A whole-genome scan for quantitative trait loci affecting teat number in pigs[1]

H. Hirooka*, D. J. de Koning†, B. Harlizius†, J. A. M. van Arendonk†, A. P. Rattink†, M. A. M. Groenen†, E. W. Brascamp†, and H. Bovenhuis†[2]

*Faculty of Economics, Ryukoku University, Kyoto, Japan, 617-8577 and
†Animal Breeding and Genetics Group, Wageningen Institute of Animal Sciences,
Wageningen University, 6700 AH Wageningen, The Netherlands

ABSTRACT: A whole-genome scan was conducted using 132 microsatellite markers to identify chromosomal regions that have an effect on teat number. For this purpose, an experimental cross between Chinese Meishan pigs and five commercial Dutch pig lines was used. Linkage analyses were performed using interval mapping by regression under line cross models including a test for imprinting effects. The whole-genome scan revealed highly significant evidence for three quantitative trait loci (QTL) affecting teat number, of which two were imprinted. Paternally expressed (i.e., maternally imprinted) QTL were found on chromosomes 2 and 12. A Mendelian expressed QTL was found on chromosome 10. The estimated additive effects showed that, for the QTL on chromosomes 10 and 12, the Meishan allele had a positive effect on teat number, but, for the QTL on chromosome 2, the Meishan allele had a negative effect on teat number. This study shows that imprinting may play an important role in the expression of teat number.

Key Words: Genome Analysis, Pigs, Quantitative Trait Loci, Teat Number

Introduction

Because the number of teats is an important trait with regard to the mothering ability of sows, the pig industry has traditionally applied selection pressure to teat number (Pumfrey et al., 1980). In particular, teat number plays an important role when the number is less than the litter size. Nevertheless, information about the inheritance of teat number is limited in comparison to other reproductive traits of pigs.

Recently, molecular genetic markers have made it possible to dissect quantitative trait variation and identify individual loci controlling economically important traits. Whole-genome scans with genetic markers offer the opportunity to reveal chromosome regions contributing to genetic variation and provide insight into the form of gene action. Using a whole-genome scan, Rathje et al. (1997) and Wilkie et al. (1999) reported QTL for reproduction traits in pigs. Wada et al. (1998) reported a putative QTL affecting teat number on *Sus scrofa* chromosome 3 (**SSC3**). More recently, Rohrer (2000) identified one significant and two suggestive QTL for teat number on SSC1, 3, and 10.

The Meishan breed has reproductive characteristics that differ from those of Western pigs. It is well known that Meishan pigs have more piglets per litter. Further, Haley et al. (1995) reported that the mean number of teats in the purebred Meishan breed was about 17.0, whereas mean teat numbers of about 14 are reported for the Large White breed (Clayton et al., 1981; Haley et al., 1995).

The objective of this study was to identify regions of the genome that contain genes affecting teat number and evaluate the effect of these genes.

Materials and Methods

Crossbreds (F_1 and F_2) between Chinese Meishan pigs and Dutch pig lines were available from an experiment involving five Dutch pig-breeding companies. Randomly selected boars and sows from the F_1 population were mated to produce the F_2 population (Janss et al., 1997; de Koning et al., 1999). A total of 1,173 F_2 animals with records on the number of morphological

normal teats (referred to as *teat number* in the remainder of this article) were used for a whole genome scan. The number of teats was established shortly after birth. The F$_2$ animals, their 291 F$_1$ parents, and 19 Meishan grandsires were typed for 132 microsatellite markers, which cover over 90% of the porcine genome.

DNA for genotyping was isolated from frozen blood or tissue. Genotyping of microsatellite markers was as previously described (Groenen et al., 1996; De Koning et al., 1999). Details about PCR reaction mixtures, PCR conditions, and multiplexes can be found in Groenen et al. (1996). Multipoint recombination fractions were calculated using CriMap version 2.4 (Green et al., 1990). Recombination fractions were transformed into map distances using the Haldane mapping function.

Preliminary analyses to assess the importance of genetic effects were performed. The phenotype data were analyzed assuming a polygenic inheritance model that included the fixed effect of sex. The heritability and variances were estimated using an animal model REML procedure (Gilmour et al., 1998) and using phenotypic observations on F$_1$ and F$_2$ animals.

