BIOLOGY OF REPRODUCTION **68,** 2172–2179 (2003)
Published online before print 22 January 2003.
DOI 10.1095/biolreprod.102.012955

Mapping Quantitative Trait Loci Affecting Female Reproductive Traits on Porcine Chromosome 8[1]

Annemarie H. King,[2,4] Zhihua Jiang,[5] John P. Gibson,[3,5] Chris S. Haley,[4] and Alan L. Archibald[4]

Roslin Institute (Edinburgh),[4] Roslin, Midlothian, EH25 9PS, United Kingdom
Centre for Genetic Improvement of Livestock,[5] Department of Animal and Poultry Science, University of Guelph, Guelph, Canada N1G 2W1

ABSTRACT

An understanding of the genetic control of porcine female reproductive performance would offer the opportunity to utilize natural variation and improve selective breeding programs through marker-assisted selection. The Chinese Meishan is one of the most prolific pig breeds known, farrowing three to five more viable piglets per litter than the European Large White breed. This difference in prolificacy is attributed to the Meishan's superior prenatal survival levels. The present study utilized a three-generation cross in which the founder grandparental animals were purebred Meishan and Large White pigs in a scan for quantitative trait loci (QTL) on porcine chromosome 8 (SSC8) associated with reproductive performance. Reproductive traits, including number of corpora lutea (ovulation rate), teat number, litter size, and prenatal survival, were recorded for as many as 220 F_2 females. Putative QTL for the related traits of litter size and prenatal survival were identified at the distal end of the long arm of SSC8. A physiological candidate gene, *SPP1*, was found to lie within the 95% confidence interval of these QTL. A suggestive QTL for teat number was revealed on the short arm of SSC8. The present study demonstrates, to our knowledge, the first independent confirmation of QTL for fecundity on SSC8, and these QTL regions provide a crucial starting point in the search for the causal genetic variants.

embryo, gene regulation, gonadotropin-releasing hormone receptor, mammary glands, ovulation

INTRODUCTION

The key porcine reproductive traits, including ovulation rate, prenatal survival, and litter size, are expressed only in females and display low heritabilities [1]. Therefore, improvement of these traits in pigs by selective breeding has proved to be difficult. However, the substantial differences in reproductive performance between pig breeds indicate that useful genetic variation is available for investigation. For example, the European Large White and the Chinese Meishan breeds show marked differences in fecundity. The Meishan is one of the most prolific pig breeds known [2], farrowing three to five more viable piglets per litter than European breeds. Thus, Meishan pigs are assumed to carry alleles with the potential to enhance the reproductive performance of Western breeds [3].

Because prolificacy is expressed in one sex relatively late in life, it is a trait for which marker-assisted selection could be particularly beneficial [4, 5]. Information from genetic markers could be used to select males carrying desirable alleles for female reproductive performance and to select females without waiting for them to reach sexual maturity and have their first litter.

Two approaches have been pursued to identify genetic markers for reproduction traits. First, genome scans employing anonymous DNA markers have been used to identify quantitative trait loci (QTL) influencing these traits [6–8]. Second, physiological candidate gene approaches have utilized polymorphisms within or close to genes that are known to play a role during reproduction in tests for associations with fecundity [9–13].

Our strategy combines these two approaches, identifying QTL through genome scans using interval mapping and testing genes identified as candidates on both positional and physiological grounds. The proportion of pig genes that have been mapped is small; consequently, the number of positional candidate genes is limited. However, extensive conservation of genome organization exists between mammalian genomes, so the selection of positional candidates can be supplemented by genes predicted from comparative genome mapping information to map to the region of interest [14]. For example, porcine chromosome 8 (SSC8) is homologous to much of human chromosome 4 [15, 16], regions of sheep chromosome 6 [17], and regions of mouse chromosome 5 [18].

In the present study, we have focused on identifying QTL for reproductive performance on SSC8. Although earlier studies have provided evidence for QTL on this chromosome influencing several reproductive traits, none of these QTL has yet been confirmed across populations [6–8, 19–21]. We report here, to our knowledge, the first independent confirmation of reproductive QTL on SSC8 and eliminate some candidate genes on the basis of their map locations.

MATERIALS AND METHODS

Animals

Three separate Meishan × Large White cross-populations were developed at Roslin Institute over a period of 8 yr. These groups were defined as QTL 1, QTL 2.1, and QTL 2.2. The last two populations had a small number of grandparental individuals in common.

[1]The reproductive QTL project was funded by the U.K. Ministry of Agriculture, Fisheries and Food. A.H.K. was supported by a BBSRC Industrial CASE studentship with Sygen/PIC as the industrial partner; the Natural Sciences and Engineering Research Council of Canada and the GENEX Swine Group, Canada, also provided financial assistance. A.H.K. and Z.J. contributed equally to the genotyping work.
[2]Correspondence: Annemarie King, Department of Genomics and Bioinformatics, Roslin Institute, Roslin, Midlothian, EH25 9PS, U.K.
FAX: 44 131 440 0434; e-mail: annemarie.king@bbsrc.ac.uk
[3]Current address: International Livestock Research Institute, Kenya.

Received: 8 November 2002.
First decision: 13 December 2002.
Accepted: 9 January 2003.
© 2003 by the Society for the Study of Reproduction, Inc.
ISSN: 0006-3363. http://www.biolreprod.org

The purebred Meishan pigs were derived from an importation of 11 males and 21 females from the Jiadan county pedigree on the Lou Tang research farm in China in 1987 [22]. The first animals used in the present study were second-generation descendants of these imports. The purebred Large White pigs were from a control population derived from a broad sample of genotypes in 1982 [23].

In all populations, F_1 reciprocal crosses were produced (Meishan male × Large White female, and Large White male × Meishan female), and all F_0 animals were unrelated [24]. From the subsequent F_1 generation, boars were mated to sows of a different grandparental pairing. The resulting F_2 female offspring were mated to one of a few selected purebred Large White boars, and various reproductive traits were recorded. In total, the present study included 35 F_0 (13 males and 22 females), 94 F_1 (14 males and 80 females), and 220 F_2 (all female) individuals.

The animals had a minimum live weight of 85 kg at the start of each trial, and they were reared indoors on standard commercial growth rations provided ad libitum until the time scheduled for first mating (for more detail, see [22]).

Matings for each of the F_2 individuals in the three different year groups took place in two 6-wk periods. Gilts in the first age group (age group 1) were 8–11 mo of age, corresponding to the animal's first parity. They were then remated at 13–17 mo of age. Individuals in this second age group (age group 2) mostly had their second parity; a few who had an unsuccessful first mating had their first litter at this later age. All sows were observed daily for signs of estrus and were mated on the same day as detection.

