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Identification of quantitative trait loci affecting reproduction in pigs[1,2,3]

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ABSTRACT: The objective of this research was to identify chromosomal regions harboring QTL affecting reproduction in pigs. A three-generation resource population was developed by crossing low-indexing pigs from a randomly selected control line (C) with high-indexing pigs of a line selected for increased index of ovulation rate and embryonic survival (I). Differences between Lines I and C at Generation 10 were 6.7 ova and 3.3 fetuses at 50 d of gestation and 3.1 fully formed and 1.6 live pigs at birth. Phenotypic data were collected on F_2 females, born in three replicates, for ovulation rate (n = 423), age at puberty (n = 295), litter size (n = 370), and number of nipples (n = 428). Litter-size data included number of fully formed, live, stillborn, and mummified pigs. Grandparent, F_1, and F_2 animals were genotyped for 151 microsatellite markers distributed across all 18 autosomes and the X chromosome. Genotypic data were available on 423 F_2 females. Average spacing between markers was 19.3 Kosambi centimorgans. Calculations of logarithms of odds (LOD) scores were by least squares, and fixed effects for sire-dam combination and replicate were included in the models. Genome-wide significance level thresholds of 5% and 10% were calculated using a permutation approach. There was evidence ($P < 0.05$) for QTL affecting ovulation rate on SSC9, age at puberty on SSC7 and SSC8, number of nipples on SSC8 and SSC11, number of stillborn pigs on SSC5 and SSC13, and number of fully formed pigs on SSC11. There was evidence ($P < 0.10$) for additional QTL affecting age at puberty on SSC7, SSC8, and SSC12, number born live on SSC11, and number of nipples on SSC1, SSC6, and SSC7. Litter size is lowly heritable and sex-limited. Therefore, accuracy of selection for litter size may be enhanced by marker-assisted selection. Ovulation rate and age at puberty are laborious to measure, and thus marker-assisted selection may provide a practical and efficient method of selection.

Key Words: Pigs, Quantitative Trait Loci, Reproduction

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Introduction

Response to direct selection for litter size has been slow because heritability is low and litter size is sex-limited (Ollivier, 1982; Lamberson et al., 1991). Johnson et al. (1984) and Bennett and Leymaster (1989) suggested that greater response can be expected from selection for an index of ovulation rate and embryonic survival or uterine capacity than from direct selection. Johnson et al. (1999) reported increases in number of fully formed and live pigs at birth in response to 14 generations of selection for ovulation rate, embryonic survival, and litter size. Marker-assisted selection (**MAS**) may be a method of selecting for components of litter size in both sexes at a very young age and improving accuracy of selection. Simulation studies have demonstrated potential benefits of MAS (Zhang and Smith, 1992, 1993; Edwards and Page, 1994). Efficiency of

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[2]Mention of trade names is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be available.

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Table 1. Generation 10 phenotypic means for the control and index lines

Line	Ovulation rate[a]	Fetuses[b]	Fully formed[c]	Born alive[d]	Embryonic survival, %[e]	Age of puberty[df]	No. of nipples[g]
Control	13.80	10.30	9.51	9.15	76.2	182	14.8
Index	20.44	13.64	12.58	10.74	71.6	192	14.8

[a]Number of corpora lutea at 50 d of gestation.
[b]Number of fetuses at 50 d of gestation.
[c]Number of fully formed pigs at birth.
[d]Number of pigs born alive.
[e]Embryonic survival was calculated as the percentage of corpora lutea represented by number of fetuses at 50 d gestation.
[f]Age of first observed estrus in d.
[g]Number of nipples measured on pigs at birth.

MAS relative to phenotypic selection is greatest for lowly heritable and sex-limited traits (Lande and Thompson, 1990). Simulation of response to MAS has been shown to be greater than response to selection on either phenotypes or markers alone (Zhang and Smith, 1992).

To implement MAS, QTL must be identified and their effects estimated. Several QTL affecting growth and carcass composition have been identified in pigs. Relatively few QTL explaining a significant proportion of the genetic variance for litter size and its component traits have been identified. Evidence of QTL affecting number of corpora lutea on SSC3 (Rohrer et al., 1999), SSC8 (Rathje et al., 1997; Rohrer et al., 1999; Wilkie et al., 1999), and SSC10 (Rohrer et al., 1999) has been reported. Wilkie et al. (1999) also reported evidence of a QTL associated with number of stillborn pigs on SSC4. The objective of this research was to identify chromosomal regions harboring QTL that explain a portion of genetic variance associated with reproduction in pigs.

Materials and Methods

Population

An F_2 resource population was created at the University of Nebraska (Rathje et al., 1997). Grandparent animals were selected from a line that had been selected 10 generations for an index (**I**) of ovulation rate and embryonic survival or its randomly selected control (**C**). Means for Generation 10 are given in Table 1. Twelve high-indexing females and five males out of high-indexing dams were selected from Line I. Fourteen low-indexing females and four males out of low-indexing dams were selected from Line C. Line I males were randomly mated to Line C females, and Line C males were randomly mated to Line I females to create F_1 pigs. Fifty F_1 females, with at least one randomly selected per full-sib family, were mated with 10 F_1 males, with at least one randomly selected from each paternal half-sib family, to produce F_2 progeny. The F_2 progeny were born in three replicates. Replicate 1 was produced by randomly mating F_1 animals while avoiding full-sib and half-sib matings. Replicate 2 progeny were produced by a second set of random matings. To increase the number of full-sibs, the matings made in Replicate 2 were repeated for Replicate 3. In total, there were 428 F_2 females from 79 full-sib families.

Measurement of Traits

Number of nipples was recorded at birth. At 8 mo of age, F_2 gilts were naturally mated to unrelated crossbred boars. At parturition, numbers of fully formed, stillborn, live, and mummified pigs were recorded. Pigs were weaned at approximately 12 d of age.

A management constraint imposed to prevent transmission of a particular disease to Replicate 1 animals prevented measurement of age at puberty. After weaning their litters, sows of Replicate 1 were monitored daily for estrus. Between 7 and 14 d after expression of estrus, sows were slaughtered and reproductive tracts were recovered. Ovaries were dissected and number of corpora lutea was recorded as a measure of ovulation rate. Age at puberty was recorded in Replicate 2 and 3 gilts by exposing them daily to mature boars and observing for signs of estrus. Seven to 14 d after second expression of estrus, laparotomy was performed and number of corpora lutea was recorded.