The QTL analyses were based on the line-cross concept (Haley et al., 1994). This type of analysis has been applied to several crossbred pig populations (Andersson et al., 1994; Knott et al., 1998; de Koning et al., 1999). The additive QTL effect was defined as half the phenotypic differences between homozygous pigs for the QTL alleles originating from the Meishan and the Dutch lines. In this study, the additive effects were estimated for the Meishan QTL allele. Thus, positive values of the additive effects denote an increase of the trait due to the Meishan alleles. The dominant effects were estimated as the deviation of heterozygous pigs from the mean of the homozygous pigs. At every centimorgan (cM) across the genome, the following model was fitted:

$$y_i = \mu + ac_{ai} + dc_{di} + e_i \qquad [1]$$

where μ is the mean, c_{ai} is the coefficient for the additive component for individual i at the given location and c_{di} is the coefficient for the dominant component for individual i at the given location, a and d are the estimated additive and dominant effect of a putative locus at the given position, and e_i is the residual error.

Imprinting is the phenomenon that autosomal genes in the genome are inherited in a silent state from one of the two parents, causing monoallelic expression (Bartolomei and Tilghman, 1997). Based on the standard line-cross principles, systematic tests for imprinting have been suggested by Knott et al. (1998) and de Koning et al. (2000). In addition to the standard line-cross analysis, the approach of De Koning et al. (2000) was used to test the contribution of the paternally and maternally inherited effects. Under the imprinting model, the probability that the Meishan allele is transmitted from the father or from the mother was used to estimate the additive parental effect instead of the additive and

Table 1. Number of pigs of each sex in the F$_1$ and F$_2$, their mean number of teats and the standard deviation

Cross	Sex	n	Mean	SD
F$_1$	Male	38	15.5	0.9
F$_1$	Female	253	15.3	1.1
F$_2$	Male	516	15.4	1.2
F$_2$	Female	657	15.4	1.2

dominant effects in the standard line-cross analysis (see de Koning et al., 2000).

The genetic model for a putative QTL was evaluated by a standard *F*-test on the contribution of the individual variance components of the saturated model to the reduction in the residual variance component. Imprinting was inferred if only one of the parental contributions was significant and no dominance was present.

The significance thresholds were determined empirically by permutations described by Churchill and Doerge (1994). A total of 10,000 permutations were performed. The threshold used in this study was 5% genomewide threshold according to Lander and Kruglyak (1995).

Results

The mean teat number of F$_2$ individuals was 15.4 ± 1.2 (Table 1). The estimated heritability was 0.53 in the present study. This estimate was higher than published values from 0.07 to 0.42 (Clayton et al., 1981; McKay and Rahnefeld, 1990). In the present study, a cross between Meishan and Dutch lines was analyzed. If the F$_0$ animals were completely inbred, then all F$_1$ parents are expected to be identical and the estimated heritability would be low. The heritability estimate of 0.53, therefore, suggests that F$_0$ parents are not fixed at loci affecting teat number. For the interpretation of the heritability estimate of 0.53, it is further important to notice that heritability estimates for crossbreds are higher than those of purebreds, because a part of the dominance effects is attributed to additive effects (e.g., Besbes and Gibson, 1999).

The whole-genome scan using standard line-cross analysis showed genomewide evidence for QTL affecting teat number on SSC10 (Table 2). Under the line-cross model with imprinting, both the paternal and maternal components were highly significant, indicating Mendelian expression for this QTL. The most probable position on SSC10 was found around 106 cM (between markers SW920 and SW951). The results for the line-cross analysis considering imprinting showed that there was strong evidence for paternally expressed QTL on SSC2 and SSC12. The QTL on SSC2 was highly significant under the analysis considering imprinting, although this QTL did not exceed the genomewide threshold levels under the standard line-cross analysis (test statistic of 4.42). The most probable positions of the imprinted QTL on SSC2 and SSC12 were 2 cM

Table 2. Summary of estimated QTL effects for teat number

SSC	Inferred model[a]	Position, cM	df_n[b]	df_d[c]	F-ratio	Genomewide P-value[d]	Additive effect	Dominance effect	% of F_2 variance
2	Paternal expression	2	1	1,171	15.97	<0.001	−0.154 ± 0.039	—	1.3
10	Mendelian	107	2	1,170	17.14	<0.001	0.351 ± 0.063	−0.134 ± 0.110	2.8
12	Paternal expression	80	1	1,171	24.44	<0.001	0.197 ± 0.040	—	2.2

[a]Inferred genetic model for the putative QTL: only paternal alleles are expressed or both paternal and maternal alleles are expressed (Mendelian).

