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A genome scan for quantitative trait loci and imprinted regions affecting reproduction in pigs[1]

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ABSTRACT: Quantitative trait loci for reproductive traits in a three-generation resource population of a cross between low-indexing pigs from a control line and high-indexing pigs from a line selected 10 generations for increased index of ovulation rate and embryonic survival are reported. Phenotypic data were collected in F_2 females for birth weight (BWT, n = 428), weaning weight (WWT, n = 405), age at puberty (AP, n = 295), ovulation rate (OR, n = 423), number of fully formed pigs (FF, n = 370), number of pigs born alive (NBA, n = 370), number of mummified pigs (MUM, n = 370), and number of stillborn pigs (NSB, n = 370). Grandparent, F_1, and F_2 animals were genotyped for 151 microsatellite markers. Sixteen putative QTL ($P < 0.10$) for reproductive traits were identified in previous analyses of these data with single QTL line-cross models. Data were reanalyzed with multiple QTL models, including imprinting effects. Data also were analyzed with half-sib models. Permutation was used to establish genome-wide significance levels (α = 0.01, 0.05, and 0.10). Thirty-one putative QTL for reproductive traits and two QTL for birth weight were identified ($P < 0.10$). One Mendelian QTL for FF ($P < 0.05$), one for NBA ($P < 0.05$), three for NSB ($P < 0.05$), three for NN ($P < 0.05$), seven for AP ($P < 0.10$), five for MUM ($P < 0.10$), and one for BWT ($P < 0.10$) were found. Partial imprinting of QTL affecting OR ($P < 0.01$), BWT ($P < 0.05$), and MUM ($P < 0.05$) was detected. There were four paternally expressed QTL for NN ($P < 0.10$) and one each for AP ($P < 0.05$) and MUM ($P < 0.10$). Maternally expressed QTL affecting NSB ($P < 0.10$), NN ($P < 0.10$), and MUM ($P < 0.10$) were detected. No QTL were detected with half-sib analyses. Multiple QTL models with imprinting effects are more appropriate for analyzing F_2 data than single Mendelian QTL line-cross models.

Key Words: Imprinting, Pigs, Reproduction, Quantitative Trait Loci, Weight

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Introduction

Sax (1923) first proposed using the association between discrete traits and continuous variables to establish linkage with QTL. Molecular markers and interval mapping (Lander and Botstein, 1989) are now commonly used to identify genomic regions harboring QTL.

Haley et al. (1994) used regression to estimate additive (**a**) and dominance (**d**) coefficients in F_2 populations. Based on their model and line origin probabilities, de Koning et al. (2000) defined contrasts to estimate a, d, and imprinted QTL effects in F_2 line-cross data.

Rathje et al. (1997) and Cassady et al. (2001) identified 16 putative QTL for reproductive traits with single Mendelian QTL models applied to an F_2 population from lines selected for increased litter size. An assumption was that marker genotypes associated with multiple QTL were uncorrelated. If genotypes are correlated, QTL effects will be overestimated and the variance explained by QTL will not be additive, in which case single-QTL models may detect only one or two QTL explaining significant portions of the variation (Schork et al., 1993). Composite interval mapping (Zeng, 1993, 1994) with multiple QTL models was developed to increase the power to detect additional QTL.

The objectives of this study were to reanalyze the F_2 data reported by Cassady et al. (2001) with multiple QTL models applied sequentially to identify Mendelian and imprinted QTL affecting reproduction and early growth in pigs.

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Table 1. Genotype-specific means of quantitative trait loci

QTL genotype	Genotypic value[a]
QQ	a + p + m
Qq	d + p − m
qQ	d − p + m
qq	−a − p − m

[a]The QTL effects include additive (a), dominance (d), paternal expression (p), and maternal expression (m).

Table 2. Contrasts of line of origin probabilities used in regression models to estimate effects in models as defined by de Koning et al. (2000)

Effect	Calculation of coefficient[a]
Additive	$P_{CC} - P_{II}$
Dominance	$P_{CI} + P_{IC}$
Paternal expression	$(P_{CC} + P_{CI}) - (P_{IC} + P_{II})$
Maternal expression	$(P_{CC} + P_{IC}) - (P_{CI} + P_{II})$

[a]Line origin probablilities, where Pij is the probability of inheriting the ith line allele from the sire and the jth line allele from the dam. Lines referenced are Line C and Line I.

