RESEARCH REPORT
ANIMAL SCIENCE

Title: Genome-Wide Association Study for Sow Feed Efficiency and Reproduction and Genomic Selection using Low Density Panels – **NPB #11-056**

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Industry Summary

With the rise in feed costs, increasing nutritional efficiency of production is, more than ever, one of the main drivers of profitability of pork production. Over the past decades, substantial effort has been placed on increasing feed efficiency. On the genetics side, this has indirectly been accomplished by selection for growth rate and leanness, which both decrease the amount of feed required to reach market weight. It is well known, however, that a large proportion (~34%) of differences in feed intake between pigs in the growing phase are not related to growth and composition but result from differences in energy required for other processes such as maintenance, activity, and digestive and metabolic efficiency. This is known as residual feed intake (RFI), which is measure of feed efficiency computed as the difference between the amount of feed a pig consumes and what it is expected to consume based on its rate of growth and backfat. So pigs with negative RFI are more efficient. Selection on RFI as been the basis of the development of two unique selection lines of Yorkshire pigs at Iowa State University over the past decade. Now in its 8th generation, pigs from the low RFI (efficient) line consume nearly 12% less feed for the same amount of growth and backfat, compared to the high RFI (inefficient) line. Over the past years, the ISU RFI lines have been an important resource for multidisciplinary research into the genetic and biological basis of differences in efficiency during the growing phase, funded by the USDA, NPB, and IPPA. Although the emphasis of the selection lines is on efficiency during the growing phase, we have also collected extensive data on sows, including individual feed intake during lactation. In recent research funded by NPB, this has led to the important finding that pigs that are selected for increased efficiency during the growing phase also tend to have greater efficiency during lactation and have slightly better reproductive performance in terms of litter size and litter weight at birth and weaning. Lactation feed intake is an important trait in swine husbandry to maximize the productivity of the sow. Decreased feed intake during lactation results in reduction in body reserves and reduce milk output, which impacts the growth of litter and there by affects the profitability.

Recent developments in genomics have given us important tools to investigate the genetic basis of traits of interest and to develop tools, in the form of genetic markers and gene tests, that can be used to select for traits such as feed efficiency, which are difficult to improve by conventional means. Capitalizing on this, and with funds from NPB, we recently used the 60k PigChip on 720 pigs from the two lines to successfully identify genomic regions associated with RFI and component traits during the growing phase, using Genome-Wide Association Studies (GWAS). The main objective of this research was to apply this same GWAS to identify genomic regions and markers that are associated with feed efficiency during lactation and reproductive performance. The present study is the first attempt to study the genetic architecture of lactation efficiency traits using a genome wide association analysis. For this purpose, combined genotypes of over 48,000 genetic markers from across the genome on 520 sows with lactation feed efficiency and reproduction traits from first and/or second parity were used to identify regions of the genome that are associated with these important traits. Multiple regions were found for most traits, with several regions affecting multiple traits. Data on parity 1 and parity 2 were analyzed separately; the amount of overlap between regions was limited, suggesting that different genes control reproduction and efficiency in first versus later parities. Several of the identified regions were consistent with regions that were found in previous research and for most regions important candidate genes that could harbor the causative mutations were identified. Although these results are promising and provide avenues for further research, these results need to be validated in other populations before they can be recommended as markers to be used for selection. To this end, we will be able to use data from a parallel GWAS project on sow feed efficiency in two commercial breeding lines that we are collaborating on with the University of Alberta and Genesus Inc.

Keywords
Feed efficiency, Lactation efficiency, Reproduction, Genetics, Genetic markers.