[b]Numerator degrees of freedom.

[c]Denominator degrees of freedom.

[d]Obtained by permutation.

(SW2443-SWC9) and 80 cM (S0090-S0106), respectively. Graphical representations of results for SSC2, SSC10, and SSC12 under standard and imprinting analyses are shown in Figures 1 to 3, respectively.

Table 2 shows the estimated effects for the significant QTL affecting teat number. The QTL affecting teat number on SSC10 was mainly of additive nature. The additive effect of 0.348 for the QTL on SSC10 indicates that animals inheriting two Meishan alleles at this locus had 0.70 more teats than those that inherited both alleles from the Dutch lines. The negative effect of the QTL on SSC2 was surprising because Meishan animals have a larger number of teats than commercial Western pig lines. This result indicates that the genes in the Meishan breed still carry alleles that have an unfavorable effect on teat number, as compared with the alleles
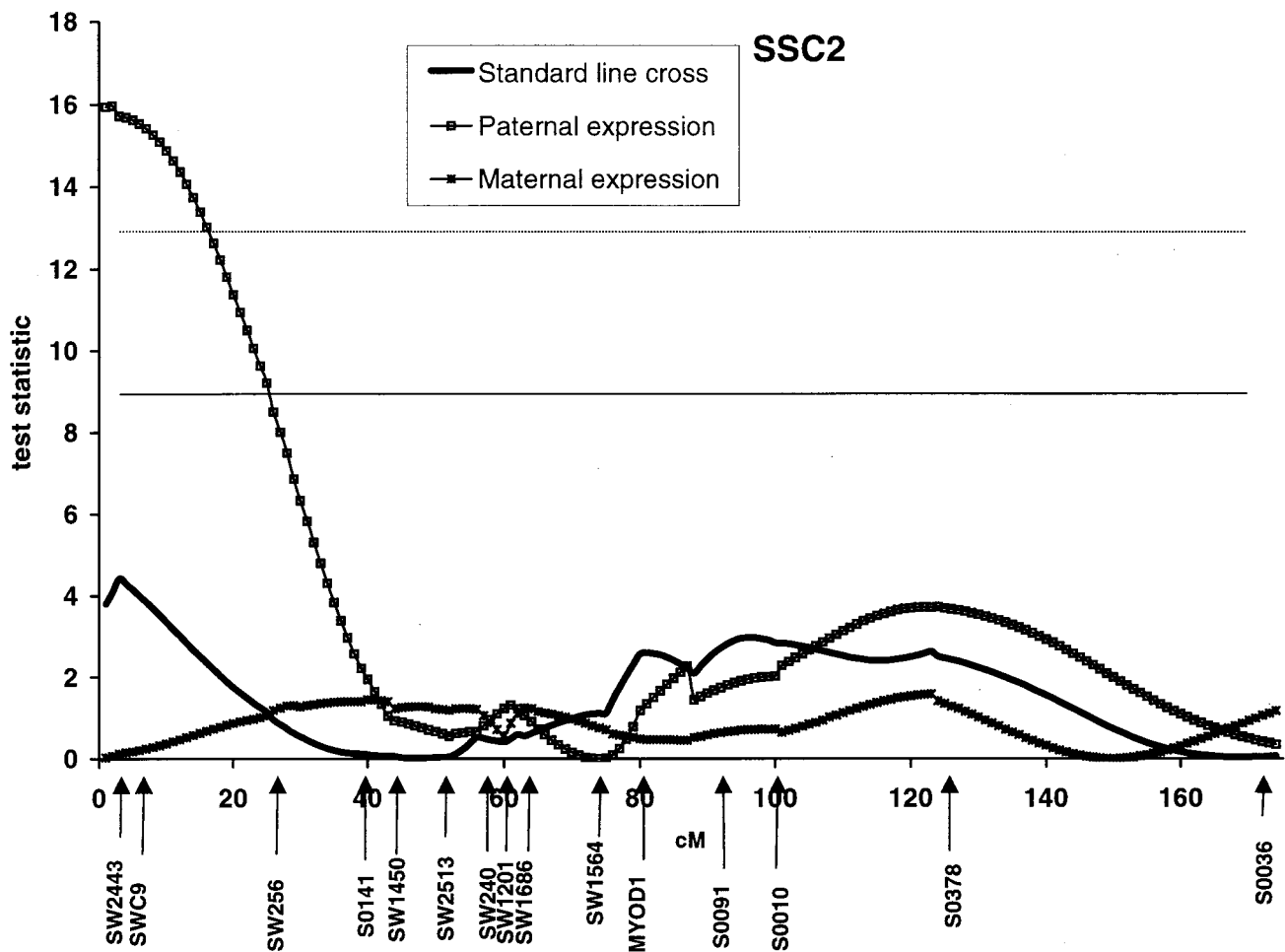