Tissue Collection

White blood cells, liver tissues, and tail tissues were all used as sources of DNA. After collection, tissues were immediately placed on ice for transport to the laboratory. Samples were stored at –20°C or colder. Whole blood samples (100 mL) were collected via jugular venipuncture from grandparents, F_1 dams, and F_2 Replicate 2 pigs. Blood samples from F_1 dams and F_2 Replicate 2 pigs were centrifuged, and white blood cells were removed and stored for DNA extraction. Tail tissues were collected from all F_1 and F_2 pigs at 1 to 3 d of age. Liver samples were collected from grandparents, F_1 boars, and F_2 Replicate 1 pigs at slaughter.

Laboratory Procedures

Microsatellite markers were preselected from the USDA swine linkage map (Rohrer et al., 1996) for testing based on their location and informativeness in two sires (white composite) in the USDA-ARS, U.S. Meat Animal Research Center (**MARC**) (Keele et al., 1994)

database. The two sires used for prescreening markers had a genetic background similar to that of the pigs in this study. Markers heterozygous in one or both of these boars were screened through a sample of grandparents to determine their informativeness. Markers known to have a null allele were not used. A total of 151 microsatellite markers were considered to be informative, genotyped in the entire population, and used in the final analysis.

Genomic DNA was obtained from tissues using a proteinase K digestion followed by phenol/chloroform extraction and precipitation with isopropanol. The DNA concentration was determined using spectrophotometry. Samples were diluted to a standardized concentration of 50 ng/μL.

Primer pair sequences for markers identified for testing in the Nebraska resource population were obtained from the USDA database (USDA, 1996). Primers were synthesized (Li-Cor, Lincoln, NE) attaching one of two fluorescent infrared dyes (IRD700 or IRD800) to the 5′ end of each forward primer.

Genotyping was done in 96-well plates using PCR and denaturing gel electrophoresis. A Li-Cor Model 4200 IR^2 System was used. A 10-μL PCR reaction was used. Ingredients included 1× supplied *Taq* buffer ($MgCl_2$-free), 0.2 m*M* each dNTP, 0.5 unit *Taq* Gold polymerase (Perkin Elmer, Foster City, CA), 2.5 m*M* $MgCl_2$, and 50 ng of genomic DNA. Primers were tested to optimize amount of forward labeled primer, which was added at 0.1, 0.2, or 0.3 pmol. Reverse primer was always added at 1 pmol. Reactions were done using a "touchdown" PCR protocol in MJ Tetrad thermal cyclers (MJ Research, Waltham, MA). For eight cycles annealing temperatures were decreased 2°C per cycle ranging

Table 2. Microsatellite markers used in the Nebraska resource population

C[a]	Marker[b]	Pos[c]	I[d]	C[a]	Marker[b]	Pos[c]	I[d]	C[a]	Marker[b]	Pos[c]	I[d]	C[a]	Marker[b]	Pos[c]	I[d]
1	SW1514	0	60	5	SW413	0	46	9	SW21	0	82	14	SW1631	0	77
	SW1515	23	58		SWR453	67	36		S0024	21	39		SW1027	10	69
	SW64	36	21		SW2	89	35		SW827	47	53		SW2612	33	27
	SW952	79	40		SW191	105	24		SW511	62	44		SWR84	41	49
	SW307	96	42		S0018	120	55		SW727	77	25		SW761	71	49
	SW745	118	49		SWR1112	152	24		SW2093	109	33		SWC27	103	56
	SW373	165	59		SW378	175	31		SW2116	134	75	15	SW1416	0	38
	SW1301	196	59	6	SW2535	0	12	10	SW767	0	29		SW919	11	89
2	SWC9	0	69		SW2406	14	61		SW497	21	32		SW964	40	50
	SW2623	16	47		SW1353	26	31		SW2491	32	30		SW1989	47	55
	S0141	38	54		SW1067	74	26		SWR198	50	41		SW1945	61	38
	FSHBMS	67	70		HAL	91	24		SW1991	68	60		SW1683	69	42
	SW766	85	30		SW122	107	59		SW951	94	45		SW1983	88	58
	S0370	104	43		SW2173	117	47		SW2067	126	71		SW1119	113	41
	SWR2157	111	59		SW1059	123	29	11	SW1460	0	61	16	SW813	0	67
	S0036	151	46		DG93	153	65		SW1632	13	9		SW2411	16	31
3	SW2021	0	84		SW322	177	32		SW151	47	39		CGT27	40	60
	SW2429	13	39		SW1328	192	62		SW435	58	48		SW81	48	36
	SW2527	33	79	7	S0025	0	69		SW1465	90	54		SW2517	71	61
	S0206	37	58		SW1873	9	27		SW13	93	32		S0105	102	57
	SW902	50	54		SW1354	26	62	12	S0143	0	57	17	SW335	0	82
	SW160	63	14		SW2155	46	13		SW957	24	18		SW1891	17	61
	SW2047	70	55		TNF	66	77		MP75	35	35		SWR1004	18	46
	S0002	101	63		SWR1928	91	39		SW1307	41	19		S0296	35	69
	SW349	111	71		S0115	111	67		SW874	55	75		SW2142	43	67
	SW2532	127	43		S0101	141	28		S0090	71	20		S0292	53	43
4	SW2404	0	67		SW2108	152	49		SWC23	84	40		S0332	91	32
	S0301	36	36		SW764	170	39		SW2180	94	64		SW2427	99	31
	SW969	68	50	8	PDE6B[e]	0	61		SWR1021	97	22	18	SW1023	0	41
	SW45	79	46		SY23[e]	9	70	13	SWR1941	0	48		SW1984	24	72
	S0107	82	65		SW905	26	41		SW344	35	81		SW787	27	53
	SW589	94	28		SW1029	63	42		SW937	56	35		S0177	63	42
	S0214	99	23		S0017	89	63		SW873	69	54	X	SW949	0	31
	SW512	104	75		SW2160	117	42		SW1030	72	65		SW980	5	63
	SW445	131	54		SW1551	139	58		SW1056	93	29		SW2470	37	66
	MP77	150	53		SW790	149	51		SW38	102	45		SW1943	104	80
					OPN	175	50		S0289	113	64		SW1608	115	69
					S0178	189	43		SW769	119	38		SW707	123	90
									S0215	123	16				

[a]Chromosome.
[b]Primer sequences may be found at http://www.marc.usda.gov/genome/swine/.
[c]Positions are reported in Kosambi centimorgans.
[d]Percentage of F_1 meioses for which allelic line of origin could be determined.
[e]Unpublished microsatellite markers (G. Rohrer and E. Campbell, unpublished data).