Scientific Abstract
The objective of this study was to conduct a genome wide association study (GWAS) to identify SNPs / chromosomal regions associated with reproductive traits and sow lactation efficiency. The Illumina porcine 60k SNP chip was used to genotype 512 purebred Yorkshire sows from the ISU Residual Feed Intake (RFI) lines, which were divergently selected for high and low RFI during finishing. After quality control, 48,521 SNPs were used for analysis. The traits included the following in each of parity 1 and parity 2: total number farrowed (TNF), number born alive (NBA); total born (TB), number weaned by sow (NWBS), number weaned from sow (NWFS), total litter birth weight of all non-mummified piglets (LBW), average litter birth weight (ALBW), total birth weight of all pigs born alive (LPBW), average live piglet birth weight (ALPBW), total weaning weight of piglets nursed by sow (WWTBS), total weaning weight of piglets born to sow (WWTFS), average weaning weight of piglets born to sow (AWWTFS) in each of the first two parities, lactation feed intake (FI), RFI, estimated maintenance requirements (MR), energy balance (EB) and lactation efficiency (LE), along with sow body weight (BWW), fat mass (FMW), and protein mass (PMW) at the time of weaning. Lactation efficiency and energy utilization of sows and piglets was calculated based on on-farm measurements of sow body weight, back fat and loin muscle area before farrowing and at the time of weaning, sow feed intake during lactation, and piglets weights at birth, death and weaning. The GWAS was implemented separately for each trait using method Bayes B of GENSEL software. The present study is the first attempt to study the genetic architecture of lactation efficiency traits using a genome wide association analysis. We report the associations of informative QTL and the genes within the QTL for each trait in different parities. These results provide evidence of gene effects having temporal impacts on reproductive traits in different parities. Different QTL and genes have different impact on reproductive and feed efficiency traits in first and second parity. Some QTL identified in this study are new for pig reproductive traits. On first parity about 50% of genes located in the identified important QTL regions were predicted to be involved in placental functions. The QTL containing a gene important for modulation of two very potent angiogenic factors responsible for blood flow, fetal growth, survival and neonatal weight (e.g. PPP3CA) was associated with TNF, TB, NWFS, LBW, ALBW, LPBW and ALPBW. The genomic regions containing genes important for maternal-fetus interface vascular development (e.g. RNU5A-1), implantation and angiogenesis (e.g. ARHGEF17), regulation of fat and energy metabolism

and parallel contribution of normal reproduction (e.g. KLF17, UCP2/3, MRPL48, GLO1, MKK6) and role in embryogenesis (e.g. CXXC) were associated with LBW, ALBW, LPBW and ALPBW. The QTL containing gene related to cooper homeostasis and embryo development (e.g. SCO1) was associated with TNF, TB, NBA and NWFS.

On second parity genomic regions containing genes important for ovarian development, still births and low birth weight (e.g. ZCCHC7), optimal endometrium development (e.g. LRIG3), oocyte and embryo competence (e.g. TIGD1) and embryonic development (e.g. member of TCP-1 family) were associated with TNF, TB, NBA and NWFS. The QTL with genes related to placental function, early embryonic lethality (e.g FTL) and maternal stress and nursing behavior
(e.g NGF1A) were associated with litter weight traits at birth and at weaning.

For feed efficiency traits, the proportion of phenotypic variance explained by markers was 0.12 for LE, 0.28 for FI, 0.09 for RFI and EB, 0.49 for MR, 0.57 for BWW, 0.51 for FMW and 0.43 for PMW. These estimates were comparable to pedigree-based estimates of heritability. Although there were no regions that explained a large proportion of variance for LE or RFI, several informative regions were identified for traits such as PMW that are components of LE. The proportion of variance explained by the most important regions varied widely by trait. E.g., for PMW, six 1 Mb windows (86 SNPs) together explained ~20% of genetic variance and for MR seven windows (166 SNPs) explained ~ 12%. Across the genome, for all traits analyzed, more than 80 1 Mb windows explained at least 1% of the genetic variance. Some regions on SSC 8 and SSC18 were associated with multiple traits. Nearly all important regions differed between parties but were little affected by removing line as a fixed effect. Overall, this GWAS revealed several genomic locations and markers associated with sow reproduction and lactation feed efficiency and associated traits, which can provide a road map for future research and application. Further validation studies on large populations are warranted to improve our understanding of the complex genetic architecture for pig reproductive traits.