Figure 1. Test statistics for SSC2 with regard to teat number under standard (Mendelian), paternal expression, and maternal expression models. The horizontal lines denote the 5% genomewide thresholds for the standard (solid line) and the imprinting (dashed lines) models, and the arrows indicate the location of the genetic markers that were used.

present at the corresponding locus in the Dutch lines. In addition to the significant QTL effects, strongly suggestive evidence for a maternally expressed QTL was found on SSC3, which has its best position at 90 cM in the S0216–S0002 interval (genomewide *P*-value ~ 0.064).

Discussion

In this study, the data of F_2 individuals from a cross between the Meishan breed and Dutch pig lines were analyzed using a genomewide scan under line-cross analyses including a systematic test for imprinting, and thereby some convincing evidence for QTL affecting teat number was obtained.

Until now, only a limited number of reports have identified QTL for reproductive traits in pigs (Rothschild et al., 1996; Rathje et al., 1997; Wilkie et al., 1999). Pumfrey et al. (1980) reported that genetic and phenotypic correlations of teat number with reproductive traits were negative, although not significant. A negative correlation would suggest that some of the QTL affecting teat number, have antagonistic effects on other reproductive traits.

Wada et al. (1998) reported a QTL affecting teat number on SSC3. Rohrer (2000) reported suggestive QTL for teat number on SSC1 and SSC3 and a siginificant QTL on SSC10. In the present study, a suggestive QTL was located on SSC3, which seems to support the findings of Wada et al. (1998) and Rohrer (2000). Wada et al. (1998) do not give any information on the location of the QTL on SSC3, but the QTL reported by Rohrer (2000) is close to the most likely position found in our study (90 cM). A highly significant QTL was detected by Rohrer (2000) on SSC10. In the same chromosomal region, a significant QTL was detected in the present study. In our study, we could not confirm the QTL on SSC1 for which Rohrer (2000) found suggestive evidence.