Phenotypic Data Recording

At 5–20 days after mating, the number of corpora lutea on the left and right ovaries was recorded by laparoscopy and used as an estimate of ovulation rate [25]. At laparoscopy, the weight of the animal was recorded. In addition, the number of teats on the right- and left-hand sides of each gilt was counted. Some sows then returned to estrus and, if they were still within the 6-wk mating period, were remated. For those animals successfully remated in this manner, no record exists of the corresponding number of corpora lutea, because the mating occurred after laparoscopy. These procedures were repeated for the same animals approximately 5 mo later. Table 1 shows the mean, range, and SD of values recorded for each trait and covariate.

Prenatal survival was calculated as the number of piglets born divided by the total ovulation rate for those individuals in which their farrowing records corresponded to the ovulation rate recorded. Gestational length (days) was calculated as the difference between the age of the sow at mating and her age at farrowing. Analyses on the trait data were carried out within age groups, and the variance within each trait was calculated to confirm that the data displayed a normal distribution, an assumption of the QTL analysis. Individuals with incomplete records were removed from the analysis.

Genotyping

The DNA was prepared by standard procedures from spleen tissue collected postmortem. The genotypes of the F_2 trait-recorded females, their F_1 parents, and their purebred grandparents were determined for 21 polymorphic genetic markers (Table 2).

For each microsatellite marker, the allelic DNA fragments were amplified from 75-ng aliquots of genomic DNA in 15-μl reaction volumes containing 7.5 pmol of each primer, 2.0 mM of each dNTP, 1.5 mM $MgCl_2$ in 1× polymerase chain reaction (PCR) buffer, and 0.375 U of *Taq* DNA polymerase. When additional magnesium was necessary in the reaction mix, the required amount of double-distilled water was substituted with 25 mM $MgCl_2$. The PCR amplifications were performed using either a Hybaid Omnigene or Touchdown thermocycler (Ashford, U.K.). Appropriate dilutions of PCR products for microsatellite markers were pooled along with a 350 Tamra size standard (Applied Biosystems, Warrington, U.K.) and fractionated on 6% polyacrylamide gels on an ABI 373 DNA sequencer (Applied Biosystems). The sizes of the allelic fragments were estimated using the ABI GeneScan 2.1 software (Applied Biosystems).

The PCR-restriction fragment length polymorphism (RFLP), bi-directional PCR amplification of specific allele (Bi-PASA), and PCR-double-stranded conformational polymorphism (DSCP) techniques were used to genotype markers in the porcine *AREG, FGG, IBSP, GNRHR, HD, QDPR, SLIT2, SPP1,* and *STE* genes. The PCR reactions were performed on approximately 50 ng of genomic DNA as templates in a final volume of 10 μl containing 3 pmol of each primer, 200 nM dNTPs, 2.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100, and 0.5 U of *Taq* polymerase. After denaturation at 94°C for 3 min, 30 amplification cycles were performed consisting of denaturation at 94°C for 30 sec, annealing at 61°C or 57°C for 30 sec, and extension at 72°C for 30 sec, followed by a further 5-min extension at 72°C. In the PCR-RFLP assays, 5 μl of PCR products were digested with 5 U of *Sty*I for the porcine *AREG* gene, *Ava*II for *HD*, *Bfa*I for *QDPR,* and *Bst*NI for *SLIT2* gene, respectively. Pairs of allele-specific primers were designed for each marker in the porcine *FGG, IBSP, GNRHR,* and *SPP1* genes for use with the technique of Bi-PASA genotyping [35]. Both PCR-RFLP and Bi-PASA products were then examined by electrophoresis on 1.5% agarose gels with 1× Tris-borate EDTA buffer. The PCR products of the *STE* gene were examined using 8% acrylamide gels. The gels were stained with ethidium bromide and photographed.

All genotypes were entered into a resSpecies database (http://www.resSpecies.org) through a data submission tool that checks for inheritance errors in the data.

Linkage Map Construction

Genotypes from all 21 markers were used to produce a linkage map of SSC8 using MultiMap [36]. The resulting linkage map was checked using the chrompic option in CRI-MAP version 2.4 (http://compgen.rutgers.edu/multimap/crimap) to highlight potential genotyping errors involving double-recombinants occurring within short map distances (i.e., $<$5 cM). When these occurred, the genotypes were corrected or removed, and the analysis was repeated. The linkage map developed was then used for the QTL scan (discussed below).

QTL Scan

The method for QTL analysis of a three-generation pedigree derived from a cross between outbred lines involving the use of regression-based interval mapping [37] was effected using the QTL Express web interface (http://qtl.cap.ed.ac.uk) [38]. A "fixed QTL allele" model, in which genetically distinct founder lines, in this case Meishan and Large White pigs, were assumed to be fixed for alternative alleles at the QTL affecting the traits of interest was used.

Each reproductive trait measured for animals in age groups 1 and 2 was investigated individually for evidence of single QTL on SSC8. Teat number was only considered for the younger age group. When traits were measured individually on the left- and right-hand sides of the animal, only the sum of left- and right-hand values were investigated. To develop the model for each QTL analysis, the effect of the covariates (i.e., age at mating, weight at laparoscopy, and length of gestation) on the individual reproductive traits measured were investigated by the use of stepwise multiple-regression analysis using Minitab statistical software (release 13.32; http://www.minitab.com).

For all QTL analyses, experimental group (i.e., QTL 1, QTL 2.1, and QTL 2.2) was included as a fixed effect. Animals classed into age group 2 also had the parity of the sow (i.e., 1 or 2) included as a fixed effect.

Initially, the additive and dominant coefficients were calculated for each marker, and the mean of these two coefficients was used to determine the information content of each individual marker. By using all the marker information simultaneously, the genetic information content (i.e., the amount of information available to determine the grandparental or breed origin of each allele) was estimated at 1-cM intervals along the whole chromosome [38]. The information content varies between 0 and 1, where a value of 1 means that the grandparental/breed origin is known with certainty.

Using ordinary least squares, the phenotypic values were regressed onto the additive and dominant coefficients to estimate the additive and dominance effects of putative QTL at 1-cM intervals through the chromosome. The ratio of regression mean square to the residual mean square provided the variance (F) ratio test statistic, and the most likely QTL position was taken at the maximum value of F along the chromosome.

A single position permutation analysis was initially carried out, using 1000 permutations of the trait data, to determine the maximum nominal significance level of potential QTL for each trait. A chromosome-wide permutation analysis with 1000 permutations was then carried out to determine whether the QTL were significant ($P < 0.05$) at the chromosomal level, which is approximately equivalent to a suggestive QTL at the genome-wide level [39]. The additive and dominance effects were fitted simultaneously in the initial QTL analyses. Either the additive or dominance effects or both must be significant to generate a significant F value for the QTL. To determine which effects underpin the QTL, the estimates of the additive and dominance effects of each QTL were tested for significance ($P < 0.05$) by the use of a two-tailed Student t-test.

TABLE 1. Range, mean (±SEM), and standard deviation (SD) of values for each trait and covariate recorded.

