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Major Genes Affect Reproduction and Early Growth

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Summary and Implications

The Nebraska Index Line is reproductively superior to its contemporary control, producing approximately four pigs more per litter. However, the genes or quantitative trait loci (QTL) that cause these differences are unknown. A previous study with an F_2 resource population created by crossing the NE Index and Control lines identified one QTL affecting ovulation rate, one QTL affecting number of fully formed fetuses, one QTL affecting number of pigs born alive, two QTL affecting number of stillborn pigs, five QTL affecting nipple number, and six QTL affecting age at puberty. However, individual birth weight and weaning weight had not been included in the analyses. In addition, improved statistical models with greater power to identify QTL and test for additional kinds of gene action have been developed. The objective of this experiment was to apply these more powerful models to the data from the F_2 resource population to identify additional chromosomal regions that contain genes that affect reproduction and early growth. Using standard statistical techniques identical to those used in the previous analyses, evidence was found for QTL ($P < 0.10$) affecting birth weight on chromosomes (C) 8 and 12. Additive effects of the C8 and C12 alleles inherited from the control line were -20 ± 17 g and -59 ± 19 g, and dominance effects were 85 ± 31 g and -73 ± 37 g, respectively. No QTL were detected for weaning weight. While fitting the largest QTL for the respective trait as a background effect to increase the statistical power, additional QTL affecting number of stillborn pigs on C12, fully formed pigs on C6, and ovulation rate on C15 and C8 were identified. No additional QTL were detected for number born alive, nipple number, age at puberty, or birth weight. Statistical procedures to test for imprinting or parent-of-origin effects were then used. Imprinting is a genetic phenomena in which an allele is expressed when inherited from one parent, but is not expressed when inherited from the other parent. Paternal imprinting describes the situation when an allele is expressed only when it is inherited from the father, whereas maternal imprinting occurs when the allele is expressed only when inherited from the mother. Partial imprinting occurred for a gene on C18 affecting number born alive ($P < 0.05$) and for a gene on C3 affecting age at puberty ($P < 0.05$). Evidence existed for paternal imprinting of a gene on C10 affecting nipple number and for maternal imprinting of a gene on C1 affecting birth weight and a gene on C4 affecting weaning weight ($P < 0.10$). Knowledge of imprinting could be used to more effectively develop the parental lines used to produce F_1 females. Selection within maternal sire lines should increase the frequency of beneficial paternally and partially imprinted QTL affecting litter size, nipple number, and age at puberty. Selection within sire lines should also increase the frequency of beneficial maternally imprinted QTL affecting birth and weaning weight in

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 live 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

Table 2. 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]Probabilities for calculations were defined as:
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.

grand-progeny. Selection within maternal dam lines should increase the frequency of beneficial maternally imprinted QTL affecting litter size, age at puberty, birth and weaning weights. F_1 gilts produced from the crossing of these two lines should produce larger litters, have greater number of nipples, reach puberty sooner and produce heavier pigs at birth and weaning.

Introduction

Identification of major genes affecting economic traits is an important research goal in the field of animal genetics. Knowledge of effects of major genes can be used to enhance selection response and more effectively design breeding systems using specialized sire and dam lines.

Previous research with an F_2 resource population produced by crossing the Nebraska Index and Control lines identified one QTL affecting ovulation rate, one QTL affecting number of fully formed fetuses, one QTL affecting number of pigs born alive, two QTL affecting number of stillborn pigs, five QTL affecting nipple number, and six QTL affecting age at puberty. Birth and weaning weights were not included in that analysis nor were parent-of-origin (or imprinting) effects tested. The objective of this study was to use these same data with a sequential statistical procedure and test for imprinting effects to identify additional genomic regions that affect reproductive traits, individual birth weight, and weaning weight.

Methods

Population

A base composite population of Large White and Landrace was formed in 1979. After three generations of random mating, the Nebraska Index Line (Line I) and Control Line (Line C) were established. Line I was selected 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 presented in Table 1.