Materials and Methods

A three-generation F_2 population was developed by crossing low-indexing pigs from a randomly selected control line (**C**) with high-indexing pigs from a line (**I**) selected 10 generations for increased index of ovulation rate and embryonic survival (Rathje et al., 1997; Cassady et al., 2001). Phenotypic data on F_2 females were collected for birth weight (**BWT**, n = 428), weaning weight (**WWT**, n = 405), age at puberty (**AP**, n = 295), ovulation rate (**OR**, n = 423), number of fully formed pigs (**FF**, n = 370), number of pigs born alive (**NBA**, n = 370), number of mummified pigs (**MUM**, n = 370), and number of stillborn pigs (**NSB**, n = 370). Genotypes for 151 microsatellite markers were determined in grandparent males (five I and four C) and females (12 I and 14 C), F_1 males (10) and females (43), and F_2 females (428). Details of development of the population, measurement of traits, tissue collection, and laboratory procedures are in Cassady et al. (2001).

Modeling of QTL

Let the quantitative phenotype, y, be a linear function of a single QTL that influences it:

$$y = \mu + \gamma + \varepsilon$$

where μ is the overall mean, γ is the effect of the QTL, and ε is the random environmental deviation. Genotypic-specific means of the QTL effect with two alleles, Q and q, are shown in Table 1. Because QTL genotypes are not exactly known, probabilities associated with QTL effects are estimated with marker information and used in regression models to identify QTL.

The program described by Haley et al. (1994) was used to calculate probabilities of allele origin for each individual in the F_2 generation at 1-cM intervals throughout the genome. Coefficients for additive, dominance, and imprinting effects were defined as described by de Koning et al. (2002) and shown in Table 2.

Statistical Models

Multiple regression models were fitted to the data with additive, dominance, and/or parent-of-origin (imprinting) coefficients as covariates. Models were developed similarly to composite interval mapping models. Alternative models were compared and tested using a method proposed by J. Dekkers (Iowa State University, personal communication), partially based on results of de Koning et al. (2002). Model comparison was by calculation of $\log_{10}$ of odds (**LOD**) scores.

Data for each trait were fitted first to a single Mendelian QTL, line-cross model. The reduced model included fixed effects of replicate, sire-dam combination (included to adjust for polygenic effects), and covariates of number of fully formed pigs in a litter for BWT and number of pigs weaned and age at weaning for WWT. The full model also included regressions on contrasts of line origin probabilities for additive and dominance effects.

Imprinting effects were tested comparing the following models:

Full model (F): $y_{ij} = \mu + c_{pi}p + c_{mi}m + c_{di}d + e_{ij}$

Paternal model (P): $y_{ij} = \mu + c_{pi}p + e_{ij}$

Maternal model (M): $y_{ij} = \mu + c_{mi}m + e_{ij}$

Null model (N): $y_{ij} = \mu + e_{ij}$

where y_{ij} is the phenotype of the ith F_2 offspring; μ is the combined fixed effects of intercept, replicate, polygenic effect defined as sire-dam combination, covariate according to trait analyzed (the number of fully formed pigs in a litter for birth weight and number of pigs weaned, and age at weaning for weaning weight); p, m, and d are the paternal, maternal, and dominance effects for the imprinted QTL, respectively; c_{pi} is the coefficient of the ith individual for the paternal component at the imprinted QTL; c_{mi} is the coefficient of the ith individual for the maternal component at the imprinted QTL; c_{di} is the coefficient of the ith individual for the dominance component at the imprinted QTL; and e_{ij} is the residual error.

The coefficients c_{pi} and c_{mi} were calculated as described in Table 2. Calculated LOD scores were used to compare F to N to detect imprinting effects. If both Mendelian and imprinting scans indicated a QTL at a position, the imprinting model was tested against the Mendelian model and, when nonsignificant, the Mende-

lian model was considered appropriate. To determine the mode of action, LOD scores for F vs. P and F vs. M were calculated. Paternal expression was indicated when only F vs. M was significant, maternal expression was indicated if only F vs. P was significant, and partial imprinting was indicated if both or neither of these contrasts were significant. As QTL scans identify chromosomal regions that may harbor a single gene or multiple genes affecting the trait, and imprinted genes have tendencies to cluster together in the genome, it is possible to identify partially imprinted QTL, indicating that both maternal and paternal imprinted genes in that region affect the trait.

A sequential multiple QTL search with forward selection model building procedures was used to find best-fitting models. From results of single Mendelian QTL models for each trait, the position of the QTL with the highest LOD score that exceeded the critical level (α = 0.10) was chosen as a background effect. An additional Mendelian QTL model was fitted with the position of background QTL fixed in the model. The model was:

$$y_{ij} = \mu + \sum_{l=1}^{n-1} (c_{ail}a_l + c_{dil}d_l) + c_{ain} + c_{din}d_n + e_{ij}$$

where y_{ijk} is the phenotype of the ith F_2 offspring; μ is the combined fixed effects of intercept, replicate, polygenic effect defined as sire-dam combination, and covariate according to trait analyzed (the number of fully formed pigs in a litter for birth weight and number of pigs weaned and age at weaning for weaning weight); a_l, d_l, a_n, and d_n are the additive and dominance effects for QTL l and n, respectively; c_{ail} is the coefficient of the ith individual for the additive effect at the lth QTL (selected by the largest significant LOD score); c_{dil} is the coefficient of the ith individual for the dominance effect at the lth QTL; c_{ain} is the coefficient of the ith individual for the additive effect at the nth QTL; c_{din} is the coefficient of the ith individual for the dominance effect at the nth QTL; and e_{ijk} is the residual error.