In this study, genomewide significant imprinted QTL were found on two chromosomes (Table 2). In particular, the QTL on SSC2 was only significant under the analysis considering imprinting (Figure 1). As pointed out by de Koning et al. (2000), although genomic im-
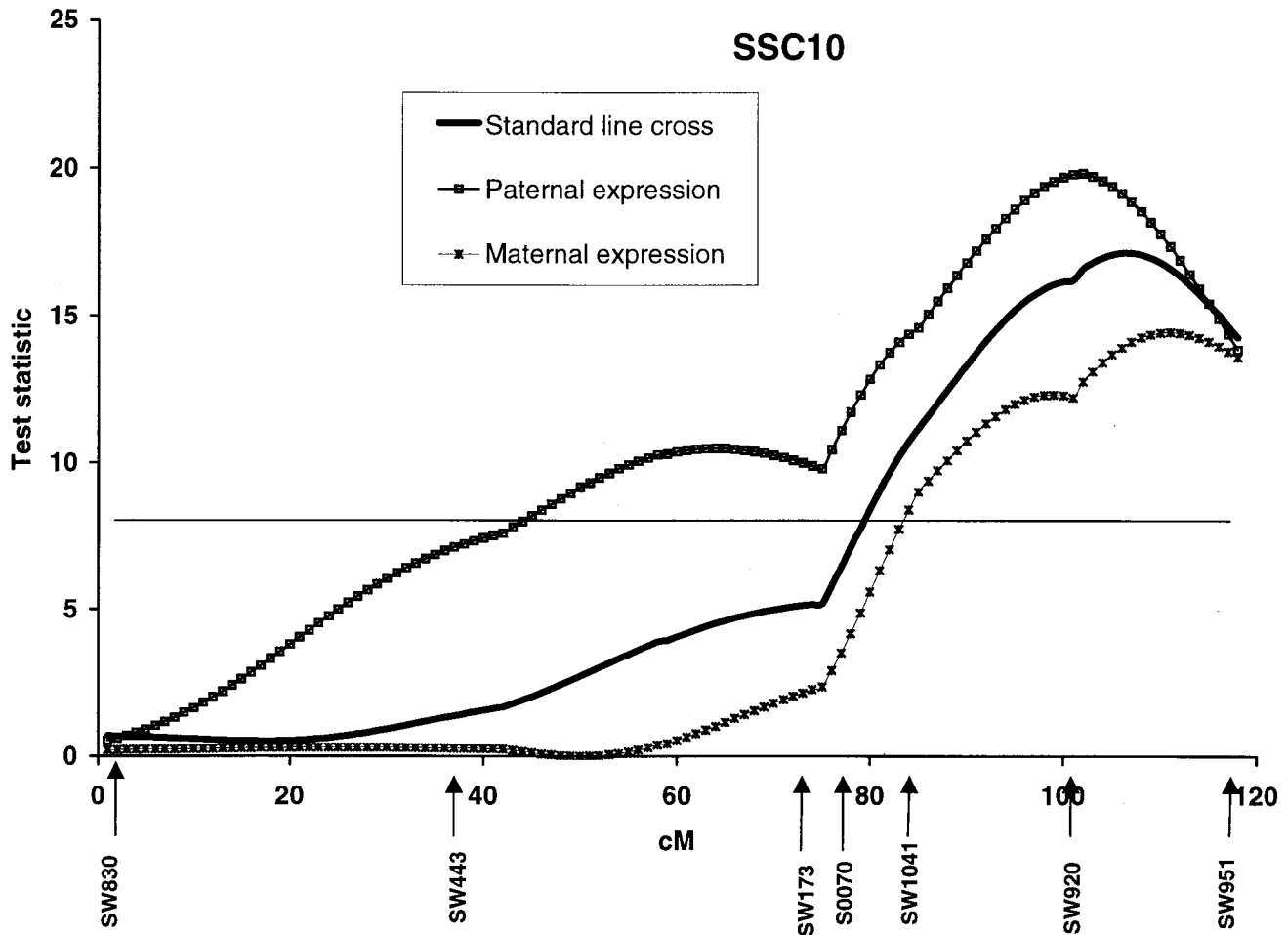