At Generation 10, pigs were sampled from both lines to create an F_2 resource population. From Line C, 14 gilts and four boars were chosen. From Line I, 12 gilts and five boars were selected. Reciprocal crossing among lines was used to create the F_1. A total of 428 F_2 gilts from three replicates were used in this study.

Data

At birth, number of nipples and birth weight were recorded for all pigs. Weaning weight was recorded at approximately 12 days of age. At 130 days of age, estrus detection was done until gilts showed a second estrus, recording the date of pubertal estrus. In Replicates 2 and 3, gilts underwent laparotomy between 7 and 14 days following second estrus to count number of corpora lutea to measure ovulation rate. Gilts were naturally mated to crossbred boars from another population. At parturition, number of fully formed, live, stillborn and mummified pigs were recorded. In Replicate 1, sows were slaughtered between 7 and 14 days after expression of post-weaning estrus and ovaries were collected and dissected to measure ovulation rate.

White blood cells, liver tissues, and tail tissues were collected from grandparents, F_1, and F_2 pigs and DNA was extracted from these tissues. Each pig was genotyped for 151 molecular DNA markers spanning all chromosomes.

Genetic Probabilities

Pedigree genotypes and estimated genetic distances between markers were used to calculate the probabilities that a particular allele was inherited from each line. These probabilities were then used in QTL analyses. Estimated genetic distances were converted to recombination fractions, the probability of a crossover between any pair of markers. 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 2.

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Statistical Analysis

All traits were analyzed by least squares. Birth weight was adjusted for number of fully formed pigs in the litter in which pigs were born and weaning weight was adjusted for number of pigs at weaning in the nurse litter and for age at weaning. At each position on every chromosome, a schedule of statistical model comparisons was completed (See Figure 1.). Models were compared by calculations of LOD scores. Rejection levels were obtained from 475 permutations of the data. Permutations randomly shuffle the data to estimate how likely an event would occur if the observations had random associations with the molecular information.

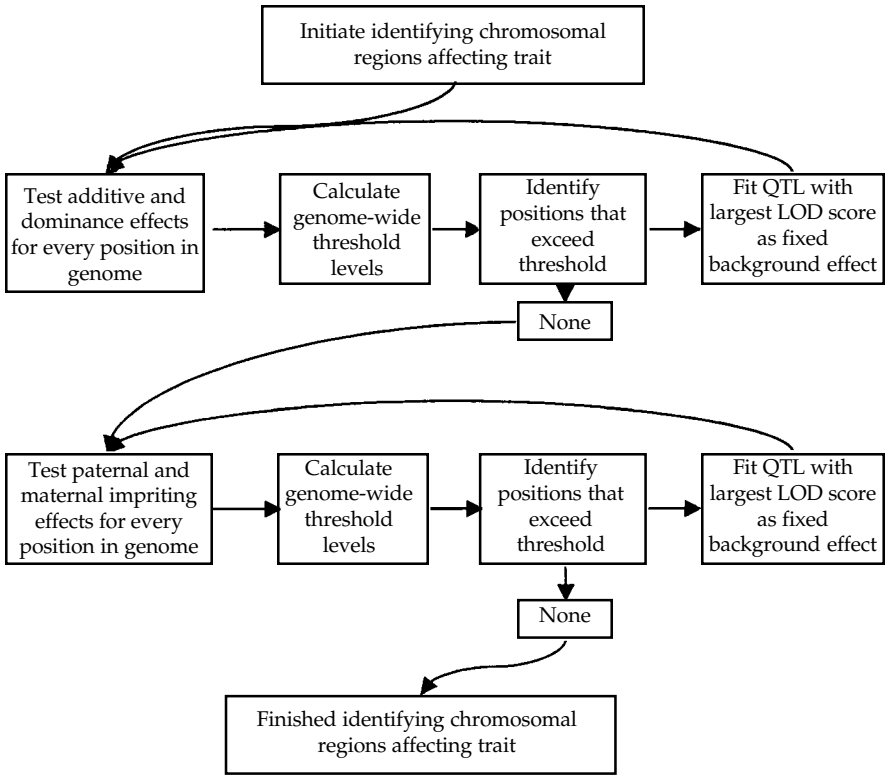

Figure 1. Schedule of model comparisons.