Tratis recorded	Age group 1 (n = 169)[a]			Age group 2 (n = 144)[a]		
	Range	Mean (±SEM)	SD	Range	Mean (±SEM)	SD
Ovulation rate (right ovary)	0:15	7.7 (0.2)	3.1	3:18	9.0 (0.3)	3.1
Ovulation rate (left ovary)	1:21	9.4 (0.3)	3.3	1:18	9.4 (0.3)	3.0
Total ovulation rate	9:31	17.1 (0.3)	3.5	10:30	18.4 (0.3)	3.7
Number teats on right side	6:9	7.5 (0.1)	0.8	—	—	—
Number teats on left side	6:9	7.5 (0.1)	0.8	—	—	—
Total number teats	12:18	15.0 (0.1)	1.3	—	—	—
Litter size	2:22	12.2 (0.3)	3.6	1:22	12.8 (0.3)	3.4
Prenatal survival	0.1:1.0	0.7 (0.0)	0.2	0.1:1.0	0.7 (0.0)	0.2
Covariates						
Age at mating (days)	248:357	300.7 (1.8)	22.8	402:559	491.6 (3.0)	35.8
Age at farrowing (days)	360:469	414.9 (1.8)	22.8	517:673	606.0 (3.0)	35.9
Gestation Length (days)	108:119	114.1 (0.1)	1.6	111:118	114.4 (0.1)	1.5
Weight at laparoscopy (kg)	90:195	141.5 (1.6)	20.6	110:245	169.4 (2.3)	27.6

[a] n = Numbers of animals. Note that calculations were only on those animals that had complete trait records.

Gene-Association Analysis

Genetic markers within or close to several genes (*GNRHR, IBSP, STE, AREG, SPP1, SLIT2, QDPR, FGG,* and *HD*) were used to test for evidence of within-breed, marker-associated variation for the reproductive traits of interest.

In the initial QTL analyses, it was assumed that the two founder breeds were fixed for alternative alleles at the QTL. To the extent that the gene-associated markers have allele frequencies that differed between the two founder breeds, an analysis looking for trait associations with a gene-associated marker may be declared to be significant just because it explains some of the effect of a breed-associated QTL. However, some of the gene-associated polymorphisms are not fixed for alternative alleles in the founder breeds and, therefore, can be used as an additional fixed effect in a QTL analysis to test for within-breed variation associated with the gene marker. These tests for within-breed variation were achieved by including the genotypes of each gene-associated marker as a fixed effect in the ''fixed QTL allele'' model as used previously. A resulting significant reduction in the residual mean square (i.e., a better fit of the data to the revised model) would indicate variation in the trait of interest associated with the gene marker over and above any resulting from the QTL. All gene-associated markers investigated were biallelic. Individuals homozygous for one of the alleles were coded as 1, heterozygous individuals as 2 and homozygous animals for the alternative allele as 3.

In theory, similar analyses could also be implemented for each of the microsatellite markers. However, there are many more genotypic classes for multiallelic markers such as microsatellites, and the number of animals in each class would be too small.

An *F* test was used to determine whether the model with these gene-associated markers fitted as fixed effects gave a significantly better fit to the data than the initial ''fixed QTL allele'' model. The value for the residual sum of squares calculated for the full model of interval mapping (i.e., the effect of the QTL, covariates, and fixed effects) with the gene fitted as a fixed effect was compared to the equivalent value for the ''fixed QTL allele'' model. The following calculation was used:

$$F = \frac{(RSS_f - RSS_g)/(df_f - df_g)}{RSS_g/df_g}$$

where RSS = the residual sum of squares for the full model of interval mapping for the ''fixed QTL allele'' model ($_f$) and the gene fitted as fixed effect model ($_g$) and df = degrees of freedom (numerator: $df = df_f - df_g$; denominator: $df = df_g$).

For those models that revealed evidence of trait variation associated with the gene of interest, it was possible to investigate whether significant variation in the effect on the trait occurred between any two genotypic classes for the gene of interest (e.g., whether homozygous animals differed from heterozygous animals at a particular locus). The significance of variation between genotype classes was tested using a two-tailed Student *t*-test.

RESULTS

Linkage Map

The linkage map developed (Fig. 1) was in close agreement, in terms of length and marker order, with the map of SSC8 produced by Rohrer et al. [40]. The individual information content of each marker along the chromosome and the information content at 1-cM intervals using simultaneous marker analysis are shown in Figure 1. For the QTL analysis, simultaneous marker information was used, and it can be seen that the information available to determine the QTL genotype at any one point along the chromosome was greater than 0.5, with the lowest area of information content on the p arm of the chromosome, around the marker *SW268.*

QTL Scan

The stepwise multiple-regression analysis revealed that the weight of the animal had a significant effect on the total ovulation rate for animals in both age group 1 ($P < 0.001$) and age group 2 ($P < 0.01$). Animals of greater weight had increased ovulation rates. For animals in age group 1 only, the weight of the animal and the length of gestation significantly ($P < 0.05$) affected litter size. Individuals with shorter lengths of gestation had larger litters, and in accordance with ovulation rate, heavier animals had larger litters. Again, for animals in age group 1 only, length of gestation had a significant effect ($P < 0.05$) on prenatal survival. Individuals with shorter gestation periods also had higher levels of prenatal survival. None of the other variables for animals in either age group had a significant effect on the other traits.

The QTL analyses were carried out separately for each trait, for animals in both age groups, fitting the relevant covariates and fixed effects. The number of animals with genotype data and information available for each trait varied, depending, for example, on whether the ovulation rate recorded for an F_2 individual corresponded to the subsequent litter that was born (Table 3). The estimated QTL locations and the corresponding significance levels are summarized in Table 3. The chromosome-wide permutation analyses revealed two putative QTL for prenatal survival ($P < 0.05$) and number of teats ($P < 0.05$) for animals in age group 1 (i.e., first-parity animals mated at 8–11 mo of age). A QTL for litter size, which is significant only at the nominal level ($P < 0.01$), was colocated with the prenatal survival QTL. Table 4 shows the estimates of the genetic effects for those QTL as being significant above the nominal level.

The interval mapping plots for prenatal survival and the related trait of litter size are shown in Figure 2 for animals

TABLE 2. PCR conditions for each primer pair.

