mapping results are presented by chromosome in Table 2. PCR amplified fragments of 24 microsatellites mostly fell in the corresponding sizes range in the USDA database. In all cases but one, map order of the markers was the same as that in the USDA map. The exception was a switch in order for SSC4 between SW841 and SW270. Map lengths for these chromosomes were considerably longer in the present study. This difference between the maps may be a result of unorganized typing errors which are known to increase map lengths (Marklund et al., 1996), as well as different reference population used.

QTL analysis

In the present study, the population including 140 F_2 offsprings was subjected to QTL analysis for eleven meat quality traits. In all, three QTLs covering SSC3, SSC4, SSC7 respectively, were detected at 5% chromosome-wise level, of which one were significant at the 1% chromosome-wise level, and on SSC4, one QTL which had an nearly significant effect on IMF were detected (Table 3). The QTL graphs for chromosomes with evidence for QTL at the 5% or 1% chromosome-wise level are presented in Figure 1.

The most convincing result in the analysis was evidence for a QTL affecting moisture on SSC3, for which the chromosome significant level of 0.01 was obtained (Figure 1A), the highest probability of QTL position was found between markers SWR1637 and SW1443. In general, only QTL with fairly large effects were expected to reach statistic significance, but the additive fraction of phenotype variation was only 0.11%. It might be spurious or the estimation of additive effects biased lower. This QTL seemed to be significantly dominantiaction, because the estimation of dominant effect was considerably larger than that of additive effect (Table 3). So far, there were no reports about QTL affecting moisture on SSC3.

Ultimate pH of pork is the most commonly used trait to assess pork quality. It is correlated with the quality traits of colour, drip loss, and water-holding capacity. A higher level of acidity within the muscle (lower pH) causes muscle protein to denature and lose their ability to hold water. Therefore, meat with higher pH will tend to have more desirable characteristics (Malek et al., 2001). In our resource population, we detected two QTL controlling pH (SC) on SSC4 and SSC7. The largest F-value was estimated at 102cM (SW270-SW841) on SSC4 and 20cM (SW1343-SW2155) on SSC7, respectively (Figure 1C; 1D). This marker region represents the most possible location for QTL. These two QTL explained 22.84% of total variance altogether. Allele came from LargeWhite breed were associated with lower pH value, which is in agreement with expectation based on breed observations. De Koning et al. (2000a, 200b) also found QTL affecting pH on SSC4, but no evidence about QTL on SSC7 was detected.

Table 2. Characterizations of microsatellites in this study

Marker	Allele number	Allele size, bp	Chromosome	Map position, cM	T_A, °C
SW2021	3 (8)	120-132 (110-132)	3	0 (12.4)	65
SWR1637	3 (4)	127-160 (118-152)	3	20.4 (27.6)	58
SW1443	2 (8)	180-200 (172-192)	3	38.1 (40.1)	58
SW2618	3 (8)	119-127 (105-127)	3	53.5 (50.8)	58
SW2408	4 (9)	174-190 (168-184)	3	83.2 (75.3)	62
SW349	3 (5)	130-170 (130-178)	3	108.6 (94.2)	58
SW717	3 (8)	157-177 (149-177)	3	133.5 (112.6)	65
SW0165	4 (7)	141-157 (141-155)	3	142.3 (116.6)	62
SW2047	3 (5)	144-174 (142-170)	3	161.0 (129.3)	60
SW2404	3 (6)	130-149 (132-174)	4	0 (0)	62
SW835	4 (8)	120-240 (218-240)	4	37.4 (27.1)	60
SW752	3 (4)	112-124 (108-124)	4	71.7 (51.2)	60
SW270	3 (8)	137-145 (139-163)	4	101.0 (71.2)	60
SW841	2 (9)	158-170 (158-188)	4	119.3 (79.3)	60
SW445	3 (11)	181-203 (181-203)	4	154.7 (105.8)	58
S0161	4 (5)	130-160 (137-163)	4	178.5 (121)	65
SWR1343	4 (4)	120-150 (122-142)	7	0 (12.2)	60
SW2155	6 (6)	135-151 (135-149)	7	26.4 (32.9)	65
SW1856	4 (5)	173-197 (180-200)	7	63.4 (61.5)	58
SW859	3 (4)	101-119 (85-123)	7	85.1 (75.3)	60
SW352	4 (3)	104-112 (107-111)	7	104.1 (87.7)	55
SW252	4 (7)	143-191 (149-179)	7	122.3 (99.4)	62
SW581	2 (3)	201-205 (199-205)	7	154.5 (123.8)	57
S0212	5 (8)	232-250 (229-249)	7	179.1 (141.2)	55