Table 3. Phenotypic mean, standard deviation, and range for grandparents, F_1, and F_2 gilts

Trait	n	Mean	SD	Range	n	Mean	SD	Range
	Index line, grandparent females				Control line, dams of grandparent males			
Number of nipples	12	14.1	1.4	12–17	4	14.5	—	13–16
Age at puberty	12	182.5	22.8	145–227	4	162.5	—	130–204
Ovulation rate	12	32.1	16.8	14–65	4	11.3	—	9–14
Pigs born alive	12	9.5	3.2	6–14	4	7.0	—	6–8
Stillborn pigs	12	1.8	1.1	0–3	4	0.5	—	0–2
Mummified pigs	12	1.58	1.2	0–3	4	0.5	—	0–1
Fully formed pigs	12	11.3	3.3	6–16	4	7.5	—	6–8
	Control line, grandparent females				F_1 females			
Number of nipples	14	14.4	.8	13–16	43	14.5	1.1	12–17
Age at puberty	14	182.4	39.6	145–277	43	187.2	28.2	141–231
Ovulation rate	14	13.5	2.2	11–18	43	15.9	2.5	13–21
Pigs born alive	14	8.3	2.1	5–11	39	10.1	2.8	4–15
Stillborn pigs	14	0.4	0.6	0–2	39	0.7	1.0	0–4
Mummified pigs	14	0.1	0.4	0–1	39	0.5	1.4	0–8
Fully formed pigs	14	8.6	2.3	5–11	39	10.9	3.0	4–16
	Index line, dams of grandparent males				F_2 females			
Number of nipples	5	15.4	—	14–18	428	14.3	1.3	11–19
Age at puberty	5	169.0	—	150–220	295	181.3	23.6	134–231
Ovulation rate	5	53.0	—	38–79	423	15.8	3.3	8–44
Pigs born alive	5	9.0	—	5–11	370	10.5	2.7	0–17
Stillborn pigs	5	0.6	—	0–2	370	0.9	1.4	0–11
Mummified pigs	5	1.8	—	0–4	370	0.5	0.9	0–8
Fully formed pigs	5	9.6	—	5–13	370	11.4	2.7	1–19

from 68°C to 54°C. This was followed by 30 cycles with an annealing temperature of 54°C. The PCR product was diluted 2:1 with stop buffer and denatured for 2.5 m at 95°C. The product was then placed on ice and loaded into a 7% denaturing polyacrylamide gel.

Each genotype was manually scored by two independent technicians using RFLPscan Plus version 3.0 (Scanalytics, Fairfax, VA). Genotypes were then compared and those not in agreement were re-evaluated. If a clear agreement could not be reached, genotypes were regenerated or entered as missing data. Occurrence of missing genotypes was 1.4%. Marker data were analyzed to identify deviations from Mendelian segregation. This analysis revealed that tissue samples collected for the grandsires were incorrectly identified. Therefore, all grandsires were genotyped retrospectively using information from granddams and F_1 progeny. Grandsires that passed the same allele to each of their progeny were scored as having one allele known and one allele missing. Thus, grandsires were never scored as homozygous. Power was lost only in cases in which an F_1 progeny and its dam were like-heterozygotes and the sire had one known and one unknown allele. This situation occurred only 7% of the time. Loss of power was actually less than 7% because some grandsires and granddams were probably like-heterozygotes, in which case the marker would not be informative. In addition, the program of Haley et al. (1994), used to calculate coefficients of additive and dominance effects, considers the entire linkage group and continues along it until a marker with known line of origin is found. Thus, some information lost at a single locus would have been recovered using adjacent markers.

Genotypes for one F_1 sire did not match those of any of the grandparents. The cause was incorrect identification during cross-fostering to standardize litter size. This boar came from the same population, but its exact parentage was not known. This boar sired 53 F_2 females. A fictitious sire and dam were created for the boar and their genotypes were entered as missing data. Therefore, this sire was completely uninformative, although line of origin information can still be partially attained for these pigs through their dams.

Statistical Analysis

Genotypic data were first analyzed using CRIMAP version 2.4 (Green et al., 1990) to estimate distance between markers. The CHROMPIC option of CRIMAP was used to identify potential genotyping errors. Cases in which three crossovers were indicated within a linkage group were rechecked for accuracy.

The method and program described by Haley et al. (1994) were used to calculate coefficients of additive and dominance effects. A least squares approach was then used to regress phenotypic data using the coefficients of additive and dominance effects as covariates. Two models were compared. Both models included fixed

effects of replicate and sire-dam combination. Sire-dam combination was included as a fixed effect to adjust for polygenic effects.

One model included covariate coefficients of additive and dominance effects, and the other excluded the coefficients of additive and dominance effects. The reduced model can be written as $\mathbf{y} = \mathbf{Xb} + \mathbf{e}$, where **y**, **b**, and **e** are vectors of phenotypic, fixed, and residual effects, respectively, and **X** is a known design matrix. The full model was $\mathbf{y} = \mathbf{ac}_1 + \mathbf{dc}_2 + \mathbf{Xb} + \mathbf{e}$, where **y**, **b**, **e**, and **X** are as previously described and $\mathbf{c}_1$ and $\mathbf{c}_2$ are vectors of coefficients of the additive (**a**) and dominance (**d**) effects, respectively. The additive coefficient was computed as the difference between the probability that a homozygous individual inherited both alleles from Line C and the probability that it inherited both alleles from Line I. The dominance coefficient is the probability that an individual is heterozygous. The program of Haley et al. (1994) produces the regression of phenotype on the additive coefficient as the deviation of individuals that are homozygous for the allele inherited from line C from the mean of the two homozygous genotypes. The regression of phenotype on the dominance coefficient is the deviation of heterozygous individuals from the mean of the two homozygous genotypes.

Genome-wide critical $\alpha = 0.05$ and $\alpha = 0.10$ levels were estimated using a permutation analysis with 1,000 random data shuffles as described by Churchill and Doerge (1994). While shuffling data, associations between fixed effects and phenotypes were retained.

A preliminary least squares regression analysis was done to determine whether a single permutation threshold could be used for all traits or whether permutation thresholds differed significantly among traits. At the time of the preliminary analysis only SSC15 data were available. Phenotypic data from each trait and genotypic data from SSC15 were shuffled 7,300 times and analyzed. Data from all seven traits were combined (n = 51,100) and LOD scores were ranked from greatest to least. The LOD exceeding the 95th percentile was LOD = 2.55. Next, data from each trait (n = 7,300) were ranked from greatest to least, and the number of observations exceeding LOD = 2.55 was determined. Chi-square was used to test differences between expected and observed number of observations exceeding LOD = 2.55. It was determined that thresholds for each trait should be estimated independently. A total of 19,000 LOD scores for each trait were calculated, with 1,000 permutations for each chromosome. The 19,000 LOD scores were then ranked. Thresholds for a critical value of $\alpha = 0.05$ and $\alpha = 0.10$ were the LOD scores that exceeded the 95th and 90th percentiles, respectively. These are values that a LOD score would exceed by chance only 5% or 10% of the time, respectively, when considering the entire genome.