Figure 2. Test statistics for SSC10 with regard to teat number under standard (Mendelian), paternal expression, and maternal expression models. The horizontal lines denote the 5% genomewide thresholds for the standard (solid line) model, and the arrows indicate the location of the genetic markers that were used.
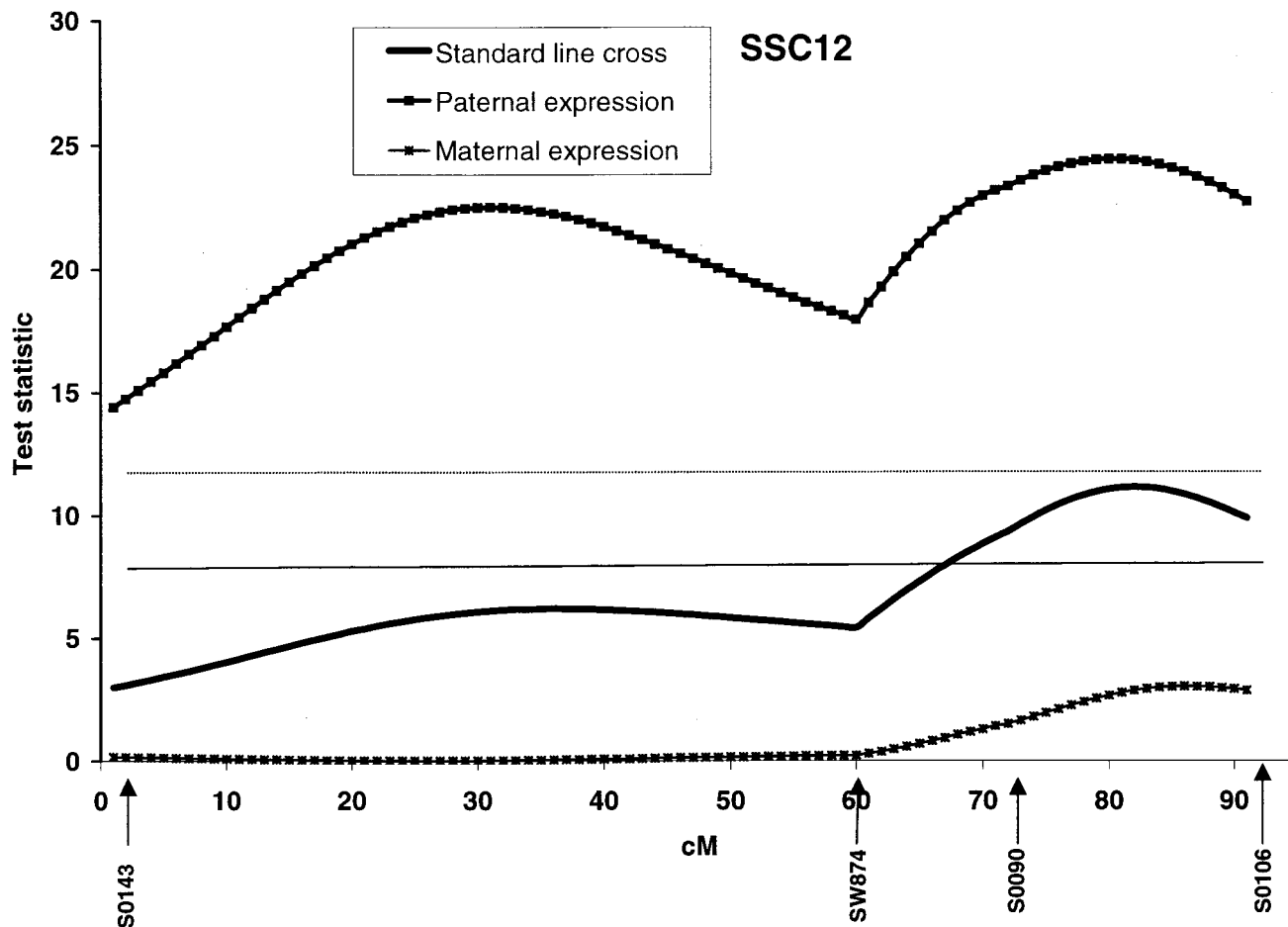
Figure 3. Test statistics for SSC12 with regard to teat number under standard (Mendelian), paternal expression, and maternal expression models. The horizontal lines denote the 5% genomewide thresholds for the standard (solid line) and the imprinting (dashed lines) models, and the arrows indicate the location of the genetic markers that were used.

printing has been regarded as a rare phenomenon and ignored in most studies, it might be more common than previously thought. Furthermore, de Koning et al. (2000) mentioned that analysis under different modes of expression (i.e., paternal and maternal) can increase the power of finding genes. Only a few reports have provided information on genomic imprinting effects in domestic animals. Using only phenotypic data, de Vries et al. (1994) were the first to show that genomic imprinting may influence the rate and composition of growth in pigs. Maternal imprinting could explain 5 to 7% of the phenotypic variation. Note that "maternal imprinting" as defined by de Vries et al. (1994) is actually paternal imprinting and maternal expression. De Vries et al. (1994) pointed out that it was difficult to discriminate between a maternal imprinting effect and a genetic maternal effect using quantitative genetic analyses because these two effects are almost completely confounded. Only analysis at the molecular level permit investigation of imprinting effects because it allows separating the maternally inherited alleles within a litter.

Recently, Jeon et al. (1999) and Nezer et al. (1999) found imprinted QTL affecting carcass traits in pigs in

the same region on SSC2 from experiments with an intercross between European wild boar and Large White and an intercross between Large White and Piétrain, respectively. The region on SSC2 detected in these studies was the same as that of the QTL on SSC2 for teat number found in the present study. More recently, de Koning et al. (2000) and Rattink et al. (2000) also found a paternally expressed QTL on SSC2 for backfat thickness in the same cross between the Meishan and commercial Dutch lines as described in this study. It is interesting to note that for different traits (i.e., carcass traits and teat number) paternally expressed QTL have been identified on SSC2. This might point toward the presence of one QTL influencing both traits or to different QTL that are both imprinted because all the genes located in this region are imprinted.