Marker	Marker type	Reference
Anonymous DNA markers		
S0017	Microsatellite	[26]
S0178	Microsatellite	[27][a]
S0225	Microsatellite	[28][b]
SW7	Microsatellite	[29]
SW61	Microsatellite	[29][c]
SW268	Microsatellite	[29]
SW905	Microsatellite	[29]
SW2410	Microsatellite	[30]
SW2611	Microsatellite	[30]
Gene-associated markers		
AREG	PCR-RFLP (*Sty*I)	
FGG-2	Bi-PASA	[31]
IBSP	Bi-PASA	[31]
GNRHR-1	Bi-PASA	[32]
GNRHR-2	Bi-PASA	[11]
HD	PCR-RFLP (*Ava*II)	[11]
QDPR	PCR-RFLP (*Bfa*I)	[32]
SLIT2	PCR-RFLP (*Bst*NI)	[32]
SPP1–1	Microsatellite	[33][d]
SPP1–4	PCR-RFLP	[34]
SPP1–5	Bi-PASA	[32]
STE	PCR-DSCP	[31]

[a] Conditions that gave more consistent results for individual markers varied from the published data as follows: 1.5 mM magnesium; denaturation cycle of 94°C for 5 min; annealing phase of 30 cycles of 94°C, 58°C , and 72°C, each for 30 sec; and extension cycle of 72°C for 5 min.
[b] Conditions that gave more consistent results for individual markers varied from the published data as follows: 1.5 mM magnesium; denaturation cycle of 94°C for 5 min; annealing phase of 30 cycles of 94°C, 55°C, and 72°C, each for 30 sec; and extension cycle of 72°C for 5 min.
[c] Conditions that gave more consistent results for individual markers varied from the published data as follows: 2.0 mM magnesium; and 35 cycles of annealing at 60°C.
[d] Conditions that gave more consistent results for individual markers varied from the published data as follows: 1.5 mM magnesium; denaturation cycle of 94°C for 5 min, 57°C for 30 sec, and 72°C for 1 min; annealing phase of 30 cycles of 94°C for 45 sec, 55°C for 30 sec, and 72°C for 45 sec; and extension cycle of 72°C for 5 min.

in age group 1. A clear peak was observed in the F values calculated between markers *SW61* and *S0178*, close to the microsatellite marker 5′ of the *SPP1* gene at the distal end of the long arm of porcine chromosome 8 (SSC8q).

Figure 3 shows the interval mapping plot for the QTL controlling the number of teats (black line). The QTL, which is significant at the chromosome-wide level ($P < 0.05$), is located near the *SLIT2* gene on the short arm of porcine chromosome 8 (SSC8p).

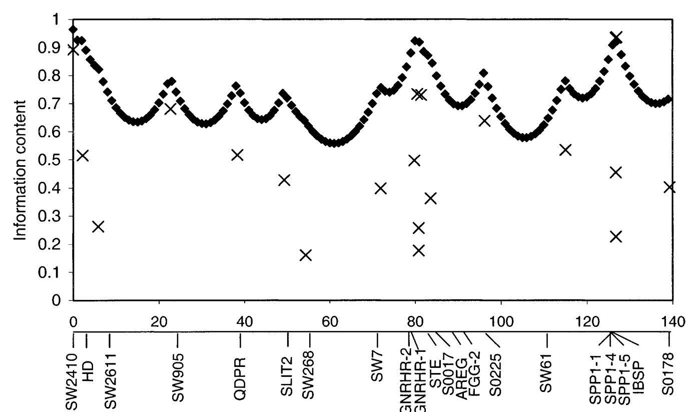

FIG. 1. Individual information content of each marker along the chromosome (×) and also at 1-cM intervals using simultaneous marker information (♦). The *x*-axis shows the position of markers on the linkage map alongside the distances (in cM) on pig chromosome 8 (total length, 139.3 cM).

Association Analyses Fitting Candidate Gene Marker Genotypes as Fixed Effects

Because most of the biallelic gene loci were not fixed in the founder breeds, they could be used to test for within-breed variation. The allele frequencies in the founder breeds at each of these loci are shown in Table 5.

For ovulation rate in age group 1 animals, *GNRHR-2* was the only genetic marker that resulted in a significant improvement ($P < 0.01$) in the fit of the model when added to the ‘‘fixed QTL allele’’ model. Under this extended model, evidence was found of a QTL for ovulation rate (F = 3.01) at approximately 3 cM. However, this QTL is only significant at the nominal level ($P < 0.05$). A second peak ($F = 2.5$) was observed at approximately 80 cM (i.e., close to the *GNRHR* gene; not shown); however, the F values calculated at this location were slightly lower than the nominal significance level ($P > 0.05$). The effect of the ovulation rate QTL at position 3 cM was additive ($P < 0.05$), with an estimate of +0.71 ova ± 0.33 (mean ± SEM) per Large White allele. The effects of the putative QTL at approximately 80 cM were also seen to be additive with the increasing allele inherited from the Large White founders. The Student *t*-test revealed that, when the *GNRHR-2* genotypes were included as a component in a fixed QTL model, individuals homozygous for allele *1* had an estimated increase of 3.41 ± 1.47 ova ($P < 0.05$) compared to individuals homozygous for allele *2*.

TABLE 3. The estimated QTL locations for all traits and the corresponding significance levels.

Trait	Number F_2 animals[a]	Position on SSC8	F-ratio	Significance level (P)
Age group 1				
Total ovulation rate	179	2 cM	1.96	N.S.[b] (> 0.05)
Total number teats	193	49 cM	5.21	Chromosome wide (< 0.05)
Litter size	152	127 cM	4.79	Nominal (< 0.01)
Prenatal survival	152	125 cM	6.84	Chromosome wide (< 0.05)
Age group 2				
Total ovulation rate	153	139 cM	1.39	N.S.[b] (> 0.05)
Litter size	134	38 cM	2.09	N.S.[b] (> 0.05)
Prenatal survival	134	38 cM	2.70	N.S.[b] (> 0.05)

[a] Number of F_2 animals = number of individuals with both genotype and phenotype records.
[b] N.S., Nonsignificant ($P > 0.05$ at nominal level).

TABLE 4. Estimates of the genetic effects for those QTL significant above the nominal level[a]

Trait	Addivtive effect[b]	(±SEM)	P	Dominance effect[c]	(±SEM)	P
Age group 1						
Total number teats	+0.25 teats	(0.14)	> 0.05	**+0.58 teats**	**(0.20)**	$< \mathbf{0.01}$
Litter size	+0.32 pigs	(0.42)	> 0.1	**−1.80 pigs**	**(0.60)**	$< \mathbf{0.01}$
Prenatal survival	+0.02 (2%)	(0.02)	> 0.1	**−0.11 (11%)**	**(0.03)**	$< \mathbf{0.001}$

[a] Those effects significant above the 95% confidence level are highlighted in bold.
[b] Additive effect is estimated as half the difference between the homozygotes for Meishan versus Large White alleles.
[c] Dominance effect is estimated as the deviation of the heterozygotes from the mean of the homozygotes.

Adding genetic markers associated with the genes *AREG* and *SLIT2* as fixed effects resulted in significant improvements ($P < 0.005$) in the model fit when compared to the ''fixed QTL allele'' model for teat number. Including *AREG* genotypes in the analysis model improved the evidence for the teat number QTL close to *SLIT2* on SSC8p, increasing the maximum F from 5.21 to 8.84 (Fig. 3). This estimate of the QTL is significant at the equivalent of a genome-wide level ($P < 0.01$ at the chromosome-wide level). The additive effect of the QTL ($P < 0.01$) was estimated as an increase in 0.49 ± 0.16 teats per copy of the Meishan allele. The dominance effect was also significant ($P < 0.01$), with an estimate of $+0.64 \pm 0.20$ teats. Individuals who were homozygous for allele *2* at the *AREG* locus were significantly different ($P < 0.05$) from individuals homozygous for allele *1*, with an estimated increase of 0.68 teats $\pm$ 0.27 teats.