Table 4. Estimates of additive (a) and dominance (d) effects and standard errors for putative QTL affecting reproduction in the pig

Trait[a] and chromosome		df[b]	cM[c]	Informativeness, %[d]	a[e]	SE	d[f]	SE	LOD[g]	Threshold LOD[h]
OR	9	340	1	78	–0.25	0.231	1.157**	0.4	2.64**	2.23/2.64
FF	11	279	52	58	–0.856**	0.268	–0.036	0.47	2.8**	2.33/2.75
NBL	11	279	71	45	–0.829**	0.295	0.663	0.607	2.54*	2.31/2.74
NSB	5	279	131	46	–0.087	0.194	1.134**	0.359	2.76**	2.31/2.76
	13	279	101	70	–0.43**	0.134	–0.502**	0.23	4.07**	
NN	1	340	155	41	0.174	0.148	0.697**	0.289	2.33*	2.24/2.65
	6	340	171	55	–0.347**	0.122	0.17	0.228	2.46*	
	7	340	62	70	0.199*	0.105	–0.407**	0.165	2.3*	
	8	340	19	59	–0.285**	0.115	0.362*	0.194	2.87**	
	11	340	46	60	–0.03	0.112	0.672**	0.192	3.25**	
AP	7	212	1	76	–2.38	2.1	10.52**	3.23	2.81**	2.15/2.57
	7	212	58	60	–3.21	2.36	–10.83**	4.13	2.43*	
	8	212	101	58	7.65**	2.85	7.44	4.58	2.41*	
	8	212	136	62	7.14**	2.39	–2.58	4.02	2.36*	
	8	212	172	53	7.22**	2.37	–10.59**	4.45	3.81**	
	12	212	9	53	–5.4**	2.34	–0.89	3.96	2.24*	

[a]OR = ovulation rate, AP = age at puberty, NN = number of nipples, and FF, NBL, and NSB = number of fully formed, stillborn, and live pigs at birth, respectively.

[b]Degrees of freedom equal N – the rank of the incidence matrix **X** in $\mathbf{y} = \mathbf{Xb} + \mathbf{e}$.

[c]Relative position in Kosambi centimorgans, based on the map reported in Table 2.

[d]Informativeness is the percentage of F_1 meioses that could be traced back to the line of origin at the putative QTL position.

[e]Additive effects are estimates of the value of pigs homozygous for the allele inherited from the control line deviated from the mean of the two homozygous genotypes, expressed as pigs for litter traits, corpora lutea for ovulation rate, nipples for nipple number, and days for age at puberty.

[f]Dominance effects are estimates of the value of the heterozygous genotype compared to the mean of the two homozygous genotypes.

[g]Significance was determined using a likelihood-ratio test statistic (LOD = $\log_{10}$ of odds), where thresholds were calculated using the method described by Churchill and Doerge (1994).

[h]$\alpha = 0.10/\alpha = 0.05$ thresholds calculated using a permutation approach.

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

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

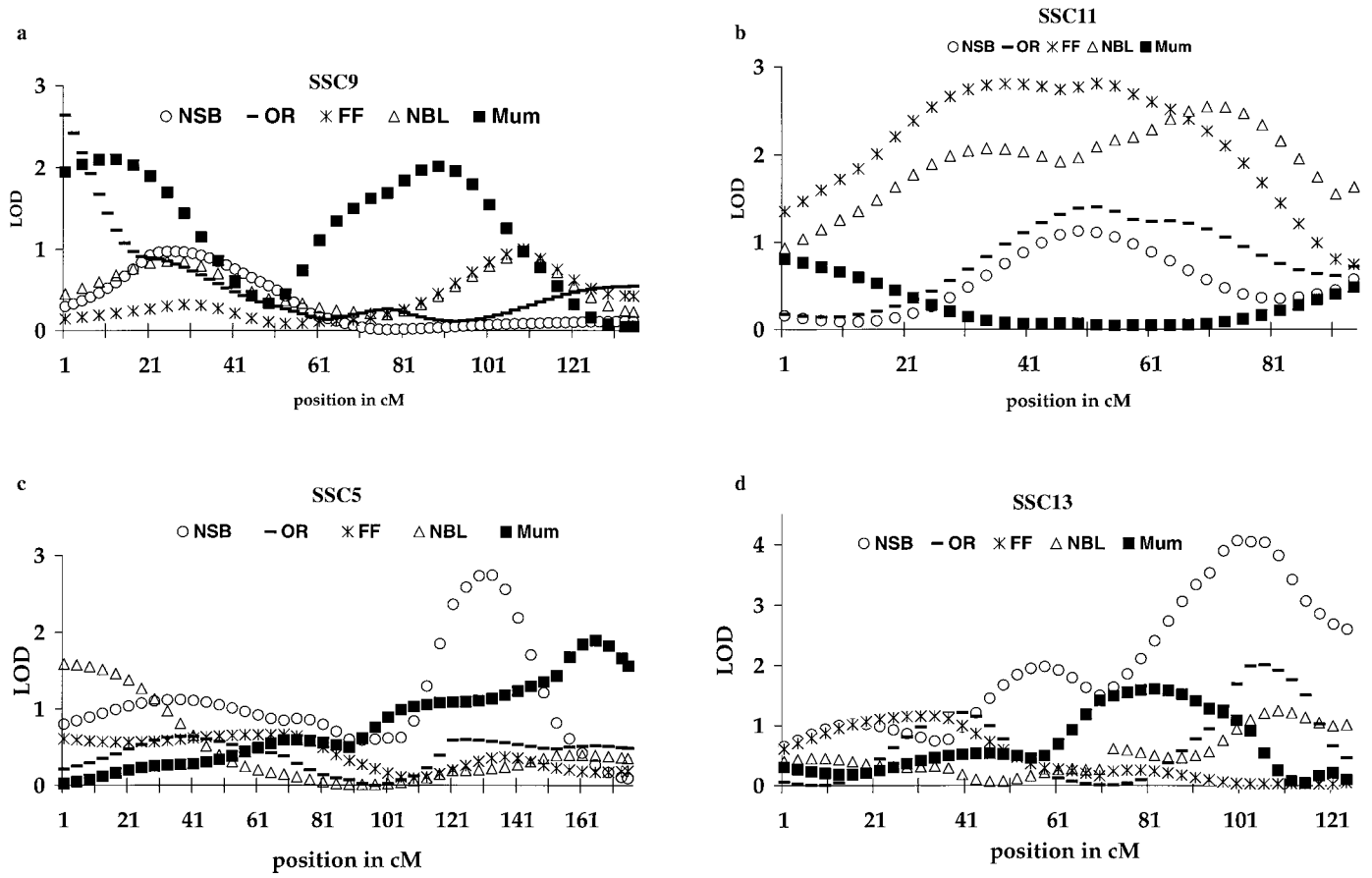

Figure 1. Likelihood ratio test statistic (LOD = $\log_{10}$ of odds) for ovulation rate and number of stillborn (NSB), fully formed (FF), and live (NBL) pigs at birth. (a) chromosome 9, (b) chromosome 11, (c) chromosome 5, (d) chromosome 13.