Calculated LOD scores were used to compare the full model with the reduced, single-QTL model. Rounds of genome scans with $n - 1$ identified Mendelian and imprinted QTL as background effects in the reduced model were completed until the largest LOD score corresponding to the nth QTL was less than the genome-wide threshold level (α = 0.10). The effects of QTL incorporated into multiple QTL models as background effects were estimated in the full model. The effects of QTL identified but not used as background effects were estimated in the model in which it was last significant.

Data also were fitted to half-sib QTL models. Reduced models included fixed effects of replicate, sire-dam combination, and appropriate covariates for BWT and WWT. Full models also included covariate coefficients of probability of inheriting the control line allele within an F_1 sire family.

Thresholds

Genome-wide significance levels (α = 0.01, 0.05, and 0.10) were estimated from 475 permutations of the data, the number required to estimate $\hat{\alpha}_{0.05}$ with a SE of 0.01 for y, the number of times that the test statistic exceeded the critical value (i.e., distributed binomially with parameters N and α). When shuffling data, associations between effects in the reduced model were retained. Thresholds for critical value of α = 0.01, 0.05, and 0.10 were LOD scores that exceeded the 99th, 95th, and 90th percentiles, respectively, of the ranked scores.

Results

Genome-wide threshold levels increased slightly as additional QTL were added to models because residual degrees of freedom decreased. Thus, LOD scores for minimum thresholds for inclusion of multiple-QTL models were larger than for single-QTL models.

Progression of genome scans with putative QTL, locations, LOD scores, mode of action, and selection of background loci are reported in Table 3. Estimates of additive, dominance, and imprinting effects are in Table 4. Additive effects were estimated as the mean of individuals homozygous for the allele inherited from Line C minus the mean of those homozygous for alleles inherited from Line I. Mean genotype specific deviations from the average of the two homozygotes were calculated and are also in Table 4. Deviations for Mendelian QTL are a, d, d, and –a for the CC, CI, IC, and II genotypes, respectively. Mean deviations for imprinted QTL are (p + m), (d + p – m), (d – p + m), and (–p – m) for the CC, CI, IC, and II genotypes.

Mendelian QTL

Scans with single Mendelian QTL models produced evidence for 18 Mendelian QTL, one each for FF, NBA, MUM, and BWT; three for NSB; five for NN; and six for AP. In later scans, there was stronger evidence that the QTL for NN on SSC1 at 155 cM and SSC6 at 171 cM were imprinted rather than Mendelian QTL. Five more Mendelian QTL were identified in later scans; four for MUM and one for AP.

Mendelian QTL in SSC11 at 52 and 71 cM affected FF and NBA, respectively (Table 3). Neither of these positions significantly affected NSB, which is the difference between FF and NBA; however, Mendelian QTL on SSC13 ($P < 0.05$), SSC5 ($P < 0.10$), and SSC12 ($P < 0.10$) affecting NSB were identified in single QTL models (Table 3). After fitting the QTL in SSC13 in the model, the one on SSC5 remained ($P < 0.10$), but the one on SSC12 was not significant after fitting both the SSC13 and SSC5 positions. Dominance expression of the SSC13 QTL (a = –0.39 ± 0.12, d = –0.53 ± 0.20), overdominance expression of the one in SSC5 (a = –0.06 ± 0.17, d = 1.00 ± 0.29), and additive expression of the one in SSC12 (a = 0.38 ± 0.13, d = –0.40 ± 0.23) were observed (Table 4).