When the genotypes of the *SLIT2* locus were included as fixed effects, the QTL was no longer evident. It can be seen from Figure 3 that the peak F value is directly above the location of the *SLIT2* gene, and the difference in frequency of *SLIT2* alleles between the lines is such that the *SLIT2* genotypes are confounded with the breed origin.

The genetic markers SPP1-5 and *IBSP*, which are located close to the peaks for the litter size and prenatal survival QTL identified under the ''fixed QTL allele'' model for age group 1 sows (Fig. 2), are fixed for alternative alleles in the founder breed (Table 5). Thus, when these markers are added as fixed effects, they are simply confounded with the breed origin, and the evidence for the QTL is eliminated.

DISCUSSION

We found evidence for QTL toward the telomere of the q arm of SSC8 controlling prenatal survival and litter size in young sows at first parity. The effects of these QTL were both negative overdominant (i.e., the heterozygotes show inferior performance to both classes of homozygotes). Although the additive effects were not significant, the beneficial alleles at this QTL appear to be from the Meishan breed. Such effects of the Meishan alleles at these QTL would be consistent with previous observations that the Meishan delivers its superior litter size through higher levels of prenatal survival for a given ovulation rate [3, 41]. However, evidence of positive overdominance has been reported in earlier studies [3].

Evidence was also found for a QTL on SSC8 for teat number, the trait for which we had the most recorded animals. A QTL, significant at the chromosome-wide level, was detected on the p arm of SSC8 around the *SLIT2* locus, with the increasing alleles coming from the Meishan breed. Interestingly, when the genotypes at the *AREG* genetic marker were added to the model, the evidence for the QTL became stronger. The *AREG* polymorphism is close to fixation for alternative alleles in the Large White and Meishan founders (Table 5), with allele *2* only being present in the Large White, and individuals homozygous for allele *2* at this locus were shown to have an increase estimate of teat number. The effects at the QTL (close to *SLIT2*) and around *AREG* are acting in opposite directions, with the increasing allele associated with the Meishan and Large White, respectively, at these two locations. Thus, the improvement in the support for the QTL in the extended model may be largely a result of fitting a two-locus model (i.e., a QTL plus *AREG* as a second locus) that reduces the interference from two QTL with opposing effects. Indeed, when a two-QTL model was investigated, evidence suggested that two QTL were acting in opposite directions, one at approxi-

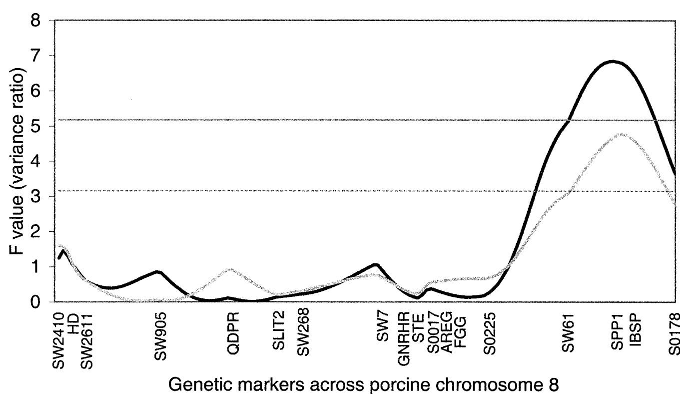

FIG. 2. Interval mapping of prenatal survival (thick black line, n = 152) and litter size (gray line, n = 152) on porcine chromosome 8 for animals in age group 1. Nominal significance level (dashed horizontal line, $P < 0.05$) and chromosome-wide significance level (solid horizontal line, $P < 0.05$).

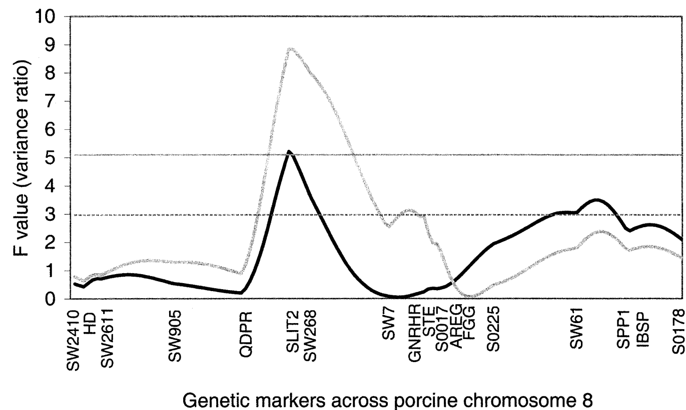

FIG. 3. Interval mapping of number of teats (thick black line, n = 193) and where genotypes at the *AREG* loci were fitted as a fixed effect (gray line, n = 193) on porcine chromosome 8 for animals in age group 1. Nominal significance level (dashed horizontal line, $P < 0.05$) and chromosome-wide significance level (solid horizontal line, $P < 0.05$).

mately 49 cM (near *SLIT2*), with the increasing allele associated with the Meishan, and one at 100 cM (near *AREG*), with the increasing allele associated with the Large White (unpublished data).

The *SLIT2* gene is of interest because it maps directly below the peak for the QTL with the positive alleles from the Meishan breed. The *SLIT2* is a homologue of the *Drosophila slit* gene, which plays a critical role in central nervous system midline formation during embryogenesis [42]. The human homologue of this gene is expressed in the spinal cord, and it is believed that mammalian SLIT proteins may participate in the formation and maintenance of the nervous and endocrine systems by protein-protein interactions [43]. Therefore, no other data suggest that this gene plays a role in determining teat number.

Number of teats has not been investigated in most previous porcine reproductive QTL studies [6–8, 21]. However, evidence for a QTL affecting number of teats also on the p arm of SSC8 at the genome-wide significance level ($P < 0.05$) has been reported [20]. Hirooka et al. [44] found strong evidence for teat number QTL on chromosomes 10 and 12 in a Meishan × Dutch cross, with the beneficial alleles from the Meishan breed and a QTL on chromosome 2 with a negative effect of the Meishan allele. In addition, Rohrer [45] found significant evidence for a QTL for teat number in a Meishan × Large White cross, also on porcine chromosome 10. Neither of these studies found evidence for QTL on SSC8 influencing teat number.