Introduction

Feed costs and the efficient use of feed are the main variable factors driving profitability of pork production in today's industry. Thus research that will allow pork producers to reduce feed costs and increase feed efficiency will have immediate impacts on their bottom line and on competitiveness of the pork industry. Efficient use of feed is not only of importance during the growing phase but also during the reproduction phase of the pork production system. In addition, the consequences of improved efficiency during the growing phase on reproductive performance are crucial to ensure that strategies are developed and implemented that will deliver improved efficiency and profitability across all levels of the production system. By utilizing the unique pig population resources and data bases that have been developed at Iowa State University in combination with mathematical models that enable a comprehensive analysis of efficiency during the reproduction phase, this project provides crucial knowledge and information that is currently lacking on the impact of selecting for feed efficiency during the growing phase on feed efficiency during lactation and on reproductive performance.

Objectives

Obj. 1 Identify genomic regions and markers that are associated with feed efficiency during lactation and with reproductive performance and to quantify the effect of markers that have been associated with feed efficiency during the growing phase.

Obj. 2 Utilize the high-density SNP genotype data that will be generated as part of this project to demonstrate the effectiveness of Genomic Selection using low-density SNP panels.

The second objective was to utilize the high-density SNP genotype data that will be generated as part of this project within two closed breeding lines to demonstrate the effectiveness of Genomic Selection using low-density SNP panels**.** The motivation for this objective was the high cost of high-density SNP genotyping, which

limits the feasibility of implementing Genomic Selection in pig breeding programs. To overcome this high costs, we (Habier, Fernando, and Dekkers, 2009) proposed that selection candidates can be genotyped with a panel of as few as 300 SNPs evenly spread over the genome, resulting in a tremendous reduction in genotyping costs (e.g. from ~$150 to less than $35 per animal with high volumes). The objective here was to demonstrate the effectiveness of this same strategy in the RFI lines. Although this was an important objective when this work was proposed, within the last year, multiple studies have been published, including our own work in chickens (Wang et al. 2013), as well as work in commercial pig breeding populations (Huang et al. 2012, Cleveland and Hickey, 2013), that has demonstrated the success of the low-density approach, including in commercial pig populations. In fact, Cleveland and Hickey (2013) showed that, with high-density SNP genotyping of all breeding males, low-density on dams and selection candidates, and sophisticated methods for imputation, this resulted in imputation accuracies of up to 97% at a dramatic reduction in genotyping costs in a commercial pig breeding population. This low-density genotyping approach has now been implemented in the routine genetic evaluation program of PIC, using a low-density panel of approximately 450 SNPs. Thus, rather than repeating this exercise with predictable results in a non-commercial population, i.e. the RFI lines, we instead focused our efforts under this objective on alternate measures of inbreeding using genomics.

Materials & Methods

Using purebred Yorkshire pigs, a line selected for decreased RFI (LRFI line) and a randomly selected control (CTRL) line were initiated in 2001 and developed as described by Cai et al. (2008). The control line was selected for increased RFI starting in generation 5. From breeding to three days before farrowing, the sows were housed in gestation crates and were fed with 2.8 kg feed per day. After farrowing, the sows were fed twice daily to appetite and the quantity offered was recorded. Excess feed was weighed and removed from the trough. The piglets were weighted at birth and weaning and all activities including cross fostering were recorded. The piglets were weaned at around 21 days and sows were weighed and scanned on the same day. A detailed account of sow and litter management practices for this herd can be found in Young et al (2012). Weight and ultrasonic backfat for the sow were recorded when entering the farrowing house and at weaning and were used to estimate fat and protein mass at the beginning and end of lactation in order to evaluate loss of fat and protein during lactation. Feed intake was recorded on the sows during lactation. All piglets born to a sow were recorded and coded for live, stillborn, or mummy. Individual birth weights of all live and stillborn piglets were recorded. At weaning, individual weights of all piglets were recorded. Farrowing and weaning dates were also recorded for all pigs.

