

QTL for Reproduction in the Nebraska Selection Lines

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This paper summarizes results of genome scans including both Mendelian QTL effects and QTL with imprinting effects for reproductive traits in the Nebraska Index Line. This line is reproductively superior to its contemporary control, producing approximately 4 pigs more per litter.

Methods

Population

The Index (Line I) and control lines (Line C) were formed from a composite population of Large White and Landrace that were reciprocally crossed in 1979. After three generations of random mating, Line I was selected 11 generations for an index of ovulation rate and embryonic survival to increase litter size. Line C was randomly selected. The phenotypic means after 10 generations are in Table 1.

Table 1. Generation 10 means for Nebraska Index (I) and Control (C) lines

Line	Ovulation rate	Number of fully formed pigs per litter	Number of live pigs per litter	Age at puberty, days	Nipple number
C	13.80	9.51	9.15	182	14.8
I	20.44	12.58	10.74	192	14.8

After farrowing their parity 1 litters, a selected sample of Generation 10 sows and boars were identified as grandparents for an F₂ resource population. The 14 sows and 4 boars from Line C with the lowest index were mated with the 12 gilts and 5 boars from Line I with the highest index to create F₁ litters. One son of each sire and 43 F₁ gilts were selected and mated, half- and full-sib matings were avoided, over three replicates (1st, 2nd, and 3rd parity) to produce 428 F₂ gilts in which phenotypic data were collected and genotypes for 151 microsatellite markers were determined.

Data

Number of nipples was recorded for all pigs. At 130 days of age, daily estrus detection was initiated in gilts to determine age at puberty. Ovulation rate was determined by counting corpora lutea either by laparotomy between 7 and 14 d following second estrus in gilts (Reps 1 and 2) or by dissection of ovaries obtained from sows slaughtered 7 to 14 d after expression of post-weaning estrus (Rep 1). At parturition, number of fully formed, live, stillborn, and mummified pigs were recorded.

Results of previous analyses

Cassady et al. (2001) previously analyzed ovulation rate, numbers of live and total pigs per litter, number of stillborn pigs per litter, nipple number and age at puberty of these same F₂ gilts with single QTL models. The QTL they identified are summarized in Table 2. Because single QTL models may overestimate the number of loci contributing to a trait, the data were re-analyzed with multiple QTL models and with models that included imprinting effects.

Table 2. Estimates of additive (a) and dominance (d) effects and standard errors from Cassady et al. (2001)

Trait ^a	Chromosome	Position, cM	a	d	LOD ^b
OR	9	1	-.25±.23	1.16±.40	2.64**
FF	11	52	-.86±.27	-.04±.47	2.8**
NBL	11	71	-.83±.30	.66±.61	2.54*
NSB	5	131	-.09±.19	1.13±.36	2.76**
	13	101	-.43±.13	-.50±.23	4.07**
NN	1	155	.17±.15	.70±.29	2.33*
	6	171	-.35±.12	.17±.23	2.46*
	7	62	.20±.11	-.41±.17	2.30*
	8	19	-.29±.12	.36±.19	2.87**
	11	46	-.03±.11	.67±.19	3.25**
AP	7	1	-2.38±2.10	10.52±3.23	2.81**
	7	58	-3.21±2.36	-10.83±4.13	2.43*
	8	101	7.65±2.85	7.44±4.58	2.41*
	8	136	7.14±2.39	-2.58±4.02	2.36*
	8	172	7.22±2.37	-10.59±4.45	3.81**
	12	9	-5.40±2.34	-.89±3.96	2.24*

^aOR = Ovulation rate, FF = number of fully formed pigs per litter, NBL = number of live pigs per litter, NSB = number of stillborn pigs per litter, NN = nipple number, and AP = age at puberty, days.

^bLikelihood-ratio test statistic.

*Genome wide significance threshold of $P < 0.10$.

** Genome wide significance threshold of $P < 0.05$.

Statistical analyses

Pedigrees and estimated genetic distances between markers were used to calculate the probabilities that a particular allele was inherited from each line. Four probabilities of inheriting the paternal allele from a specific line (I or C) and inheriting the maternal allele from a specific line (I or C) were calculated (i.e., Prob[Paternal = Line I, Maternal = Line C] = P_{IC}). Genetic coefficients (additive, dominance, paternal imprinting, and maternal imprinting) were calculated from contrasts of the four probabilities and are illustrated in Table 3.

All traits were analyzed by least squares. At each position on every chromosome, a schedule of statistical model comparisons was completed. The reduced model included fixed effects of replicate and sire-dam combination (included to adjust for polygenic effects). In addition to the effects in the reduced model, the full model included covariate coefficients of additive and dominance effects.