Comparative mapping between human (and also mouse) and pigs has been conducted in recent years to search for candidate genes for traits in pigs using the information derived from the human genome map (Pinton et al., 2000). The distal tip of porcine chromosome SSC2 has shown to be homologous to human chromosome (**HSA**) 11p, where a large cluster of imprinted genes is located. Among those, IGF2 has been mapped

to SSC2 and shown to be imprinted in pigs as well (Jeon et al., 1999; Nezer et al., 1999). The SSC12 is homologous to HSA17 in human and chromosome 11 in mouse. Although no imprinted genes have been reported for this chromosome in human, Sapienza et al. (1992) reported that the ovum mutant gene on mouse chromosome 11 is paternally expressed. Furthermore, although not significant at the genomewide 5% level, strong evidence for a paternally imprinted QTL was found on SSC3. The SSC3 shows homologies to HSA2, 7, and 16. Imprinted genes have been reported up to now, for only two areas on HSA7, a cluster on HSA7p21-p11 and on 7q32 (Morison and Reeve, 1998; Blagitko et al., 1999). Genes from these regions, however, map to SSC18, whereas the centromeric part of HSA7 in between is located on SSC9 (Pinton et al., 2000). Due to the low resolution of the comparative map for SSC12, the imprinted genes from HSA7 cannot be excluded as possible candidates for the QTL on SSC3.

There is no final answer about why some genes are imprinted and how the genes become imprinted. It is only known that methylation of cytosine in CpG islands plays an important role during development and is essential for genomic imprinting (Bartolomei and Tilghman, 1997). However, the presence of imprinting may lead to some important consequences for the practice of animal breeding. De Vries et al. (1994) pointed out that when a commercial product is based on a crossbreeding system with special sire and dam lines, selection in each line should take imprinted genes into account.

Implications

Teat number plays a significant role when there are more piglets than teats. The selection on litter size may require improvement of teat number. Evidence for quantitative trait loci (QTL) for teat number was found on chromosomes 2, 10, and 12, of which QTL on chromosomes 2 and 12 were imprinted. The markers associated with the significant QTL can be used in marker-assisted selection for teat number. The region on chromosome 2, for which a paternally expressed QTL was detected in this study, is reported as the IGF2 area with imprinted QTL affecting muscle mass and fat deposition. The result suggests that using the IGF2 locus for marker-assisted selection may affect traits other than the ones targeted for by marker-assisted selection. Furthermore, the existence of imprinted QTL opens new perspectives for the optimization of crossbreeding programs with specialized sire and dam lines.

Literature Cited

Andersson, L., C. S. Haley, H. E. Ellegren, S. A. Knott, M. Johansson, K. Anderson, L. Andersson-Eklund, I. Edfors-Lilja, M. Fredholm, I. Hasson, J. Hakansson, and K. Lundstrom. 1994. Genetic mapping of quantitative trait loci for growth and fatness in pigs. Science (Wash. DC) 263:1771–1774.

Bartolomei, M. S., and S. M. Tilghman. 1997. Genomic imprinting in mammals. Annu. Rev. Genet. 31:493–525.

Besbes, B., and J. P. Gibson J. P. 1999. Genetic variation of egg production traits in purebred and crossbred laying hens. Anim. Sci. 68:433–439.

Blagitko, N., U. Schulz, A. A. Schinzel, H. Ropers, and V. M. Kalscheuer. 1999. γ2-COP, a novel imprinted gene on chromosome 7q32, defines a new imprinting cluster in the human genome. Hum. Mol. Genet. 8:2387–2396.

Churchill, G.A., and R. W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138:963–971.

Clayton, G. A., J. C. Powell, and P. G. Hiley. 1981. Inheritance of teat number and teat inversion in pigs. Anim. Prod. 33:299–304.

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 (Sus Scrofa). Genetics 152:1679–1690.

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

De Vries, A. G., R. Kerr, B. Tier, and T. Long. 1994. Gametic imprinting effects on rate and composition of pig growth. Theor. Appl. Genet. 88:1037–1042.

Gilmour, A. R., B. R. Cullis, S. J. Welham, and R. Thompson. 1998. ASREML. Program User Manual. Oregon Agricultural Institute, Forest Road, OR.

Green, P., K. Fallis, and S. Crooks. 1990. Documentation for CRI-MAP version 2.4, Washington University School of Medicine, St. Louis, MO.

Groenen, M. A. M., B. J. de Vries, and J. J. van der Poel. 1996. Alignment of the PiGMap and USDA linkage maps of porcine chromosomes 3 and 9. Anim. Genet. 27:355–357.