Table 3. Progression of genome scans using background quantitative trait loci

	Single-QTL model scan				Two-QTL model scan				Three-QTL model scan				Four-QTL model scan			
Trait[a]	SSC	Pos, cM[b]	LOD[b]	Action[c]	SSC	Pos, cM[b]	LOD[b]	Action[c]	SSC	Pos, cM[b]	LOD[b]	Action[c]	SSC	Pos, cM[b]	LOD[b]	Action[c]
OR	9[d]	1	4.79***	I	—				—				—			
FF	11[d]	52	2.80**	M	—				—				—			
NBA	11[d]	71	2.54*	M	—				—				—			
NSB	5	131	2.76*	M	5[d]	130	2.42*	M	14[d]	104	3.25*	I	—			
	12	37	2.52*	M	14	104	3.02*	I	—				—			
	13[d]	100	4.07**	M	—				—				—			
	14	104	3.12*	I	—				—				—			
MUM	6	81	4.03**	I	6	81	3.83**	I	6	64	2.42*	M	2[d]	6	2.53*	M
	12[d]	98	2.65*	M	12[d]	70	3.59**	M	6[d]	81	3.61**	I	2	29	3.14*	I
	—				—				—				6	165	2.46*	M
	—				—				—				6	191	3.06*	I
NN	1	155	2.33*	M	1	155	2.29*	M	1	155	2.72*	I	1	156	2.86*	I
	6	171	2.46*	M	6	169	2.32*	M	6	85	3.57**	I	6[d]	85	4.01**	I
	7	62	2.30*	M	7	62	2.45*	M	6	161	3.4**	I	6	163	3.36*	I
	8	20	2.87**	M	8[d]	20	3.34**	M	7[d]	62	2.9**	M	15	64	2.81*	I
	11[d]	47	3.25**	M	15	108	3.04*	I	15	67	3.07*	I	15	106	2.97*	I
	15	109	3.63**	I	—				15	107	3.57**	I	—			
AP	7	1	2.81**	M	7[d]	1	2.93**	M	7[d]	51	2.54*	M	18[d]	40	2.64*	M
	7	58	2.43*	M	7	57	2.31*	M	—				—			
	8	101	2.41*	M	—				—				—			
	8	136	2.36*	M	—				—				—			
	8[d]	172	3.81**	M	—				—				—			
	12	56	2.22*	M	—				—				—			
	15	98	3.25**	I	—				—				—			
BWT	6	155	3.61**	I	6[d]	155	3.65**	I	—				—			
	12[d]	17	2.53*	M	—				—				—			

*$P < 0.10$.
**$P < 0.05$.
***$P < 0.01$.
[a]OR = ovulation rate (ova); FF = number of fully formed pigs at birth; NBA = number of pigs born alive; NSB = number of stillborn pigs; MUM = number of mummified pigs; NN = number of nipples; AP = age at puberty; BWT = pig birth weight.
[b]Relative position (Pos) in Kosambi centimorgans on the swine chromosome (SSC); LOD = $\log_{10}$ of odds.
[c]M = Mendelian QTL; I = imprinted QTL.
[d]QTL chosen as background effect for subsequent genome scans.