The data in brackets were cited from the USDA database (http://sol.marc.usda.gov/genome/swine/swine.htm).

Table 3. Analytical results of QTL significant at the 5% or 1% chromosome-wise level and estimation of gene effects

Trait	Chromosome	Location (cM)	Marker interval	F-value	Threshold		Additive effect	Dominant effect	Variance%
					P=0.05	P=0.01			
pH(SC)	4	102	SW270-SW841	5.88*	5.62	8.19	-0.08±0.02	-0.06±0.03	12.9
	7	20	SW1343-SW2155	5.68*	5.30	7.28	-0.07±0.04	0.19±0.06	9.94
Moisture	3	33	SWR1637-SW1443	9.4**	5.70	7.35	-0.03±0.12	0.77±0.18	0.11
IMF	4	71	Sw752	5.23	5.43	7.78	-0.19±0.10	-0.25±0.13	5.1

* Represent significance at the 5% chromosome-wise level.

** Represent significance at the 1% chromosome-wise level.

Intramuscular fat (IMF) content is a major determinant of meat quality. After the elimination of the halothane genes, the next limiting factor for meat quality would be IMF (Webb, 1998). At present, IMF has been the major focus of QTL mapping for meat quality traits. For IMF, the most probable position of a QTL on SSC4 was found around 71cM (Figure 1B). However, this test statistic did not exceed chromosome-wise significance level. Although this QTL did not significantly contribute to the variation in IMF content in this population, the effect of this locus was still considerable. This QTL can explain 5.1% fraction of phenotype variation. Individuals homozygous for the Meishan alleles had an average 0.38% more IMF content than those homozygous for Largewhite alleles, and Meishan alleles were causing a increase in IMF content. It is currently observed that IMF content is positively correlated with subcutaneous fatness. Many experiments have succeeded in identifying chromosome regions associated with backfat thickness (BFT) (Wu et al., 2001, 2002). In the same studies, chromosome-wise evidence for QTL affecting BFT was found around 53cM, close to marker SW752 (Zuo et al., 2003). It is likely that the QTL affecting BFT and IMF are the same gene, as they are located close together in the chromosome. However, owing to the low precision of the mapping of this QTL, it is difficult to decide whether there is only one QTL with pleiotropic effects or several linked QTL. This QTL was localised to the region where *adipocyte fatty acid-binding protein* (A-FABP) were previously located. The porcine A-FABP gene was located between S0001 and S0073 that have been assigned to SSC4p12-13 (Marklund et al., 1993) and SSC4q15-16 (Robic et al., 1996). Functionally, FABP are intracellular proteins that transport fatty acid from the cell membrane to sites of fatty acid oxidation or phospholipid or triacylglycerol synthesis. De Koning et al. (1999) reported the suggestive QTL for IMF in the S0001-S0073 interval. In