Results

Markers

All markers, their relative positions, and informativeness are listed in Table 2. Informativeness of a marker is the proportion of F_1 meioses for which the allelic line of origin could be determined. The program by Haley et al. (1994) uses all available marker information within a linkage group to calculate coefficients of additive and dominance effects. Therefore, the marker informativeness values presented in Table 2 represent informativeness only of that individual marker, and not informativeness at that location.

Phenotypic Means and Standard Deviations

Phenotypic means, standard deviations, and ranges are reported in Table 3. The F_1 generation is expected to have less genetic variance than the F_2 generation. However, phenotypic standard deviations for the F_1 and F_2 generations were similar.

Putative QTL

All putative QTL, their most probable positions, informativeness and LOD scores at those positions, and estimates of additive effects of the control-line alleles are reported in Table 4. Corresponding plots of LOD ratios are shown in Figures 1, 2, and 3.

Evidence was found for a QTL affecting ovulation rate near marker SW21 on SSC9 ($P < 0.05$; Figure 1a; Table 4). The dominance effect was estimated to be 1.16 ova, which was different from zero ($P < 0.05$). The LOD ratio for number of mummified pigs for a QTL in this same region on SSC9 also approached significance (Figure 1a).

Putative QTL for number of fully formed ($P < 0.05$, Figure 1b, Table 4) and live pigs ($P < 0.10$, Figure 1b, Table 4) at birth were identified on SSC11. Additive effects of the allele inherited from the Control line were estimated to be -0.86 ± 0.27 ($P < 0.05$) and -0.83 ± 0.3 ($P < 0.05$) pigs, respectively. Maximum LOD ratios for ovulation rate and number of stillborn pigs also occurred in this same region of SSC11, supporting evidence for a QTL on SSC11 affecting litter size.

For number of stillborn pigs, putative QTL were identified on SSC5 ($P < 0.05$, Figure 1c, Table 4) and SSC13 ($P < 0.05$, Figure 1d, Table 4). The estimated dominance effect for a QTL at position 131 cM on SSC5 was 1.13 ± 0.36 ($P < 0.05$). There was little supporting evidence from correlated traits for this QTL (Figure 1c). However, the LOD ratio for number of mummified pigs did

peak in this same region of SSC5. At position 101 cM on SSC13, the estimated additive and dominance effects for number of stillborn pigs were –0.43 ± 0.13 ($P < 0.05$) and –0.5 ± 0.23 ($P < 0.05$). Figure 1d also shows nonsignificant peaks for ovulation rate and number of live and mummified pigs at birth in this same region of SSC13.

Several putative QTL were identified for number of nipples and age at puberty. Means of Lines I and C did not differ for age at puberty or number of nipples. Evidence exists for QTL associated with number of nipples on SSC1 ($P < 0.10$, Figure 2a), SSC6 ($P < 0.10$, Figure 2b), SSC7 ($P < 0.10$, Figure 2c), SSC8 ($P < 0.05$, Figure 2d), and SSC11 ($P < 0.05$, Figure 2e). Putative QTL associated with age at puberty were identified on SSC7 at positions 1 cM ($P < 0.05$, Figure 3a) and 58 cM ($P < 0.10$, Figure 3a). On SSC8 there were three peaks associated with age at puberty (Figure 3b) at positions 101 ($P < 0.05$), 136 ($P < 0.05$), and 172 ($P < 0.05$). At position 9 cM on SSC12, there was evidence ($P < 0.10$, Figure 3c) of a QTL affecting age at puberty.

Discussion

Rathje et al. (1997) reported preliminary results from this project. They found evidence for a QTL affecting ovulation rate on SSC4, SSC8, SSC13, and SSC15. Those QTL were not confirmed in this study. Rathje et al. (1997) used fewer markers and data from Replicate 1 females only. Differences in results of the current analysis and those of Rathje et al. (1997) may be due to sampling variance and different marker data. This highlights the need for powerful QTL analysis, in regard to numbers of meioses and markers, before potential application of results.