Females were recorded for only two parities and hence the traits recorded in this study were restricted to parities one and two. Following parity 1, sows were culled based on performance and for logistic restrictions. Tail tissue from a total of 524 sows with sufficient data were sent to GeneSeek, Inc. (Lincoln, NE, USA) for genotyping using Illumina (San Diego, CA, USA) PorcineSNP60 Bead Chip. Details of the number of records included in each parity x line x generation combination are in table 1. In total there were 512 sows of first parity and 350 sows with records for second parity used in this study. The experimental protocols for this study were approved by the Iowa State University Institutional Animal Care and Use Committee.

Table 1: Details of the genotyped sows

Generation	Parity	Selection Line	
		Low RFI	High RFI
0	1	67*	
	2	33*	
2	1	45	18
	2	25	16
3	1	35	20
	2	21	12
4	1	43	27
	2	28	20
5	1	48	52
	2	40	31
6	1	37	35
	2	40	35
7	1	41	44
	2	24	25

* Generation 0 litters were prior to separation into two lines

A total of 62,163 SNPS were genotyped for each animal. SNPs with a minor allele frequency ≤ 0.025 and missing genotypes ≥ 0.05 % were excluded from the analysis. After the quality control steps 48,521 SNPs were included in the final analysis.

Reproductive Performance Traits

Traits analyzed were split into reproductive performance traits and sow feed efficiency traits. The reproductive traits analyzed included TNF - Total number farrowed (nba + nsb + nm); NBA - Number born alive; TB - Total born (nba + nsb); NWBS - Number weaned by sow (number of piglets the sow had on her at weaning); NWFS - Number weaned from sow (number of the sow's piglets that survived to weaning, regardless of whether she nursed them or not); LBW - Total litter birth weight of all non-mummified piglets; ALBW - Average litter birth weight (lbw/tb); LPBW - Total birth weight of all pigs born alive; ALPBW - Average live piglet birth weight (lpbw/nba); WWTBS - Total weaning weight of piglets nursed by the sow; WWTFS - Total weaning weight of piglets born to the sow; AWWTFS - Average weaning weight of piglets born to the sow (wwtfs/nwfs) were also recorded in these two parities.

Sow Feed Efficiency Traits

The feed efficiency traits analyzed were split into three groups, viz. traits measured at the time of farrowing, traits measured during or at the end of lactation, and overall feed intake / efficiency traits. The traits included in the first group were Sow body weight at farrowing, Sow back fat at farrowing, Sow fat mass at farrowing, Sow protein mass at farrowing, and litter weight at farrowing. The second group, the traits measured during and / or at the end of lactation, included Body weight loss, Back fat loss, Fat mass loss, Protein mass loss, and litter weight at weaning. The third group included traits such as total feed intake during lactation, Total energy input (energy from feed and body tissues mobilization above the maintenance requirement of the sow), Total energy output (energy utilized for piglet growth and maintenance), Net energy balance (the difference between the energy retained by the sow at weaning and farrowing), Sow lactation efficiency (the ratio of energy output to input expressed in percentage), and Sow residual feed intake (defined as the difference between the observed daily feed intake and predicted daily feed intake based on sow metabolic mid weight, litter growth rate sow weight loss and sow back fat loss (Gilbert et al., 2012)). The sow body weight at farrowing was adjusted for total fetal weight, placental weight and intra uterine fluid weight. The sow body weight at weaning was adjusted for the inta-mammary water content. All these traits were calculated based on the on-farm measurements of sow body weight, back fat and loin muscle area before farrowing and at the time of weaning, sow daily feed intake

during lactation and piglet weight at birth and weaning. The body protein mass and fat mass were calculated from the body weight and back fat measured. The traits in groups one and two were used to estimate the overall efficiency of the sow during lactation. A detailed description of methods and equations used for the calculating sow lactation efficiency and its component traits can be found in Bergsma et al. (2009). The individual values for the traits described above were obtained from a phenotypic study conducted on the same population by Young et al. (2012).