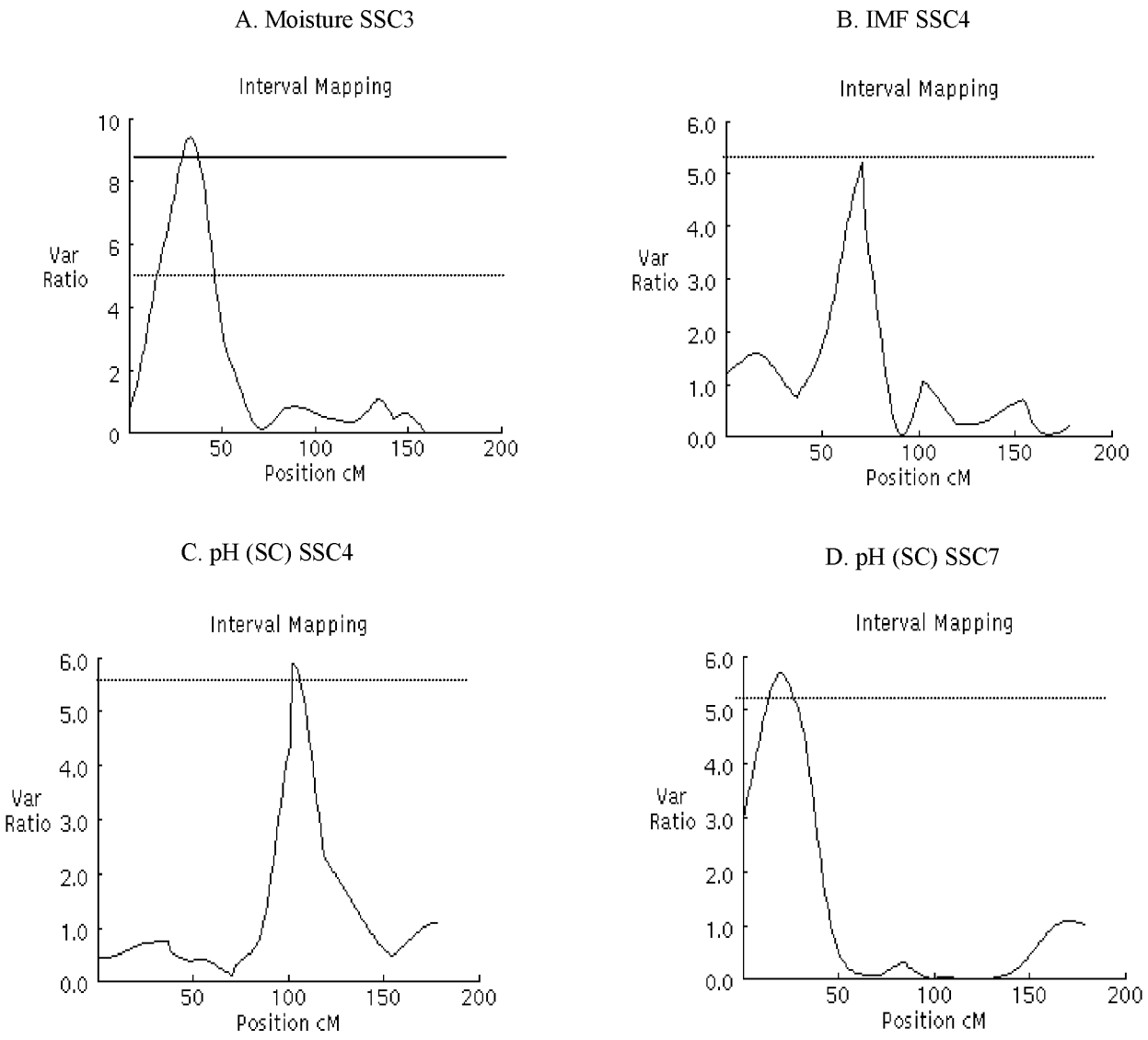

Figure 1. F ratio curves for evidence of QTL. The X-axis indicates the relative position on the linkage map, the Y-axis represents the F-ratio. (·····) and (——) represent 5% and 1% chromosome-wise significance obtained by permutation with 1000 replicates, respectively.

addition. Rattink et al. (2000) also found a QTL for IMF at the same region, with additional A-FABP typed in this part of the chromosome. Gerbens et al. (1998) reported that A-FABP was showed to be associated with IMF content, BFT, and growth in Duroc population, but no evidence was found for an effect of A-FABP gene on IMF or BFT in the later studies (Gerbens et al., 2000). Further studies are needed to determine whether the observed effects are due to the A-FABP genes or closely linked genes in our resource population.