Table 4. Estimated effects of quantitative trait loci

Trait[a]	SSC	Pos, CM[b]	Scans sig.[c]	QTL action[d]	a[e]	d[e]	p[e]	m[e]	CC[f]	CI[f]	IC[f]	II[f]
OR	9	1	1[g]	Partial	—	1.30 ± 0.40	−0.42 ± 0.12	0.18 ± 0.12	−0.25	0.71	1.90	0.25
FF	11	52	1[g]	M	−0.86 ± 0.27	−0.04 ± 0.47	—	—	−0.86	−0.04	−0.04	0.86
NBA	11	71	1[g]	M	−0.83 ± 0.30	0.66 ± 0.61	—	—	−0.83	0.66	0.66	0.83
NSB	13	100	1[g]	M	−0.39 ± 0.12	−0.53 ± 0.20	—	—	−0.39	−0.53	−0.53	0.39
	5	131	1,2[g]	M	−0.06 ± 0.17	1.00 ± 0.29	—	—	−0.06	1.00	1.00	0.06
	14	104	1,2,3[g]	Mat	—	0.44 ± 0.28	—	−0.30 ± 0.08	−0.30	0.74	0.14	0.30
	12	37	1	M	0.38 ± 0.13	−0.40 ± 0.23	—	—	0.38	−0.40	−0.40	−0.38
MUM	12	98	1[g]	M	−0.32 ± 0.09	−0.31 ± 0.12	—	—	−0.32	−0.31	−0.31	0.32
	12	70	2[g]	M	0.16 ± 0.09	0.45 ± 0.14	—	—	0.16	0.45	0.45	−0.16
	6	81	1,2,3[g]	Partial	—	−0.53 ± 0.17	−0.16 ± 0.07	0.13 ± 0.06	−0.03	−0.82	−0.24	0.03
	2	6	4[g]	M	−0.27 ± 0.08	0.02 ± 0.13	—	—	−0.27	0.02	0.02	0.27
	2	29	4	Mat	—	0.04 ± 0.14	—	−0.21 ± 0.04	−0.21	0.25	−0.17	0.21
	6	64	3	M	−0.06 ± 0.10	−0.77 ± 0.23	—	—	−0.06	−0.77	−0.77	0.06
	6	165	4	M	0.21 ± 0.09	0.40 ± 0.16	—	—	0.21	0.40	0.40	−0.21
	6	191	4	Pat	—	0.28 ± 0.12	0.16 ± 0.05	—	0.16	0.44	0.12	−0.16
NN	11	47	1[g]	M	−0.05 ± 0.10	0.72 ± 0.17	—	—	−0.05	0.72	0.72	0.05
	8	20	1,2[g]	M	−0.31 ± 0.10	0.49 ± 0.17	—	—	−0.31	0.49	0.49	0.31
	7	62	1,2,3[g]	M	0.22 ± 0.09	−0.51 ± 0.15	—	—	0.22	−0.51	−0.51	−0.22
	6	85	3,4[g]	Pat	—	0.28 ± 0.21	−0.34 ± 0.08	—	−0.34	−0.06	0.62	0.34
	1	155	1,2,3,4	Mat	—	0.58 ± 0.25	—	0.19 ± 0.07	0.19	0.39	0.77	−0.19
	6	171	1,2,3,4	Pat	—	0.08 ± 0.19	−0.27 ± 0.07	—	−0.27	−0.19	0.35	0.27
	15	64	3,4	Pat	—	−0.10 ± 0.16	−0.23 ± 0.07	—	−0.23	−0.33	0.13	0.23
	15	109	1,2,3,4	Pat	—	0.16 ± 0.20	−0.26 ± 0.08	—	−0.26	−0.10	0.42	0.26
AP, d	8	172	1[g]	M	6.36 ± 2.12	−11.96 ± 4.01	—	—	6.36	−11.96	−11.96	−6.36
	7	1	1,2[g]	M	−0.90 ± 2.04	10.86 ± 2.82	—	—	−0.90	10.86	10.86	0.90
	7	58	1,2,3[g]	M	−3.19 ± 2.30	−11.41 ± 3.74	—	—	−3.19	−11.41	−11.41	3.19
	18	40	4[g]	M	1.80 ± 2.25	−7.11 ± 4.16	—	—	1.80	−7.11	−7.11	−1.80
	8	101	1	M	7.65 ± 2.85	7.44 ± 4.58	—	—	7.65	7.44	7.44	−7.65
	8	136	1	M	7.14 ± 2.39	−2.58 ± 4.02	—	—	7.14	−2.58	−2.58	−7.14
	12	56	1	M	5.51 ± 1.88	−3.86 ± 2.87	—	—	5.51	−3.86	−3.86	−5.51
	15	98	1	Pat	—	3.13 ± 4.58	−2.80 ± 1.68	—	−2.80	0.33	5.93	2.80
BWT, g	12	17	1[g]	M	−5.32 ± 1.92	−8.62 ± 3.65	—	—	−5.32	−8.62	−8.62	5.32
	6	155	1,2[g]	Partial	—	0.44 ± 2.53	−3.70 ± 1.16	2.47 ± 1.04	−1.23	−5.73	6.61	1.23

[a]OR = ovulation rate (ova); FF = number of fully formed pigs at birth; NBA = number of pigs born alive; NSB = number of stillborn pigs; MUM = number of mummified pigs; NN = number of nipples; AP = age at puberty; BWT = pig birth weight.
[b]Relative position (Pos) in Kosambi centimorgans on the swine chromosome (SSC).
[c]Number of QTL in model, including itself, when locus is tested to be significant (sig.), $P < 0.10$.
[d]M = Mendelian; Partial = partial imprinting; Mat = maternal expression; Pat = paternal expression.
[e]Estimated effects (a = additive; d = dominance; p = paternal expression; m = maternal expression) in appropriate QTL model.
[f]Genotype-specific mean deviation from average of homozygotes.
[g]Chosen as background QTL effect in subsequent QTL scans.

One Mendelian QTL affecting MUM on SSC12 at 98 cM ($P < 0.10$) was identified in a single QTL model (Table 3). The four additional Mendelian QTL identified in multiple-QTL models were on SSC12 at 70 cM ($P < 0.05$) that entered the model after fitting the QTL at 98 cM, on SSC6 at 64 cM ($P < 0.10$), which became significant after fitting the two QTL on SSC12, and one each on SSC2 ($P < 0.10$) and SSC6 at 165 cM ($P < 0.10$) that were identified in four-QTL models. With the exceptions of the SSC2 QTL, for which the estimate of d was 0.02 ± 0.13, and the SSC6 QTL at 64 cM, for which the estimate of a was –0.06 ± 0.10, these QTL tended to be dominant with absolute values of a and d ranging from 0.16 to 0.45 (Table 4).

The Mendelian QTL for NN with the greatest LOD score in a single QTL model (Table 3) was on SSC11 ($P < 0.05$). The second and third QTL to be added were in SSC8 and SSC7, respectively, ($P < 0.05$). The LOD score for the SSC7 QTL increased from 2.30 ($P < 0.10$) in a single QTL model to 2.90 ($P < 0.05$) after fitting the positions of QTL on SSC11 and SSSC8. Overdominance was found for the SSC11 QTL (a = –0.05 ± 0.10, d = 0.72 ± 0.17), whereas the magnitude of estimates of a and d were similar for the other two QTL, indicating dominance (Table 4).