Table 3. Calculations of additive, dominance, paternal and maternal imprinting coefficients^a

Additive Coefficient	$1/2 (P_{CC} - P_{II})$
Dominance Coefficient	$P_{CI} + P_{IC}$
Paternal Coefficient	$(P_{CC} + P_{CI}) - (P_{II} + P_{IC})$
Maternal Coefficient	$(P_{CC} + P_{IC}) - (P_{II} + P_{CI})$

^a P_{II} is probability of paternal allele from Line I and maternal allele from Line I, P_{IC} is probability of paternal allele from Line I and maternal allele from Line C, P_{CI} is probability of paternal allele from Line C and maternal allele from Line I, and P_{CC} is probability of paternal allele from Line C and maternal allele from Line C.

A sequential multiple QTL search was then done using a forward selection model building procedure. From results of Cassady et al. (2001), the QTL with the highest LOD score was chosen as a fixed background effect and a scan was done for each trait fitting a second QTL. Calculated LOD scores were used to compare the full model with the reduced, single QTL model. If the largest LOD score exceeded the genome-wide threshold level, the first and second QTL were included in the reduced model and a model with an additional QTL was fitted. When the largest LOD score did not exceed the genome-wide threshold level, the reduced model was considered the best model with the appropriate number of QTL. This model was used as the reduced model for scanning the genome for the presence of imprinting effects.

Parent-of-origin effects were tested using a method proposed by J. Dekkers (personal communication). The following models were compared:

Full model (F): $y_{ijk} = \mu + r_i + p_j + c_{ak1}a_1 + c_{dk1}d_1 + c_{pk}p + c_{mk}m + c_{dk}d + e_{ikj}$,

Paternal model (P): $y_{ijk} = \mu + r_i + p_j + c_{ak1}a_1 + c_{dk1}d_1 + c_{pk}p + e_{ikj}$,

Maternal model (M): $y_{ijk} = \mu + r_i + p_j + c_{ak1}a_1 + c_{dk1}d_1 + c_{mk}m + e_{ikj}$, and

Null model (N): $y_{ijk} = \mu + r_i + p_j + c_{ak1}a_1 + c_{dk1}d_1 + e_{ikj}$, where

y_{ijk} is the phenotype of the i^{th} F_2 offspring,
 \mathbf{m} is the intercept,
 r_i is the fixed replicate effect,
 p_j is the fixed polygenic effect defined as the sire-dam combination,
 a_1, d_1 are, respectively, the additive and dominance effects for background QTL,
 c_{ak1} is the coefficient of the i^{th} individual for the additive component at the background QTL,
 c_{dk1} is the coefficient of the i^{th} individual for the dominance component at the background QTL,
 p, m, d are, respectively, the paternal, maternal, and dominance effects for the imprinted QTL,

c_{pk}	is the coefficient of the i^{th} individual for the paternal component at the imprinted QTL,
c_{mk}	is the coefficient of the i^{th} individual for the maternal component at the imprinted QTL,
c_{dk}	is the coefficient of the i^{th} individual for the dominance component at the imprinted QTL,
e_{ijk}	is the residual error.

Calculated LOD scores were used to compare F to N to initially detect parent-of-origin effects. To determine the mode of action, LOD scores were calculated for F versus P and F versus M. If only F versus M was significant, no maternal effect was detected, indicating paternal expression. No paternal effect was found if only F versus P was significant, indicating maternal expression. A conclusion of partial expression was made if both or neither contrasts were significant.

Genome-wide critical $\alpha = 0.01$, $\alpha = 0.05$, and $\alpha = .10$ levels were estimated using a permutation analysis with 475 random data shuffles for each trait. When shuffling data, associations between effects in the reduced model were retained. LOD scores for each permutation were calculated and ranked. Thresholds for critical values of $\alpha = 0.01$, $\alpha = 0.05$, and $\alpha = .10$ were LOD scores that exceeded the 99th, 95th, and 90th percentiles, respectively.

Results

Quantitative trait loci for the reproductive traits identified from the sequential statistical procedure are reported in Table 4. After fitting the QTL explaining the most variation for the trait, evidence for additional QTL affecting number of stillborn pigs (C12), number of fully formed pigs (C6), and ovulation rate (C15 and C8) were identified. No additional QTL were found for number born alive. Cassady et al. (2001) found evidence for five QTL affecting nipple number and six QTL affecting age at puberty. After fitting the QTL explaining the most variation for nipple number (C11) and age at puberty (C8, 172 cM) and holding these effects constant in models, no additional QTL for either trait were found. Therefore, evidence supporting only the QTL explaining the most variation for nipple number and age at puberty as reported by Cassady et al. (2001) was found and the effects of the additional QTL that they reported were probably the result of correlations between phenotypes and genotypes for these markers that could not be detected in single QTL models.

Imprinted regions, LOD scores, and estimates of imprinting effects are presented in Table 5. There was evidence of imprinting for number born alive ($P < 0.05$) on C18, age at puberty ($P < 0.05$) on C3, nipple number ($P < 0.10$) on C10, birth weight ($P < 0.10$) on C1, and weaning weight ($P < 0.10$) on C4.