In agreement with the study by Rohrer et al. [8], which also utilized a Meishan × Large White cross, we found some evidence for a QTL for ovulation rate at the p telomere of SSC8 when *GNRHR-2* genotypes were fitted as fixed effects in the QTL analysis. In both studies, an additive increasing effect from the Large White breed was seen. Our data provide insufficient evidence for this QTL to merit reporting the finding on its own; however, the estimated location of the QTL and the direction of the effect are consistent with the data of Rohrer et al. [8]. Because the power to detect minor QTL was limited in the present study as well as in most previous studies of reproductive traits, it is important to report QTL for which the evidence is weak, because confidence in such QTL can be strengthened by comparisons across studies. There would also be considerable benefit in analyzing data pooled from several small to medium-sized studies (for example, see [46]).

We found no evidence for a QTL for ovulation rate at the telomere of the q arm, as reported by Rathje et al. [6]. However, when this research group included additional animals in a more comprehensive study, the previously reported QTL for ovulation rate at the telomeric end of the q arm of SSC8 was not confirmed [20]. Cassady et al. [20] described a QTL for age at puberty at SSC8q-ter, a trait that was not investigated in the present study.

Wilkie et al. [7] reported a putative QTL for ovulation rate around the centromere of SSC8, with a positive additive effect from the Yorkshire breed within the University of Illinois Meishan × Yorkshire Swine Resource Family. In a follow-up study, in which more markers were scored in these animals, the SSC8 centromeric QTL for number of corpora lutea was confirmed with increased confidence [21]. In the present study, a region was observed around the centromere of SSC8 that appeared to display an additive increasing effect from the Large White breed when *GNRHR-2* genotypes were added as fixed effects to the QTL model. Under this extended model, two peaks were seen, one at 3 cM ($F = 3.01$) and one at 80 cM, close to the *GNRHR* locus ($F = 2.5$), both displaying positive additive effects from the Large White breed.

TABLE 5. The frequency of allele *1* in the founder breeds at each of the gene marker loci.

	Meishan purebreed F_0 animals	Large White purebreed F_0 animals
GNRHR-1	1.00	0.19
FGG-2	0.84	0.35
IBSP	1.00	0.00
GNRHR-2	0.97	0.39
STE	0.28	0.23
SPP1–5	1.00	0.00
SPP1–4	1.00	0.46
AREG	1.00	0.04
HD	0.63	0.00
QDPR	0.94	0.31
SLIT2	0.69	0.12

It is recognized that the model in which it is assumed that the founder breeds are fixed for alternative QTL alleles is often an oversimplification in outbred species such as pigs. Including within-breed variation at the *GNRHR-2* marker represents a modest improvement in the sophistication of the model and provides a better fit for the ovulation rate data.

In an earlier study on the same animals, associations between the number of corpora lutea and *GNRHR* genotypes were tested [11]. The allele most prevalent in the Meishan breed for the polymorphism identified at position 1721 of the gene was shown to be associated with increased numbers of corpora lutea for animals at first parity. The genotypes for this polymorphism were included in this study as *GNRHR-2,* with the same allele being coded as allele *1.* Although allele *1* is present in both founder breeds, it is much more abundant in the Meishan (Table 5), and as already mentioned, animals with the *1,1* genotype at the *GNRHR-2* locus had an estimated 3.41 ±1.47 ova more than *2,2* homozygotes ($P < 0.05$). Thus, the effects associated with *GNRHR* that maps close to the centromere appear to differ from those of the QTL reported for the Illinois population, in which the Meishan allele has a decreasing effect on the number of corpora lutea [7, 21]. Both studies lacked the power to determine whether two QTL acting in opposite directions may be present in close proximity to one another around the centromere of SSC8.

Milan et al. [19] reported preliminary evidence for putative QTL for ovulation rate and litter size, with positive effects from the Meishan breed resulting in an increase of one or two ova or piglets on chromosomes 7 and 8. However, these authors did not provide information regarding the location of the QTL on these chromosomes.

The statistical support for QTL, for all the traits examined across both age groups of sows, was more compelling for the younger age group. The main reason for this could be the loss of power resulting from the smaller number of F_2 animals available in this second age group with trait data recorded.

One of the reasons to search for reproductive QTL on SSC8 was that the pig homologue of the Booroola fecundity gene (*BMPR1B*) was predicted to, and is now known to map to the q arm of SSC8, relatively close to *SPP1* [47]. Alleles at the *BMPR1B* locus are known to improve litter size in sheep through increases in ovulation rate [48, 49]. However, because we found no evidence for an ovulation rate QTL on the q arm of SSC8, where pig *BMPR1B* maps, and because the litter-size QTL effects appear to be attrib-

utable to improvements in embryo survival, it seems unlikely that *BMPR1B* is the gene responsible for the pig litter-size QTL reported here.

Interestingly, however, variation in a microsatellite repeat 5′ of the *SPP1* gene was previously shown to be associated with an increase in litter size in a Meishan × Large White cross [10]. This marker lies within the 95% confidence intervals for the litter-size and embryo-survival QTL. More recently, Korin-Kossakowska et al. [50] reported associations between the presence of a SINE in the *SPP1* gene [34] and litter size of the second and subsequent parities for 519 sows from a commercial Polish line.

Also known as osteopontin, *SPP1* is a physiological as well as a positional candidate gene. Studies of this gene have revealed that it is expressed in a variety of tissues, including the epithelial cells of the endometrium and the metrial gland cells of the decidua within the uterus, the placenta, and the invading trophoblast, during the defined window of receptivity of the peri-implantation period of pregnancy in several mammalian species [51, 52]. These studies indicate that this gene has an important role to play in embryo implantation and placentation.

The SPP1 protein is secreted into the uterine lumen during early pregnancy in humans and ewes, and it binds to integrin heterodimer receptors expressed on luminal epithelial cells in response to increased levels of progesterone [53]. This induces adhesion between the luminal epithelium of the endometrium and the trophectoderm of the blastocyst, and it triggers the cascade of molecular events leading to successful implantation and placental function [54, 55]. The same process is believed to occur in pigs, in that *SPP1* mRNA has been shown to be expressed by the porcine uterine luminal epithelium during the early implantation period and to induce cytoplasmic reorganization and focal adhesions in the uterus and on the conceptus [56]. It has been reported that the significant differences in prenatal survival levels between the Meishan breed and U.S. as well as European commercial breeds can be mainly attributed to the marked reduction in peri-implantation conceptus loss seen in the Meishan breed [57, 58]. Even when the uterus size and the ovulation rate of the two breeds has been observed to be similar, the Meishan breed farrows three to five more viable piglets per litter [2, 3].

Although the *SPP1* gene is a candidate for the litter size and prenatal survival traits based on positional and physiological arguments, the confidence intervals for the QTL identified in the present study are large, harboring hundreds of genes. Although a DNA test based on the causal genetic variation provides the most powerful tool for marker-assisted selection, the markers that define the litter-size and prenatal-survival QTL can be used for this purpose in the meantime.