Three of the QTL for AP identified in single-QTL models (Table 3) were on SSC8 at 101 ($P < 0.10$), 136 ($P < 0.10$), and 172 cM ($P < 0.05$); two were on SSC7 at 1 ($P < 0.05$) and 58 cM ($P < 0.10$); and one was on SSC12 ($P < 0.10$). After fitting the position on SSC8 at 172 cM, only the two QTL on SSC7 remained significant; however, a fourth scan in which these three positions were fixed identified another Mendelian QTL on SSC18 ($P < 0.10$). These QTL tended to be dominant as estimates of d were similar to or greater than estimates of a in each case (Table 4).

There was evidence for a dominant Mendelian QTL affecting BWT (a = –5.32 ± 1.92 g, d = –8.62 ± 3.65 g, Table 4) on SSC12 ($P < 0.10$). No Mendelian QTL for WWT or OR were found.

Imprinted QTL

Six imprinted QTL were identified in single, imprinted-QTL models, one each for OR, NSB, MUM, NN, AP, and BWT (Table 3). Those for OR (SSC9, $P < 0.01$), MUM (SSC6 at 81 cM, $P < 0.05$), and BWT (SSC6, $P < 0.05$) were partially imprinted (Table 4). The QTL on SSC14 for NSB was maternally expressed ($P < 0.10$) and those for AP (SSC15 at 98 cM, $P < 0.05$) and NN (SSC15 at 109 cM, $P < 0.05$) were paternally expressed.

Six more imprinted QTL were found in multiple-QTL models (Table 3). Using three QTL as background effects, a maternally expressed QTL affecting MUM on SSC2 at 6 cM ($P < 0.10$) and a paternally expressed QTL on SSC6 at 191 cM ($P < 0.10$) were found. Using two QTL as background effects, there was evidence for four more imprinted QTL affecting NN. These included paternally expressed QTL on SSC6 at 85 cM ($P < 0.05$) and SSC15 at 64 cM ($P < 0.10$) and QTL on SSC1 ($P < 0.10$) and SSC6 (171 cM in single-QTL model, 163 cM in four-QTL model, $P < 0.10$) that were classified as Mendelian in single- and two-QTL models but were identified as imprinted in three- and four-QTL models.

Half-Sib Analyses

No QTL were identified with half-sib analyses.

Discussion

Cassady et al. (2001) analyzed these same data with single-QTL models and found evidence ($P < 0.10$) for 16 QTL affecting the reproductive traits studied herein. Fifteen of these QTL were confirmed; however, an imprinted model fitted the QTL for OR on SSC9 and QTL for NN on SSC1 and on SSC6 (171 cM) better than the Mendelian model used by Cassady et al. (2001) because of the differences between heterozygotes. However, de Koning et al. (2002) showed that Mendelian QTL may be incorrectly identified as imprinted QTL. The test used herein to compare the Mendelian model to an imprinted model was shown by de Koning et al. (2002) to be more conservative in identifying imprinted QTL when the QTL is actually Mendelian.

Although the power to identify QTL in segregating populations is decreased due to the combination of differences in gene frequencies and size of genetic effects (de Koning et al., 2002), the model-building procedures resulted in 15 additional Mendelian or imprinted QTL that were not detected by Cassady et al. (2001). With the exception of the QTL on SSC12 affecting MUM at 98 cM and NSB at 37 cM, results of single QTL scans were identical to those of Cassady et al. (2001). The discrepancies on SSC12 were due to an error in the marker data in the earlier analysis resulting in misidentification of QTL regions. By using imprinted QTL models or by fixing background QTL to decrease residual variation, two additional QTL for NSB, three for NN, two for AP, and seven for MUM were detected.

The power of multiple-QTL models compared with a single-QTL model is best illustrated for MUM. A QTL in SSC12 close to marker *SWR10* at 98 cM was located with a single QTL model; a nonsignificant local peak existed in the 60- to 75-cM interval near marker *S0090* (Figure 1). A second model in which the QTL near *SWR10* was fixed identified another QTL on SSC12 within the interval of the local peak at 70 cM. Linkage between these positions was expected to create a correlation between them such that the QTL at 70 cM may not be detected after fixing the one at 98 cM. Apparently, adequate recombination had occurred so that the variation explained by the QTL near marker *S0090* was significant. By fixing two QTL in models for MUM, a Mendelian QTL on SSC6 (64 cM) and an imprinted QTL on SSC6 (81 cM) were identified. However, the QTL at 64 cM was not significant in a four-QTL model in which the imprinted QTL on SSC6 was included as the third