As for other meat quality traits, Wang et al. (1998) reported suggestive QTL affecting meat colour on SSC4 and SSC7. De Koning et al. (2000a, 2000b) detected QTL for drip loss with maternal imprinted effects on SSC4. We are not able to confirm the QTL by Wang et al. (1998) and De Koning et al. (2000a, 2000b). Moreover, in order to test whether there could be more than a single QTL on a chromosome affecting the trait of interest, a grid search was used with two QTL fitted at all possible combination of 5 cM intervals on chromosome. No evidence for two QTL was found for any trait.

ACKNOWLEDGEMENTS

We wish to thank for the contribution of microsatellites primers provided by USDA-MARC freely. This study was supported financially by the National "973" Projects of P. R. China. (G2000016105) and National High Technology Development Project. The authors are also gratefully acknowledging teachers and graduate students in

Agricultural Ministry Key Laboratory of Swine Genetics and Breeding and Swine Breeding Center of China for cooperation.

REFERENCES

Andersson-Eklund, L., L. Marklund, K. Lundstrom, C. S. Haley, K. Andersson, I. Hansson, M. Moller and L. Andersson. 1998. Mapping quantitative trait loci for carcass and meat traits in a Wild boar×Large White intercross. J. Anim. Sci. 76:694-700

De Koning, D. J., L. L. G. Janss, A. P. Rattink, P. A. M. Van Oers, B. J. De Vries, M. A. M. Groenen, J. J. van der Poel, P. N. de Groot, E. W. Brascamp and J. A. M. van Arendonk. 1999. Detection of quantitative trait loci for backfat thickness and intramuscular fat content in pigs. Genetics 152:1679-1690.

De Koning, D. J., B. Harlizius, A. P. Rattink, J. A. M. van Arendonk and E. W. Brascamp. 2000a. Detection and characterization of quantitative trait loci for carcass and meat quality traits in pigs. Proc. Eur. Assoc. Anim. Prod. 6, 3

De Koning, D. J., A. P. Rattink, B. Harlizius, J. A. M. van Arendonk and E. W. Brascamp. 2000b. Genome-wide scan for body composition in pigs reveals important role of imprinting. Proc. Natl. Acad. Sci. USA. 97:7947-7950.

Gerbens, F., A. Jansen, A. J. M. Van Erp, F. L. Harders and T. H. E. Meuwissen, G. Rettenberger, J. H. Veerkamp and M. F. W. Tepas. 1998. The *adipocyte fatty acid-binding protein* locus: characterization and association with intramuscular fat content in pigs. Mamm. Genome. 9:1022-1026.

Gerbens, F., D. J. de Koning, F. L. Harders, T. H. E. Meuwissen, L. L. G. Janss, M. A. M. Groenen, J. H. Veerkamp, J. A. M. Van Arendonk and M. F. W. Tepas. 2000. The effect of *adipocyte fatty acid-binding protein* genes on intramuscular fat and backfat content in Meishan crossbred pigs. J. Anim. Sci. 78:552-559.

Green, P., K. Falls and S. Crooks. 1990. Document for CRIMAP, wersion 2.4. Washington Uni. School of Medicine. St. Louis, MO.

Haley, C. S., S. A. Knott and J. M. Elsen. 1994. Mapping quantitative trait loci in cross between outbred lines using least square. Genetics 136:1195-1207.

Malek, M., J. C. M. Dekkers, H. K. Lee, T. J. Bass and M. F. Rothchild. 2001. A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. Ⅱ: Meat and muscle composition. Mamm. Genome. 12:637-645.