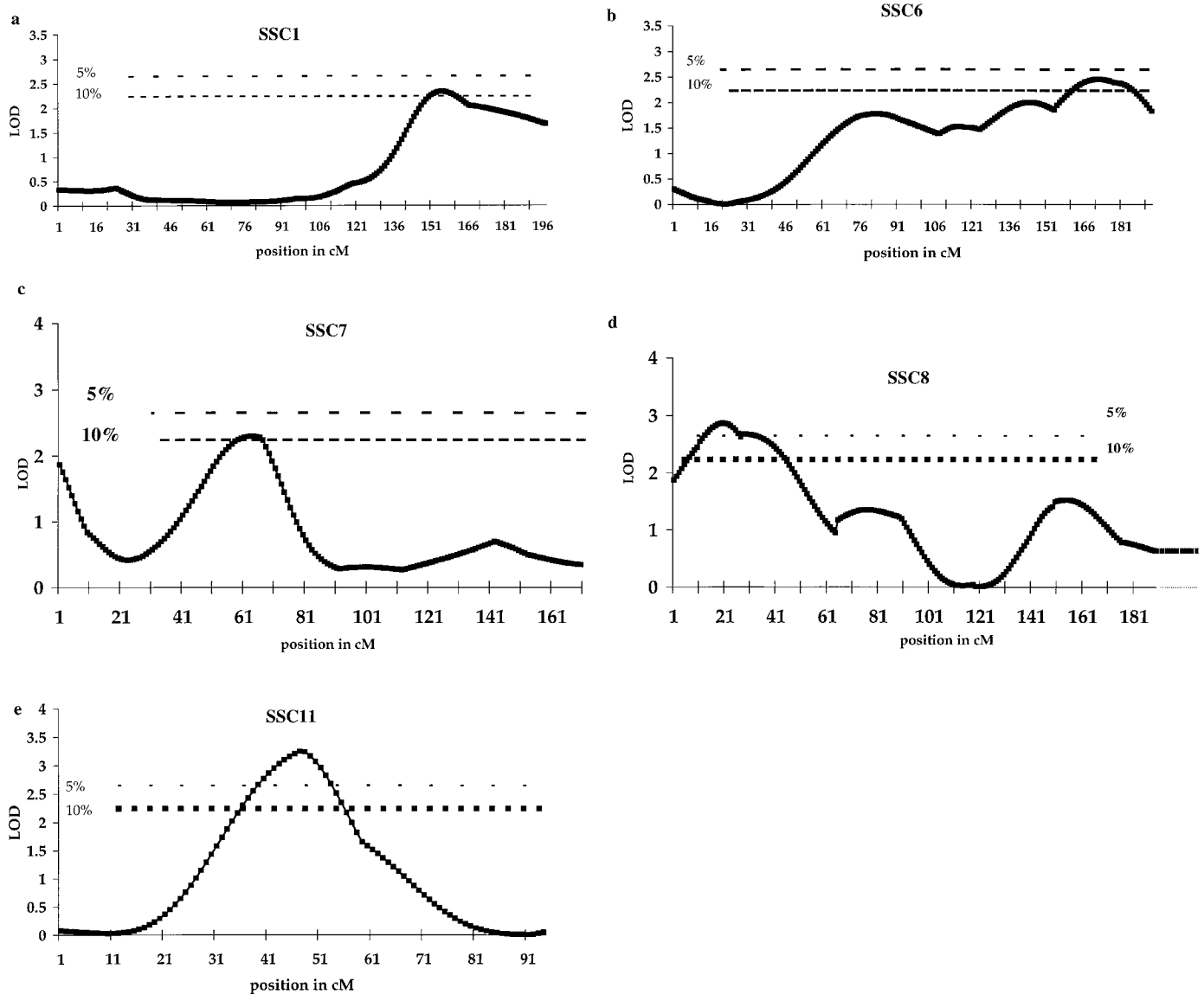

Figure 2. Likelihood ratio test statistic (LOD = $\log_{10}$ of odds) for number of nipples. (a) chromosome 1, (b) chromosome 6, (c) chromosome 7, (d) chromosome 8, (e) chromosome 11.

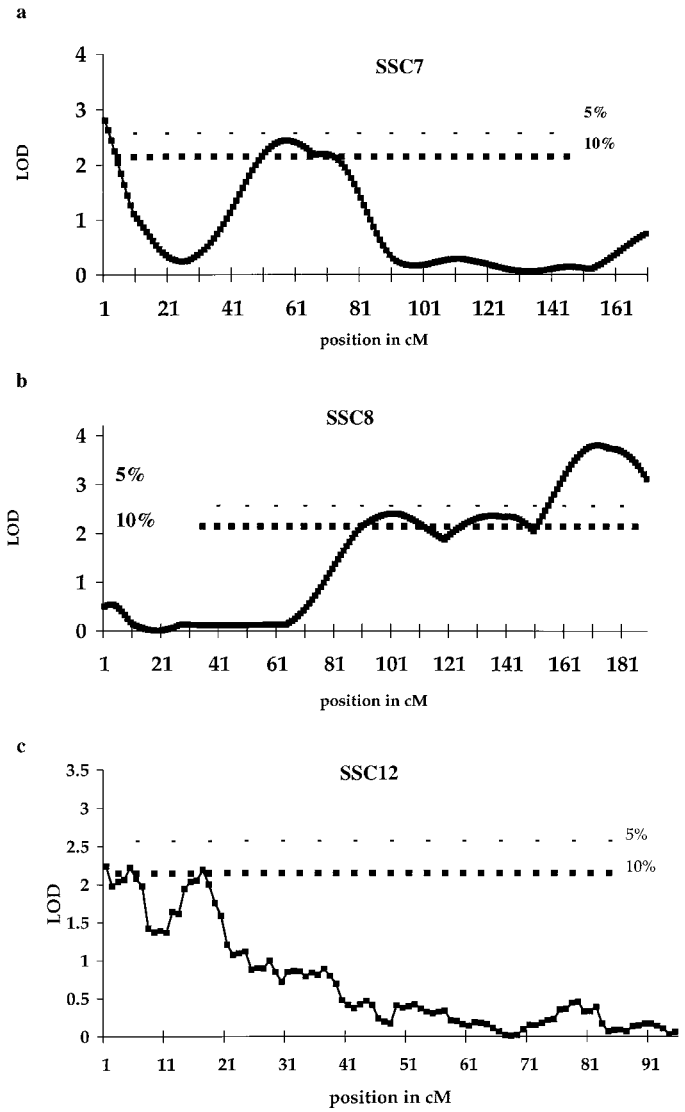

Figure 3. Likelihood ratio test statistic (LOD = $\log_{10}$ of odds) for age at puberty. (a) chromosome 7, (b) chromosome 8, (c) chromosome 12.

Estimates of QTL for reproduction in pigs reported elsewhere and those from the present study are summarized in Table 5. The purpose of Table 5 is to compare results from several studies on a common statistical basis and map. Most probable position of each QTL was adjusted to the map published by Rohrer et al. (1996). In addition, test statistics were adjusted to an *F*-statistic for comparison. Expected number of false positives per genome scan was calculated using the methods of Lander and Kruglyak (1995).

In this study, the strongest evidence for a QTL associated with litter size was on SSC13 affecting number of stillborn pigs with most probable position at 101 cM. Both the additive effect of –0.43 ± 0.13 pigs and dominance effect of –0.5 ± 0.23 pigs were significant. LOD scores for other litter-size traits did not reach significance on SSC13. However, LOD scores for both ovulation rate and number of pigs born live were maximized in this same region of SSC13. For this putative QTL the additive effect is in the direction expected, and animals inheriting a Line C allele had fewer stillborn pigs than those inheriting a Line I allele. Knott et al. (1998) reported evidence of a QTL on SSC13 at position 61 cM affecting birth weight. Because the most probable positions of these putative QTL are 40 cM apart, it is unlikely that they are the same QTL.

Wilkie et al. (1999) and Paszek et al. (1999) reported QTL on SSC4 at positions 4 cM and 8 cM affecting number of stillborn pigs and birth weight, respectively. Johnson et al. (1999) reported a decrease in average pig birth weight in Line I relative to Line C. In addition, stillborn pigs weighed significantly less than live pigs in both Lines I and C. Johnson et al. (1999) suggested that selection for increases in litter size and pig birth weight may decrease number of stillborn pigs. Evidence of linked QTL affecting number of stillborn pigs and pig birth weight supports that conclusion.