ACKNOWLEDGMENTS

We thank the farm staff at Mountmarle for animal husbandry and on-farm data recording. We thank the staff of the Roslin Large Animal Unit for the laparoscopy data as well as Dr. Andy Law and others for developing and maintaining the QTL database (resSpecies). We also thank Heather Finlayson for preparing the DNA samples and for assistance with the genotyping techniques.

REFERENCES

1. Bennett GL, Leymaster KA. Integration of ovulation rate, potential embryonic viability and uterine capacity into a model of litter size in swine. J Anim Sci 1989; 67:1230–1241.
2. Haley CS, Lee GJ, Ritchie M. Comparative reproductive performance in Meishan and Large White pigs and their crosses. Anim Sci 1995; 60:259–267.
3. Haley CS, Lee GJ. Genetic basis of prolificacy in Meishan pigs. J Reprod Fertil Suppl 1993; 48:247–259.
4. Avalos E, Smith C. Genetic-improvement of litter size in pigs. Anim Prod 1987; 44:153–164.
5. Lande R, Thompson R. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 1990; 124:743–756.
6. Rathje TA, Rohrer GA, Johnson RK. Evidence for quantitative trait loci affecting ovulation rate in pigs. J Anim Sci 1997; 75:1486–1494.
7. Wilkie PJ, Paszek AA, Beattie CW, Alexander LJ, Wheeler MB, Schook LB. A genomic scan of porcine reproductive traits reveals possible quantitative trait loci (QTLs) for number of corpora lutea. Mamm Genome 1999; 10:573–578.
8. Rohrer GA, Ford JJ, Wise TH, Vallet JL, Christenson RK. Identification of quantitative trait loci affecting female reproductive traits in a multigeneration Meishan-White composite swine population. J Anim Sci 1999; 77:1385–1391.
9. Rothschild M, Jacobson C, Vaske D, Tuggle C, Wang L, Short T, Eckardt G, Sasaki S, Vincent A, McLaren D, Southwood O, van der Steen H, Mileham A, Plastow G. The estrogen receptor locus is associated with a major gene influencing litter size in pigs. Proc Natl Acad Sci U S A 1996; 93:201–205.
10. Van der Steen H, Southwood O, Vries A, Short T, McLaren D, Wei M, Plastow G. Evidence of a new genetic marker for litter size in Meishan synthetic pigs. In: Proceedings of International Conference on Animal Biotechnology; 1997; Beijing, China: 17–20.
11. Jiang ZH, Gibson JP, Archibald AL, Haley CS. The porcine gonadotropin-releasing hormone receptor gene (GNRHR): genomic organization, polymorphisms, and association with the number of corpora lutea. Genome 2001; 44:7–12.
12. Drogemuller C, Hamann H, Distl O. Candidate gene markers for litter size in different German pig lines. J Anim Sci 2001; 79:2565–2570.
13. Kmiec M, Dybus A, Terman A. Prolactin receptor gene polymorphism and its association with litter size in Polish Landrace. Arch Tierzucht 2001; 44:547–551.
14. Archibald AL. Comparative genome mapping—the livestock perspective. In: Clark AJ (ed.), Animal Breeding—Technology for the 21st Century, 1st ed. Amsterdam, The Netherlands: Harwood Academic Publishers; 1998: 137–164.
15. Goureau A, Yerle M, Schmitz A, Riquet J, Milan D, Pinton P, Frelat G, Gellin J. Human and porcine correspondence of chromosome segments using bidirectional chromosome painting. Genomics 1996; 36: 252–262.
16. Rettenberger G, Klett C, Zechner U, Kunz J, Vogel W, Hameister H. Visualisation of the conservation of synteny between humans and pigs by heterologous chromosomal painting. Genomics 1995; 26:372–378.
17. Montgomery GW, Lord EA, Penty JM, Dodds KG, Broad TE, Cambridge L, Sunden SL, Stone RT, Crawford AM. The Booroola fecundity (FecB) gene maps to sheep chromosome 6. Genomics 1994; 22: 148–153.
18. Crosby AH, Lyu MS, Lin K, McBride OW, Kerr JM, Aplin HM, Fisher LW, Young MF, Kozak CA, Dixon MJ. Mapping of the human and mouse bone sialoprotein and osteopontin loci. Mamm Genome 1996; 7:149–151.
19. Milan D, Bidanel JP, Le Roy P, Chevalet N, Woloszyn N, Caritez JC, Gruand H, Bonneau M, Lefaucheur L, Renard C, Vaiman M, Mormede P, Desautes C, Amigues Y, Bourgeois F, Gellin J, Olliver L. Current status of QTL detection in Large White × Meishan crosses in France. In: Proceedings of the 6th World Congress on Genetics Applied to Livestock Production; 1998; Armidale, NSW, Australia: 414–417.
20. Cassady JP, Johnson RK, Pomp D, Rohrer GA, Van Vleck LD, Spiegel EK, Gilson KM. Identification of quantitative trait loci affecting reproduction in pigs. J Anim Sci 2001; 79:623–633.
21. Braunschweig MH, Paszek AA, Weller JI, Da Y, Hawken RJ, Wheeler MB, Schook LB, Alexander LJ. Generation and exploration of a dense genetic map in a region of a QTL affecting corpora lutea in a Meishan × Yorkshire cross. Mamm Genome 2001; 12:719–723.
22. Haley CS, D'agaro E, Ellis M. Genetic components of growth and ultrasonic fat depth traits in Meishan and Large White pigs and their reciprocal crosses. Anim Prod 1992; 54:105–115.
23. Cameron ND, Curran MK, Thompson R. Estimation of sire with feeding regime interaction in pigs. Anim Prod 1988; 46:87–95.
24. Walling GA, Archibald AL, Cattermole JA, Downing AC, Finlayson HA, Nicholson D, Visscher PM, Walker CA, Haley CS. Mapping of