Haley, C. S., S. A. Knott, and J. Elsen, 1994. Mapping quantitative trait loci in crosses between outbred lines using least squares. Genetics 136: 1195–1207.

Haley, C. S., G. J. Lee, and M. Ritchie. 1995. Comparative reproductive performance in Meishan and Large White pigs and their crosses. Anim. Sci. 60:259–267.

Janss, L. L. G., J. A. M. van Arendonk, and E. W. Brascamp. 1997. Baysian statistical analyses for presence of single genes affecting meat quality traits in a crossbred pig population. Genetics 145:395–408.

Jeon, J. T., O. Carlborg, A. Tornsten, E. Griuffra, V. Amarger, P. Chardon, L. Andersson-Eklund, K. Andersson, I. Hansson, K. Lundstron, and L. Andersson. 1999. A paternally expressed QTL affecting skeletal and cardiac muscle mass in pig maps to the IGF2 locus. Nature Genet. 21:157–158.

Knott, S. A., L. Marklund, C. S. Haley, K. Anderson, W. Davies, H. Ellegren, M. Fredholm, I. Hansson, B. Hoyheim, K. Lundstrom, M. Moller, and L. Andersson. 1998. Multiple marker mapping of quantitative trait loci in a cross between outbred wild boar and large white pigs. Genetics 149:1069–1080.

Lander, E. S., and L. Krugylak, 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nature Genet. 11: 241–247.

McKay, R. M., and G. W. Rahnefeld. 1990. Heritability of teat number in swine. Can. J. Anim. Sci. 70:425–430.

Morison, M., and A. E. Reeve. 1998. A catalogue of imprinted genes and parent-of origin effects in humans and animals. Hum. Mol. Genet. 7:1599–1609.

Nezer, C., L. Morean, B. Brouwers, W. Coppieters, J. Detilleux, R. Hanset, L. Karim, A. Krasz, P. Leroy, and M. Georges. 1999. An imprinted QTL with major effect on muscle mass and fat deposition maps to the IGF2 locus in pigs. Nature Genet. 21:155–156.

Pinton, P., L. Schibler, E. Cribiu, J. Gellin, and M. Yerle. 2000. Localization of 113 anchor loci in pigs: improvement of the com-

parative map for humans, pigs, and goats. Mamm. Genome. 11:306–315.

Pumfrey, R. A., R. K. Johnson, P. J. Cunningham, and D. R. Zimmerman. 1980. Inheritance of teat number and its relationship to maternal traits in swine. J. Anim. Sci. 50:1057–1060.

Rathje, T. A., G. A. Rohrer, and R. K. Johnson. 1997. Evidence for quantitative trait loci affecting ovulation rate in pigs. J. Anim. Sci. 75:1486–1494.

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.

Rohrer, G. A. 2000. Identification of quantitative trait loci affecting birth characters and accumulation of backfat and weight in a Meishan-White composite resource population. J. Anim. Sci. 78:2547–2553.

Rothschild, M., C. Jacobson, D. Vaske, C. Tuggle, L. Wang, T. Short, G. Eckardt, S. Sasaki, A. Vincent, D. McLaren, O. Southwood, H. van der Steen, A. Mileham, and G. Plastow. 1996. The estrogen receptor locus is associated with a major gene influencing litter size in pigs. Proc. Nat. Acad. Sci. USA 93:201–205.

Sapienza, C., J. Paquette, P. Pannunzio, S. Albrechtson, and K. Morgan. 1992. The polar-lethal ovum mutant gene maps to the distal portion of mouse chromosome 11. Genetics 132:241–246.

Wada, Y., T. Akita, T. Furukawa, N. Hisamatsu, Y. Ito, E. Kobayashi, M. Komatsu, H. Kusumoto, H. Mikami, S. Mikawa, M. Minezawa, M. Miyake, S. Shimanuki, T. Sugiyama, Y. Uchida, S. Yanai, and H. Yaue. 1998. Quantitative trait loci (QTL) analysis in a Meishen × Goettingen cross population. In: Proc. 6th World Cong. Genet. Appl. To Livest. Prod., Armidale, Australia 26:320–323.

Wilkie, P. J., A. A. Paszek, C. W. Beattie, L. J. Alexander, M. B. Weller, and L. B. Schook. 1999. A genome scan of porcine reproductive traits reveals possible quantitative trait loci (QTL) for number of corpora lutea. Mamm. Genome. 10:573–578.