A putative QTL affecting ovulation rate was found near SW21 on SSC9. Initial estimates of effects of this QTL were an additive effect of –0.25 ± 0.23 and a dominance effect of 1.16 ± 0.4 (Table 4). The first marker evaluated on SSC9 was SW21. The LOD score for ovulation rate seems to be increasing at that point (Figure 1a). Thus, the most probable location for a QTL affecting ovulation rate on SSC9 is between markers CCKBR and SW21. Adding more markers may help to better locate the position of this QTL. Rohrer et al. (1999) also reported evidence of a QTL on SSC9 affecting ovulation rate. The most probable locations of the two QTL differed by 56 cM; therefore, it is unlikely that these are the same QTL.

Evidence existed for a QTL affecting number of fully formed and live pigs at birth on SSC11. LOD scores for ovulation rate and number of stillborn pigs are maximized in the same region of SSC11. However, informativeness of markers in this region was low. Thus, location of this putative QTL is not well defined. Estimates of the additive effects of these QTL are –0.85 ± 0.27 fully formed pigs and –0.83 ± 0.3 pigs born alive (Table 4). These effects are in the direction that would be expected with the allele from Line C decreasing numbers of fully formed and live pigs at birth. Because these effects are approximately equal, selection for the favorable QTL allele is expected to be equally effective at increasing number of fully formed and live pigs at birth. This evidence seems to be the first for a QTL affecting litter size on SSC11.

Putative QTL associated with ovulation rate have been reported on SSC15 at position 79 cM (Rohrer et al., 1999, Table 5) and at position 100 cM (Wilkie et al., 1999). These QTL are in close proximity to the QTL for ovulation rate reported by Rathje et al. (1997). In the present study the LOD score for ovulation rate on SSC15 was maximized at position 88 cM (LOD = 1.7). These results from three independent studies support evidence for a QTL in this region. Wilkie et al. (1999) found evidence for a QTL at position 42 cM on SSC5

Table 5. Location, significance, and estimates of additive (a) and dominance (d) effects of reported putative QTL affecting reproduction in the pig

Trait	Chr[a]	cM[b]	*F*-ratio[c]	df_n[df]	df_d[ef]	*P*-value[g]	Genome[h]	a	d	Reference
Birth weight, g	1	19	6.4	2	168	2.1×10^{-3}	2.05	–59.5 ± 22.1	74.1 ± 32.8	Knott et al., 1998
Age at puberty, d	1	105	6.67	2	344	2.2×10^{-4}	0.44	9.35	–5.49	Rohrer et al., 1999
Number of nipples	1	115 (155)	5.45	2	340	4.7×10^{-3}	3.91	0.17 ± 0.15	0.7 ± 0.29	Present study
Gestation length, d	1	94 (166)	5.22	2	103	6.9×10^{-3}	5.56	1.18 ± 0.55	0.62 ± 1.09	Wilkie et al., 1999
Number of corpora lutea	3	36	12.72	1	288	4.0×10^{-4}	0.55	–2.2	0	Rohrer et al., 1999
Number of stillborn pigs	4	4 (1)	9.97	2	98	1.0×10^{-4}	0.17	–0.31 ± 0.11	–0.57 ± 0.18	Wilkie et al., 1999
Birth weight, g	4	8 (33)	8.04	2	170	4.6×10^{-4}	0.56	–46 ± 17	85 ± 28	Paszek et al., 1999
Uterine length, cm	5	42 (1)	5.13	2	104	7.5×10^{-3}	5.92	87.2 ± 27.4	–31.5 ± 54.1	Wilkie et al., 1999
Number of stillborn pigs	5	85 (131)	6.5	2	279	1.7×10^{-3}	1.73	–0.87 ± 0.19	1.13 ± 0.36	Present study
Number of fully formed pigs	6	104 (102)	5.15	2	98	7.5×10^{-3}	5.91	–0.81 ± 0.49	1.94 ± 0.65	Wilkie et al., 1999
Number of nipples	6	145 (171)	5.76	2	340	3.4×10^{-3}	3.06	–0.35 ± 0.12	0.17 ± 0.23	Present study
Age at puberty, d	7	4 (1)	6.67	2	212	1.5×10^{-3}	1.6	–2.4 ± 2.1	10.5 ± 3.2	Present study
Age at puberty, d	7	55 (58)	5.75	2	212	3.7×10^{-3}	3.27	–3.2 ± 2.4	–10.8 ± 4.1	Present study
Number of nipples	7	59 (62)	5.38	2	340	5.0×10^{-3}	4.14	–0.2 ± 0.11	–0.41 ± 0.17	Present study
Uterine length, cm	7	154 (148)	5.71	2	104	4.4×10^{-3}	3.88	20.5 ± 33.5	–180.9 ± 57.6	Wilkie et al., 1999
Number of corpora lutea	7	156 (150)	6.22	2	104	2.8×10^{-3}	2.67	2.57 ± 0.73	1.33 ± 1.21	Wilkie et al., 1999
Number of corpora lutea	8	5	26.71	2	288	4.4×10^{-7}	0	–2.87	0	Rohrer et al., 1999
Number of nipples	8	19 (15)	6.74	2	340	1.3×10^{-3}	1.39	–0.29 ± 0.12	0.36 ± 0.19	Present study
Number of corpora lutea	8	50 (101)	8.89	2	104	2.7×10^{-4}	0.37	–1.20 ± 0.37	–1.76 ± 0.63	Wilkie et al., 1999
Age at puberty, d	8	70 (101)	5.7	2	212	3.9×10^{-3}	3.4	7.7 ± 2.9	7.4 ± 4.6	Present study
Uterine capacity	8	71	7.87	2	187	5.2×10^{-4}	0.83	1.99	1.43	Rohrer et al., 1999
Number of corpora lutea	8	110	7.38	2	75	1.2×10^{-3}	1.35	3.07	–5.35	Rathje et al. 1997
Age at puberty, d	8	110 (136)	5.58	2	212	4.4×10^{-3}	3.73	7.1 ± 2.4	–2.6 ± 4	Present study
Age at puberty, d	8	120 (172)	9.15	2	212	1.5×10^{-4}	0.21	7.2 ± 2.4	–10.6 ± 4.5	Present study
Weight of ovary, g	8	122	6.55	3	270	2.7×10^{-4}	0.54	1.04	0.26	Rohrer et al., 1999
Number of corpora lutea	9	11 (1)	6.19	2	340	2.3×10^{-3}	2.17	–0.25 ± 0.23	1.16 ± 0.4	Present study
Number of corpora lutea	9	67	5.78	3	286	7.6×10^{-4}	1.32	–1.98	0.10	Rohrer et al., 1999
Gestation length, d	9	130 (135)	9.3	2	103	1.9×10^{-4}	0.27	1.52 ± 0.44	–2.34 ± 0.75	Wilkie et al., 1999
Number of corpora lutea	10	89	7.62	2	287	6.0×10^{-4}	0.92	–2.26	–1.22	Rohrer et al., 1999
Age at puberty, d	10	125	6.92	3	344	1.6×10^{-4}	0.33	–27.58	–11.20	Rohrer et al., 1999
Number of nipples	11	45 (46)	7.65	2	340	5.6×10^{-4}	0.65	–0.03 ± 0.11	0.68 ± 0.19	Present study
Number of fully formed pigs	11	51 (52)	6.6	2	279	1.6×10^{-3}	1.6	–0.86 ± 0.27	–0.04 ± 0.47	Present study
Number of pigs born alive	11	67 (71)	5.97	2	279	2.9×10^{-3}	2.64	–0.83± 0.3	0.66± 0.61	Present study
Birth weight, g	12	0	5.4	2	168	5.3×10^{-3}	4.42	–36.6± 37.1	–303.3± 94.6	Knott et al., 1998
Age at puberty, d	12	15 (9)	5.29	2	212	5.7×10^{-3}	4.67	–5.4± 2.3	–0.89± 3.9	Present study
Birth weight, g	13	61	6.5	2	168	1.9×10^{-3}	1.9	75.4± 21.1	11.8± 28.5	Knott et al., 1998
Number of stillborn pigs	13	101 (101)	9.7	2	279	8.5×10^{-5}	0.12	–0.43± 0.13	–0.5± 0.23	Present study
Number of corpora lutea	15	79	5.73	3	286	7.4×10^{-4}	1.4	2.44	0.25	Rohrer et al., 1999
Gestation length, d	15	89 (96)	6.79	2	103	1.7×10^{-3}	1.76	1.86± 0.54	1.01± 1.06	Wilkie et al., 1999
Number of corpora lutea	15	100 (107)	6.2	2	104	2.9×10^{-3}	2.71	–0.81± 0.59	3.84± 1.13	Wilkie et al., 1999