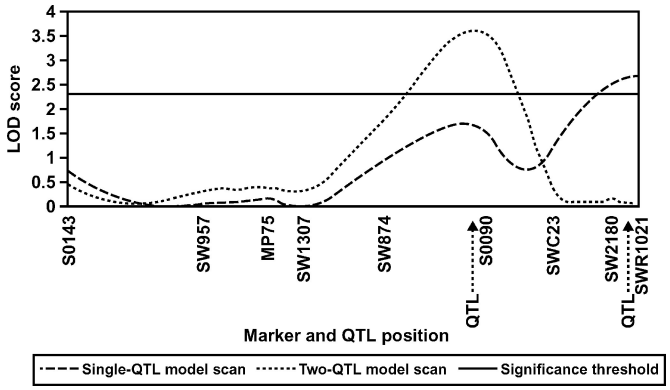

Figure 1. A QTL scan for number of mummified piglets (MUM) on SSC12. Marker names and relative positions of markers and QTL are shown on the x-axis.

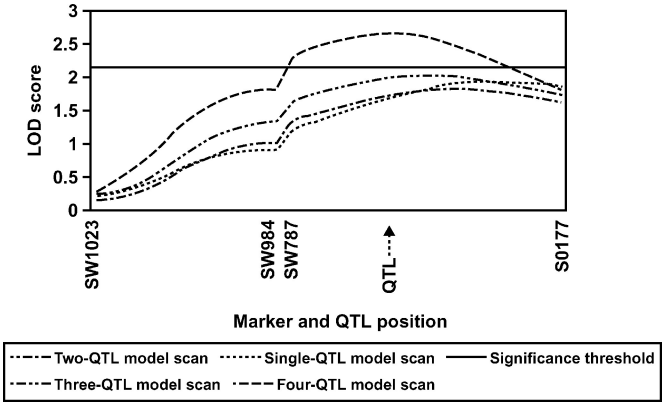

Figure 2. A QTL scan for age at puberty (AP) on SSC18. Marker names and relative positions of markers and QTL are shown on the x-axis.

background position, probably due to correlation between these positions caused by linkage. Four additional QTL for MUM, two on SSC2 at 6 and 29 cM and two on SSC6 at 165 and 191 cM, were identified in the four-QTL model.

Estimates of QTL effects (Table 4) are expressed as the effect of the allele inherited from Line C. Interpretation of these estimates is illustrated for MUM because Mendelian, partially imprinted, paternally expressed, and maternally expressed QTL were found.

Mendelian QTL for MUM on SSC12, SSC2, and SSC6 were found. Fixing the QTL on SSC12 (98 cM, a = –0.32 ± 0.09) and SSC2 (6 cM, a = –0.27 ± 0.08) for Line C alleles was estimated to cause a decrease of 1.18 mummified pigs at birth compared with fixing Line I alleles. However, fixing the Line I alleles for the QTL on SSC12 (70 cM, a = 0.16 ± 0.09) and SSC6 (165 cM, a = 0.21 ± 0.09) is expected to decrease MUM by 0.74 compared with Line C alleles. Dominance was found for the Mendelian QTL in SSC6 at 64 cM (a = –0.06 ± 0.10, d = –0.77 ± 0.23). Individuals heterozygous for Line I and Line C alleles were estimated to have 0.77 fewer mummified piglets than the average of those homozygous for Line I and Line C alleles. Partial imprinting occurred for the QTL on SSC6 (81 cM). Identification of partial imprinting indicated that there may be at least two imprinted QTL, one paternally and one maternally expressed. The joint effect of inheriting a Line C allele from the sire and a Line I allele from the dam (CI genotype) was estimated to result in the least mummified pigs of any genotype at this QTL position, 0.58 mummified pigs less than the reciprocal genotype (Table 4.). Fixing either Line C or Line I alleles is expected to increase the number of mummified pigs by approximately 0.8 compared with the CI genotype. There was complete paternal expression of the QTL on SSC6 (191 cM) affecting MUM. Fixing Line I alleles was estimated to decrease mummified pigs by at least 0.28 pigs compared with other genotypic means. The CI genotype was estimated to have the largest number of mummified pigs.

Similar application of estimates of QTL effects for other traits produces the mean genotypic differences in Table 4. Interpretations are straightforward for Mendelian QTL, but are less so for imprinted QTL. For OR and BWT, the joint effect of inheriting a Line I allele from the sire and a Line C allele from the dam was estimated to maximize response compared with other genotypes at these QTL. However, in the case of the OR QTL, the dominance effect exceeded other effects and resulted in both heterozygotes with greater OR than either homozygote. In contrast, the dominance effect for the BWT QTL was small compared with other effects; thus, the IC heterozygote was predicted to have the greatest BWT and the CI heterozygote the least.