quantitative trait loci on porcine chromosome 4. Anim Genet 1998; 29:415–424.
25. Wildt DE, Fujimoto S, Spencer JL, Dukelow WR. Direct ovarian observation in the pig by means of laparoscopy. J Reprod Fertil 1973; 35:541–543.
26. Coppieters W, Vandeweghe A, Peelman L, Depicker A, Vanzeveren A, Bouquet Y. Characterization of porcine polymorphic microsatellite loci. Anim Genet 1993; 24:163–170.
27. Ellegren H, Chowdhary BP, Johansson M, Marklund L, Fredholm M, Gustavsson I, Andersson L. A primary linkage map of the porcine genome reveals a low-rate of genetic recombination. Genetics 1994; 137:1089–1100.
28. Robic A, Dalens M, Woloszyn N, Milan D, Riquet J, Gellin J. Isolation of 28 new porcine microsatellites revealing polymorphism. Mamm Genome 1994; 5:580–583.
29. Rohrer GA, Alexander LJ, Keele JW, Smith TP, Beattie CW. A microsatellite linkage map of the porcine genome. Genetics 1994; 136: 231–245.
30. Alexander LJ, Troyer DL, Rohrer GA, Smith TP, Schook LB, Beattie CW. Physical assignments of 68 porcine cosmid and lambda clones containing polymorphic microsatellites. Mamm Genome 1996; 7:368–372.
31. Jiang ZH, Robinson JAB, Verrinder Gibbins AM, Gibson JP, Archibald AL, Haley CS. Mapping of QTL for prolificacy traits on SSC8 using a candidate gene approach. In: Proceedings of the 7th World Congress on Genetics Applied to Livestock Production; 2002; Montpellier, France (paper 8-02).
32. Jiang ZH, He H, Hamasima N, Suzuki H, Gibbins AMV. Comparative mapping of Homo sapiens chromosome 4 (HSA4) and *Sus scrofa* chromosome 8 (SSC8) using orthologous genes representing different cytogenetic bands as landmarks. Genome 2002; 45:147–156.
33. Moran C. Microsatellite repeats in pig (*Sus domestica*) and chicken (*Gallus domesticus*) genomes. J Hered 1993; 84:274–280.
34. Knoll A, Stratil A, Cepica S, Dvorak J. Length polymorphism in an intron of the porcine osteopontin (SPP1) gene is caused by the presence or absence of a SINE (PRE-1) element. Anim Genet 1999; 30: 466.
35. Liu Q, Thorland EC, Heit JA, Sommer SS. Overlapping PCR for bidirectional PCR amplification of specific alleles: a rapid one-tube method for simultaneously differentiating homozygotes and heterozygotes. Genome Res 1997; 7:389–398.
36. Matise TC, Perlin M, Chakravarti A. Automated construction of genetic-linkage maps using an expert-system (Multimap)—a human genome linkage map. Nat Genet 1994; 6:384–390.
37. Haley CS, Knott SA, Elsen JM. Mapping quantitative trait loci in crosses between outbred lines using least squares. Genetics 1994; 136: 1195–1207.
38. Seaton G, Haley CS, Knott SA, Kearsey M, Visscher PM. QTL Express: mapping quantitative trait loci in of simple and complex pedigrees. Bioinformatics 2002; 18:339–340.
39. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 1995; 11:241–247.
40. Rohrer GA, Alexander LJ, Hu Z, Smith TP, Keele JW, Beattie CW. A comprehensive map of the porcine genome. Genome Res 1996; 6: 371–391.
41. Bidanel JP, Caritez JC, Legault C. Estimation of crossbreeding parameters between Large White and Meishan porcine breeds. 1. Reproductive-performance. Genet Sel Evol 1989; 21:507–526.
42. Rothberg JM, Jacobs JR, Goodman CS, Artavanistsakonas S. Slit—An extracellular protein necessary for development of midline glia and commissural axon pathways contains both EGF and LRR domains. Genes Dev 1990; 4:2169–2187.
43. Itoh A, Miyabayashi T, Ohno M, Sakano S. Cloning and expressions of three mammalian homologues of *Drosophila* slit suggest possible roles for Slit in the formation and maintenance of the nervous system. Mol Brain Res 1998; 62:175–186.
44. Hirooka H, De Koning DJ, Harlizius B, van Arendonk JAM, Rattink AP, Groenen MAM, Brascamp EW, Bovenhuis H. A whole-genome scan for quantitative trait loci affecting teat number in pigs. J Anim Sci 2001; 79:2320–2326.
45. Rohrer GA. Identification of quantitative trait loci affecting birth characters and accumulation of backfat and weight in a Meishan-White Composite resource population. J Anim Sci 2000; 78:2547–2553.
46. Walling GA, Visscher PM, Andersson L, Rothschild MF, Wang LZ, Moser G, Groenen MAM, Bidanel JP, Cepica S, Archibald AL, Geldermann H, De Koning DJ, Milan D, Haley CS. Combined analyses of data from quantitative trait loci mapping studies: chromosome 4 effects on porcine growth and fatness. Genetics 2000; 155:1369–1378.
47. King AH, Jiang Z, Rohrer GA, Gibson JP, Waddington D, Archibald AL. Use of radiation hybrid mapping to locate candidate genes for female reproductive traits on porcine chromosome 8. In: Proceedings of the XXVIIIth International Conference on Animal Genetics; 2002; Göttingen, Germany. (Abstract E063). Abstracts available at http://www.isag.org.uk.
48. Wilson T, Wu XY, Juengel JL, Ross IK, Lumsden JM, Lord EA, Dodds KG, Walling GA, McEwan JC, O'Connell AR, McNatty KP, Montgomery GW. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biol Reprod 2001; 64:1225–1235.
49. Souza CJH, MacDougall C, Campbell BK, McNeilly AS, Baird DT. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. J Endocrinol 2001; 169:R1–R6.
50. Korwin-Kossakowska A, Kamyczek M, Cieslak D, Pierzchala M, Kuryl J. The estimation of the candidate genes polymorphism effect on the reproductive traits in line 990 sows. In: Proceedings of the XXVIIIth International Conference on Animal Genetics; 2002; Göttingen, Germany. (Abstract E064). Abstracts available at http://www.isag.org.uk.
51. Johnson GA, Burghardt RC, Spencer TE, Newton GCR, Ott TL, Bazer FW. Ovine osteopontin: II. Osteopontin and $\alpha_v\beta_3$ integrin expression in the uterus and conceptus during the peri-implantation period. Biol Reprod 1999; 61:892–899.
52. Nomura S, Wills AJ, Edwards DR, Heath JK, Hogan BLM. Developmental expression of 2AR (osteopontin) and SPARC (osteonectin) RNA as revealed by in situ hybridisation. J Cell Biol 1988; 106:441–450.
53. Omigbodun A, Ziolkiewicz P, Tessler C, Hoyer JR, Coutifaris C. Progesterone regulates osteopontin expression in human trophoblasts: a model of paracrine control in the placenta? Endocrinology 1997; 138: 4308–4315.
54. Fazleabas AT, Bell SC, Fleming S, Sun JH, Lessey BA. Distribution of integrins and the extracellular matrix proteins in the baboon endometrium during the menstrual cycle and early pregnancy. Biol Reprod 1997; 56:348–356.
55. Johnson GA, Bazer FW, Jaeger LA, Ka H, Garlow JE, Pfarrer C, Spencer TE, Burghardt RC. Muc-1, integrin, and osteopontin expression during the implantation cascade in sheep. Biol Reprod 2001; 65: 820–828.
56. Garlow JE, Ka HH, Johnson GA, Burghardt RC, Jaeger LA, Bazer FW. Analysis of osteopontin at the maternal-placental interface in pigs. Biol Reprod 2002; 66:718–725.
57. Ford SP. Embryonic and fetal development in different genotypes in pigs. J Reprod Fertil Suppl 1997; 52:165–176.
58. Wilson ME, Biensen NJ, Ford SP. Novel insight into the control of litter size in pigs, using placental efficiency as a selection tool. J Anim Sci 1999; 77:1654–1658.