[a]Chromosome on which the putative QTL was reported.

[b]Relative position in Kosambi centimorgans as reported based on maps developed by Rohrer et al. (1996). Numbers in parentheses are relative position as reported by the authors.

[c]*F*-ratios were taken directly from the literature when possible. In cases in which an *F*-ratio was not reported it was approximated using the reported *P*-value and appropriate degrees of freedom.

[d]If degrees of freedom were not reported in the article they were approximated based on the reported model and number of animals.

[e]Numerator degrees of freedom.

[f]Denominator degrees of freedom.

[g]When possible nominal *P*-values were taken directly from the literature. When *P*-values were not reported they were approximated using the reported test statistic and appropriate degrees of freedom.

[h]A genome-wide significance value was calculated for each putative QTL using the equation presented by Lander and Kruglyak (1995), where genome-wide significance = $(C + 2 \cdot G \cdot \rho \cdot f \cdot df_n) \times (1 - \text{prob}(f, df_n, df_d)$, where C = 19 (representing the 18 autosomes and the X chromosome), G = 25 (the length of the swine genome in morgans), ρ is the autocorrelation function ($\rho = 1$ for a backcross and 1.5 for an F_2 population), and f is the *F*-ratio, with df_n numerator degrees of freedom and df_d denominator degrees of freedom. This is the expected number of false positives per genome scan.

for uterine length. The present study provides evidence for a QTL for number of stillborn pigs at position 85 cM on SSC5. Short et al. (1997) reported a favorable association between the B allele at the estrogen receptor (**ESR**, SSC1 position 19 cM) locus and total number of pigs born and born live in a Large White-based commercial line. Knott et al. (1998) reported a QTL near the location of the ESR locus associated with pig birth weight. Rohrer et al. (1999) reported no association between the ESR locus and reproductive traits in a reference population developed from Chinese Meishan and the white line composite of Landrace, Large White, Yorkshire, and Chester White at MARC. The Nebraska reference population was screened for the ESR marker (Short et al., 1997), which was found to be uninformative. The ESR locus also did not explain the selection response in lines derived from the Nebraska index line that were selected for ovulation rate and litter size (Linville et al., 1999), and no evidence for QTL influencing litter size was found in the region of SSC1 harboring the ESR locus.

Several QTL affecting nipple number were identified. Nipple number is easily measured in both males and females and is not a likely candidate for MAS. However, QTL associated with nipple number may provide an opportunity for greater understanding of biological function.

Quantitative trait loci affecting age at puberty on SSC7, SSC8, and SSC12 were identified. In addition, Rohrer et al. (1999) has identified QTL affecting age at puberty on SSC1 and SSC10. Additive effects of these QTL ranged from –5 to 27 d. Potential may exist to change age at puberty using marker-assisted selection. Age at puberty is an economically important trait that is laborious to measure.

The present study is the first to complete a whole-genome scan using an F_2 cross between selection lines of pigs originating from a common base population. Casas-Carrillo et al. (1997) searched for growth QTL in half-sib families from F_1 sires arising as a result of the cross of lines divergently selected for growth rate. The use of crosses of selection lines for QTL discovery has inherent limitations and benefits. In contrast to other pig reproduction QTL scans (Rohrer et al., 1999; Wilkie et al., 1999), both of which used Meishan-White crosses, the power of the present experiment was limited by lower marker informativeness and less phenotypic divergence for some traits. Conversely, findings from crosses between lines with commercially viable phenotypes will have more immediate application to the industry. Favorable alleles originating from Meishan or other Chinese breeds of pigs must be slowly introgressed into commercially relevant lines or breeds.

Finally, use of selection line crosses enables examination of the nature of selection response at the QTL level. Our results indicate that few, if any, major genes for reproductive traits were segregating in the Large White × Landrace composite base population. Possible exceptions are the QTL found for litter size on SSC11. However, informativity at that point was low, and thus the QTL effect is not precisely estimated. It is likely that selection for ovulation rate, embryonic survival, and litter size in this population has acted on many loci, each with modest to small effect. Much greater experimental power is necessary to genetically dissect such polygenic traits.

Implications

Putative QTL affecting reproduction in pigs were identified. Informativeness at the most probable position of some of these putative QTL was less than 50%. In those cases, fewer than 400 meioses observed in F_2 animals were informative at the marker locus. Therefore, additional informative markers should be genotyped in this population to obtain more information and possibly identify false positives. Confirmation of these putative QTL in other populations is also needed. At present, insufficient information exists to encourage marker-assisted selection. However, putative QTL with significant effects associated with litter size, number of corpora lutea, number of nipples, and age at puberty have been identified.

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