When a QTL was imprinted and the magnitude of the dominance effect was at least twice as large as the imprinting effect, as found for maternally expressed QTL for NN, both heterozygotes had means outside the range of the two homozygotes. However, imprinted QTL for which d was small, such as paternally expressed QTL for NN and maternally expressed QTL for MUM, resulted in positive effects in one heterozygote and one homozygote and negative effects in the other heterozygote and homozygote. When the estimated effect was negative, means for CC and CI (IC) genotypes were negative for paternally (maternally) expressed QTL and means for the other genotypes were positive. The four paternally expressed QTL for NN followed this pattern, suggesting that the Line C allele inherited from the sire would decrease the number of nipples. Maternally expressed QTL for MUM indicated a decrease in mummified pigs when the Line C alleles were inherited from the dam.

Composite interval mapping was designed to remove the residual error associated with known markers or QTL affecting the trait to detect additional QTL explaining less variation. The increase in power was readily apparent in scans for QTL for MUM (Figure 1) and AP (Figure 2). Some QTL identified in single-QTL models were not significant in full models as illustrated

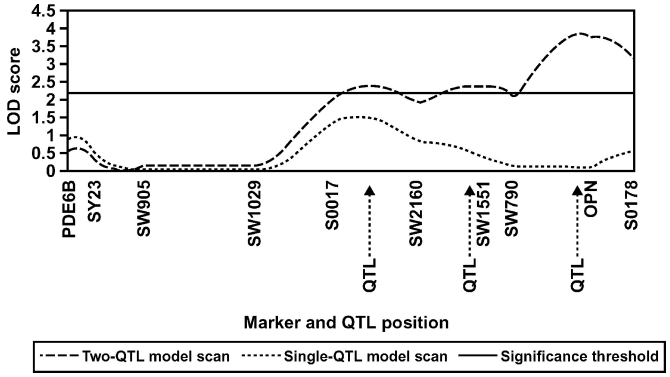

Figure 3. A QTL scan for age at puberty (AP) on SSC8. Marker names and relative positions of markers and QTL are shown on the x-axis.

for AP in Figure 3. For AP, six QTL were identified in the single Mendelian QTL scan; however, three of these QTL were not significant in subsequent scans. If several QTL are acting independently, then single- and multiple-QTL models are expected to identify the same QTL. But if epistasis exists, effects of multiple QTL are not independent (Schork et al., 1993), and QTL identified in single QTL scans may not be significant in sequential scans in which the one explaining the most variation is fixed. Another possibility is that only one QTL exists, but there is a covariance between marker genotypes at the two positions. Depending on the magnitude of the covariance, a single scan might identify two QTL, but after fixing the one explaining the greatest variation, a sequential scan may not identify additional QTL. Covariation between marker genotypes could be because chromosomal associations that occurred in base generation animals of the selection lines were maintained during the selection experiment, or be due to random sampling.

Several imprinted regions in the swine genome have been reported, although none of them is for reproductive traits. Hirooka et al. (2001) reported an imprinted region affecting NN on SSC2. Imprinting effects for backfat (de Koning et al., 2000; Rattink et al., 2000), lean growth (Jeon et al., 1999; Nezer et al., 1999), and coat color (Hirooka et al., 2002) on SSC2 have been reported. An imprinted QTL affecting MUM was identified on SSC2 in the study herein. This region comparatively maps to a highly imprinted region of human chromosome 11. Notable imprinted genes in this region include IGF_2 and H19 (Amarger et al., 2002).

Other imprinted effects reported in swine include a QTL on SSC4 affecting abdominal fat (Knott et al., 1998) and regions on SSC6 affecting i.m. fat and on SSC7 affecting muscle depth (de Koning et al., 2000). In humans, imprinted genes can be found on every chromosome and tend to cluster together (Tycko and Morison, 2002; Okita et al., 2003). Five of the 12 imprinted QTL identified in this study were on SSC6 and three were on SSC15.

Half-sib analyses have the potential to identify QTL when F_1 sires differ in QTL genotype. This can occur when founder lines are segregating, even if they do not differ in allele frequencies. Therefore, half-sib analyses may identify QTL not identified in line-cross analyses. However, if founder populations are completely inbred, a half-sib analysis requires four times the number of individuals to obtain the same power of the F_2 line-cross analysis (Weller, 2001). The power of half-sib analyses also increases with increasing family size. The LOD peaks in half-sib analyses did not reach the critical threshold levels and no QTL were indicated. However, LOD peaks for NSB and NN coincided with positions of peaks in the line-cross analyses. Family sizes in this experiment were insufficient to detect QTL with the half-sib analyses.

Implications

Multiple quantitative trait loci models with imprinting effects are more appropriate for analyzing F_2 data than single quantitative trait locus line-cross models. Knowledge of imprinting can be used to more effectively develop the parental lines used to produce F_1 females and to understand the possible effect on selection.

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