Imprinting effects occur when an allele inherited from the sire is expressed differently than when the same allele is inherited from the dam. Paternal imprinting for a gene on C10 affecting nipple number was detected. On average, inheriting an allele from a Line

C sire (CC or CI) compared with an allele from a Line I sire (II or IC) resulted in 0.07 ± 0.04 fewer functional teats. There was no detectable difference between alleles inherited from the dams.

Partial imprinting occurs when maternal and paternal imprinting are simultaneously affecting a trait. In this case, the largest difference is between the two heterozygotes (CI vs. IC). Partial imprinting of QTL on C18 affecting number born alive was detected. On average, inheriting Line C alleles from the sire (CC or CI) increased number born alive compared with Line I alleles (IC or II). Inheritance of Line I alleles from the dam (II or CI) also increased number born alive by 0.37 ± 0.10 pigs per litter compared with Line C alleles (CC or IC). On average, receiving the Line C allele from the sire and the Line I allele from the dam (CI) resulted in approximately 0.7 more live pigs per litter than the reciprocal heterozygote (IC). In addition, evidence for partial imprinting for a QTL on C3 affecting age at puberty between markers *SW2047* and *S0002* was found. On average, daughters receiving Line C alleles from sires were delayed in puberty compared with gilts receiving Line I alleles. In contrast, gilts receiving Line C alleles from their dam reached puberty sooner than daughters inheriting Line I alleles from their dam.

Although chromosomal regions have been identified that affect reproduction and early pig weights, the exact genes causing these effects are still unknown. Further research is needed to identify these genes.

Table 4. Chromosomal regions and estimation of effects by sequentially fitting QTL to the model

Trait ^a	Order ^b	C ^c	cM ^d	Flanking Marker 1	Flanking Marker 2	LOD ^e	a ^f	d ^f
OR	1	9	1	SW21	S0024	2.64**	-0.25 ± 0.21	0.49 ± 0.36
OR	2	15	48	SW1989	SW1945	2.89**	0.15 ± 0.24	-0.57 ± 0.37
OR	3	8	20	SY23	SW905	2.57*	0.23 ± 0.26	-1.58 ± 0.47
FF	1	11	52	SW151	SW435	2.80**	-0.11 ± 0.24	-0.40 ± 0.43
FF	2	6	108	SW122	SW2173	2.91**	-0.49 ± 0.22	-0.39 ± 0.34
NBL	1	11	71	SW435	SW1465	2.54*	-0.83 ± 0.30	0.66 ± 0.61
NSB	1	13	101	SW1056	SW38	4.07**	-0.26 ± 0.12	-0.51 ± 0.21
NSB	2	12	60	SW874	S0090	2.58**	0.08 ± 0.13	-0.19 ± 0.22
NN	1	11	46	SW1632	SW151	3.25*	-0.03 ± 0.11	0.67 ± 0.19
AP	1	8	172	SW790	OPN	3.81**	7.22 ± 2.37	-10.59 ± 4.45

^aNSB = number of stillborn pigs; FF = number of fully formed pigs; OR = ovulation rate.

^bOrder that QTL were added to the model.

^cChromosome number.

^dRelative position in Kosambi centimorgans.

^eLOD score corresponding to entry into model.

^fEffect estimated using full model with appropriate QTL and imprinting effects in units of pigs for litter traits and corpora luteum for ovulation rate.

* Genome-wide significance threshold of $P < 0.10$.

** Genome-wide significance threshold of $P < 0.05$.

Table 5. Results from fitting a model with imprinting.

Trait ^a	C ^b	cM ^c	Flanking Marker 1	Flanking Marker 2	LOD ^d	p ^e	m ^e
NBA	18	25	SW1984	SW787	3.48**	0.34 ± 0.11	-0.37 ± 0.10
AP	3	71	SW2047	S0002	3.51**	3.44 ± 1.10	-2.95 ± 1.03
NN	10	75	SW1991	SW951	2.81*	-0.07 ± 0.04	NA

^aNBA = number born alive; AP = age at puberty; NN = nipple number.

^bChromosome number.

^cRelative position in Kosambi centimorgans.

^dLOD score corresponding to presence of imprinting effects.

^eImprinting effect estimated using a model with appropriate significant QTL in units of pigs for litter traits, days for age at puberty, and nipples for nipple number.

* Genome-wide significance threshold of $P < 0.10$.

** Genome-wide significance threshold of $P < 0.05$.

Literature Cited

Cassady, J. P., R. K. Johnson, D. Pomp, G. A. Rohrer, L. D. Van Vleck, E. K. Spiegel, and K. M. Gilson. 2001. Identification of quantitative trait loci affecting reproduction in pigs. *J. Anim. Sci.* 79:623-633.