Results and Discussion

Analyses with a single QTL model produced suggestive evidence ($P < 0.10$) for QTL affecting birth weight on chromosomes (C) 8 and 12. The additive effects of the allele inherited from Line C were estimated to be -20 $\pm$ 17g and -59 $\pm$ 19g and dominance effects were 85 $\pm$ 31g and -73 $\pm$ 37g for the QTL on C8 and C12, respectively. The additive effect of the QTL on C8 is explained as individuals that had both alleles from the control line averaged 20g less birth weight than individuals with two alleles from the Index line. The dominance effect is explained as individuals receiving a C8 allele from each line averaged greater birth weights (by 85g) than the mean of homozygous individuals. The increased inbreeding that occurred in both parental lines likely increased the frequency of homozygotes, decreasing the average birth weight within lines. Crossing increased heterozygosity and resulted in an advantage for pigs that inherited an allele from each line. The negative additive effect on C12 is explained as individuals receiving both alleles from Line C having lower birth weight than individuals receiving both alleles from Line I. Although mean birth weight of Line I was less than Line C, Line I possessed alleles that increased birth weight compared with Line C. No QTL were detected for weaning weight.

Quantitative trait loci identified from the sequential statistical procedure are reported in Table 3. Evidence for an additional QTL affecting number of stillborn pigs was found on C12. An additional QTL affecting number of fully formed pigs was found on C6, and two additional QTL for OR on C15 and C8 were identified. No additional QTL were found for number born alive, nipple number, age at puberty, or birth weight.

Imprinted regions, LOD scores and estimates of imprinting effects are presented in Table 4. 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. In the case of paternal imprinting, alleles inherited from the sires will have different effects on a trait and the same alleles inherited from the dam will not have any effect on a trait. 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 $\pm$ 0.04 fewer functional teats. There was no detectable difference between alleles inherited from the dams.

For maternal imprinting, alleles inherited from the dams will have different effects on a trait and the alleles inherited from the sire will not have any effect. There was evidence of maternal imprinting for a gene on C1 affecting birth weight and for a gene on C4 affecting wean-

Table 3. Chromosomal regions and estimation of effects by sequentially fitting additional QTL to the model.

Trait[a]	Order[b]	C[c]	cM[d]	Flanking Marker 1	Flanking Marker 2	LOD[e]	a[f]	d[f]
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
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
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

[a]NSB = number of stillborn pigs; FF = number of fully formed pigs; OR = ovulation rate.
[b]Order that QTL were added to the model.
[c]Chromosome number.
[d]Relative position in Kosambi centimorgans.
[e]LOD score corresponding to entry into model.
[f]Effect 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 4. 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
BWT	1	91	SW952	SW307	2.88*	NA	-3.25 ± 0.91
WWT	4	149	SW445	MP77	2.94*	NA	7.67 ± 2.25

[a]NBA = number born alive; AP = age at puberty; NN = nipple number; BWT = individual pig birth weight; WWT = individual pig weaning weight.
[c]Chromosome number.
[c]Relative position in Kosambi centimorgans.
[d]LOD score corresponding to presence of imprinting effects.
[e]Imprinting effect estimated using a model with appropriate significant QTL in units of pigs for litter traits, days for age at puberty, nipples for nipple number, and grams for weight traits.
* Genome-wide significance threshold of P < 0.10.
** Genome-wide significance threshold of P < 0.05.

ing weight. On C1, piglets with maternally inherited Line C alleles (CC or IC) averaged lower birth weights than pigs with maternally inherited Line I alleles (II or CI). Although Line C piglets had larger birth weights than Line I, this QTL can only partially offset the effects of other genes that caused increased birth weight in Line C. Piglets with maternally inherited Line C alleles averaged greater weaning weights than those with maternally inherited Line I alleles. A possible explanation may be that as litter size increased with selection, the energy requirements for lactating sows also increased. There may have been a selection advantage in Line I for maternal alleles that decreased or maintained energy requirements for lactating sows with the potential for more pigs per litter. Decreased pig survival to weaning and decreased number weaned in Line I may have been affected by this QTL.

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.

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