Marklund, L., A. K. Wintero, P. D. Thomsen, M. Johansson, M. Fredholm, U. Gustafsson and L. A. Andersson. 1993. Linkage group on pig chromosome 4 comprising the loci for blood group L, GBA, ATP1B1 and three microsatellites. Anim. Genet. 24:333-338.

Marklund, L., M. Johansson-Moller, B. Hoyheim, W. Davis, M. Fredholm, R. K. Juneja, P. Marinai, W. Coppierters, H. Ellegren and L. Andersson. 1996. A comphrensive linakage map of the pig based on a wild pig -large white intercross. Anim. Genet. 27:225-269.

Meuwissen, T. H. E. and M. E. Goddard. 1996. The use of marker haplotypes in animal breeding schemes. Genet. Sel. Evol. 28(2):161-176.

Milan, D., J. P. Bidanal, P. Le Roy, C. Chevalet, N. Woloszyn, J. C. Caritez, J. Gruand, H. Lagant, M. B. Lefaucheur, C. Renard, M. Vaiman, P. Mormede, C. Desautes, Y. Amigues, F. Bourgeois, J. Gellin and L. Ollivier. 1998. Current status of QTL detection in Large White×Meishan crosses in France. Proceedings of the 6th World Congress on Genetics Applied to Livestock Production. 26:414-417.

Moser, G., E. Mueller, P. Beckmann, G. Yue and H. Geldermenn. 1998. Mapping of QTLs in F2 generations of Wild Boar, Pietrain and Meishan pig. Proceeding of the 6th World Congress on Genetics Applied to Livestock Production. 26: 478-481.

Ovilo, C., M. Perez-Enciso, C. Barragan, A. Clop, C. Rodriguez, M. A. Oliver, M. A. Toro and J. L. Noguera. 2000. A QTL for intramuscular fat and backfat thickness is located on porcine chromosome 6. Mamm. Genome. 11:344-340.

Rattink, A. P., D. J. De Koning, M. Faivre, B. Harlizius, J. A. M. van Arendonk and M. A. M. Groenen. 2000. Fine mapping and imprinting analysis for fatness trait QTLs in pigs. Mamm. Genome. 11:656-661.

Robic, A., J. Riquet, M. Yerle, D. Milan, Y. Lahbib-Mansais, C. Dubut-Fontana and J. Gellin. 1996. Porcine linkage and cytogenetic maps intergrated by regional mapping of 100 microsatellites on somatic cell hybrid panel. Mamm. Genome. 7:438-445.

Wang, L., T. P. Yu, C. K. Tuggle, H. C. Liu and M. F. Rothschild. 1998. A directed search for quantitative trait loci on chromosome 4 and 7 in the pig. J. Anim. Sci. 76:2560-2567.

Webb, A. J. 1998. Objective and strategies in pig improment and applied perspective. J. Dairy Sci. 81(Suppl. 2):36-46.

Wu, X. L., C. Lee, J. Jiang, Y. L. Peng, S. L. Yang, B. N. Xiao, X. C. Liu and Q. S. Shi. 2001. Mapping a quantitative trait locus for growth and backfat on porcine chromosome 18. Asian-Aust. J. Anim. Sci. 14:1665-1669.

Wu, X. L., C. Lee, J. Jiang, Y. L. Peng, H. F. Yan, S. L. Yang, B. N. Xiao, X. C. Liu and Q. S. Shi. 2002. Quantitative trait loci mapping for porcine backfat thickness. Asian-Aust. J. Anim. Sci. 15:932-937.

Xiong, Y. Z. and C. Y. Deng. 1999. Principle and method of swine testing. Chinese Agricultural Press, Beijing.

Zuo, B., Y. Z. Xiong, Y. H. Su, C. Y. Deng, R. Zheng, M. G. Lei and L. Yu. 2003. Mapping and genetic effect analysis of swine quantitative trait loci using 24 microsatellites. Acta Veterinaria et Zootechnica Sinica (China). In press.