Genome wide association analysis

Association of the SNP genotypes with traits were analyzed by fitting all SNPs simultaneously using the Bayes B method implemented in software Gensel Version 4.0 (Fernando & Garrick, 2009). As Gensel software does not allow repeated records, the analyses were conducted separately for parities one and two. The following statistical model was used:

Where $\boldsymbol{y}$ is the vector of phenotypes, $\boldsymbol{X}$ is an incidence matrix of fixed effects, $\boldsymbol{b}$ is a vector of fixed effects of generation, line (high or low RFI) and line by generation, k is the number of SNPs, $\boldsymbol{Z_i}$ is a column vector representing the genotypic covariate at SNP i, α_j is the random allele substitution effect of SNP i , δ_j is a random 0/1 variable indicating the absence (with probability of π) or presence (with probability of 1- π) of SNP i included in the model with in an iteration of MCMC chain and $\boldsymbol{e}$ a vector of random residual effects. For the reproductive traits, sire of the litter was included as a fixed effect and additionally total born, number born alive, lactation length, number born by litter and number born by sow were used as a covariate for some of the reproductive traits in the analyses, following Young et al. (2010). For the second and third group of sow feed efficiency traits, lactation length was added as a covariate. A total of 100,000 iterations were used, of which first 30,000 were discarded as burn-in.

Results

Obj. 1 Identify genomic regions and markers that are associated with feed efficiency during lactation and with reproductive performance and to quantify the effect of markers that have been associated with feed efficiency during the growing phase.

Reproductive traits

Least square means of the reproduction trait by line in generation 7 are in Table 2. Results show the higher reproductive performance of the low RFI line.

Table 2. Least square means of reproduction traits in generation 7

Trait	Least square means		P-value
	LRFI	HRFI	
Total born (n)	12.1	10.4	<0.01
Number born alive (n)	10.9	9.7	<0.05
Number of piglets dead at birth (n)	1.2	0.8	<0.05
Number of mummies (n)	0.24	0.21	0.76
Farrowing survival (%)	89.2	91.5	0.24
Litter birth weight (kg)	13.7	13.3	0.25
Average litter birth weight (kg)	1.27	1.2	<0.05
Total live piglet birth weight (kg)	12.8	12.6	0.55
Average live piglet birth weight (kg)	1.27	1.22	0.12
Number weaned by litter (n)	9	7.5	<0.01
Number weaned by sow (n)	8.8	8.2	<0.05
Pre-weaning survival by sow (%)	83.3	80.9	0.31
Weaning weight by litter (kg)	53.9	50.8	<0.1
Average weaning weight by litter (kg)	6.1	5.9	0.37
Weaning weight by sow (kg)	55.2	51.9	<0.1
Average weaning weight by sow (kg)	6.1	5.8	0.19
Piglet average daily gain (g/d)	192.1	183.3	0.14
Piglet growth (kg)	4.63	4.45	0.36
Piglet energy gain (MJ ME)	3.16	3.02	0.1
Litter average daily gain (g/d)	1824	1679	<0.05
Litter growth (kg)	44.2	40.6	<0.1
Litter energy gain (MJ ME)	30.1	27.7	<0.05

Estimates of variances associated with markers are in Table 3.

Table 3. Posterior means of the variance components explained by whole genome SNP markers for reproductive traits (Bayes B).

Trait	Parity	Estimated total variance	Proportion of phenotypic variance explained by markers
TNF	1	9.56	0.105
	2	12.04	0.201
NBA	1	8.39	0.095
	2	10.07	0.136
TB	1	9.31	0.097
	2	11.48	0.171
NWBS	1	3.32	0.071
	2	3.66	0.093
NWFS	1	6.88	0.074
	2	7.22	0.073
LBW	1	3.70	0.224
	2	4.12	0.165
ALBW	1	0.03	0.277
	2	0.03	0.247
LPBW	1	3.07	0.218
	2	3.65	0.155
ALPBW	1	0.03	0.276
	2	0.03	0.229
WWTBS	1	52.54	0.158
	2	76.76	0.220
WWTFS	1	58.02	0.062
	2	80.26	0.066
AWWTFS	1	0.86	0.085
	2	1.05	0.157

[1]TNF: Total number farrowed (nba + nsb + nm); NBA: Number born alive; TB: Total born (nba + nsb); NWBS: Number weaned by sow (number of piglets the sow has on her at weaning); NWFS: Number weaned from sow (number of sow's piglets that survived to weaning whether or not she nursed them); LBW: Total litter birth weight of all non-mummified piglets; ALBW: Average litter birth weight (lbw/tb); LPBW: Total birth weight of all pigs born alive; ALPBW: Average live piglet birth weight (lpbw/nba); WWTBS: Total weaning weight of piglets nursed by sow; WWTFS: Total weaning weight of piglets born to sow; AWWTFS: Average weaning weight of piglets born to sow (wwtfs/nwfs)

Reproduction traits first parity

We found 14 important informative QTL regions and the genes within the QTL for several reproductive traits on first parity (Table 4). Most of these QTL regions, 9 of 14, were associated with litter weight at birth traits – LBW, ALBW, LPBW and ALPBW, and just four regions were associated with litter size traits at birth and at weaning – TNF, TB, NBA, NWBS and NWFS. Another three regions were associated with litter weight traits at weaning – WWTBs, WWTFS and AWWTFS. Most regions are new for the specific traits studied but some could be explained and connected with previously found QTL in the regions (Table 5). The QTL region on chromosome 8 was previously found as a QTL for uterine capacity, corpus luteum number, TNB and NBA, was found to be associated with TNF, TB, NWFS, LBW, ALBW, LPBW, ALPBW. This region contains gene PPP3CA, which is important for modulation of the two most potent angiogenic factors responsible for blood flow, fetal growth, survival and neonatal weight. The QTLs on chromosome 12 contain a gene related to cooper homeostasis and embryo development (e.g. SCO1) and the QTL on chromosome 16 contain a gene important for embryo development and innate immunity to mastitis (e.g. SEMA5A) and were found to be associated with TNF, TB, NBA and NWFS. On chromosome 13, a genomic region containing a gene with effect on regulation of fat mobilization (e. g. ABHD5) was associated with litter size at weaning traits: NWBS and NWFS. Genomic

regions containing genes important for maternal-fetus interface vascular development on chromosome 13 (e.g. RNU5A-1), with implantation and angiogenesis on chromosome 9 (e.g. ARHGEF17) and with a role in embryogenesis on chromosome 8 (e.g. CXXC), were associated with litter weight traits at birth LBW, ALBW, LPBW and ALPBW. Several of the established genomic regions containing genes important for obesity and energy metabolism regulation were associated with litter size at birth – on chromosome 9 (e.g. UCP2/3, MRPL48), on chromosome 6 (e.g. KLF17), on chromosome 12 (e.g. GLO1, MKK6), and on chromosome 6 (e.g. SDC3) with litter weight at birth and at weaning. The genomic region on chromosome 8, containing genes with a role in estrogen signaling (e.g. PRMT10) and milking ability (e.g. EDNRA), was associated with litter weight traits at weaning – WWTBS, WWTFS and AWWTFS.

Table 4. Percentage of genetic variance - Important QTL regions (>0.30% genetic variance) for reproductive traits on first parity.

	Percentage of genetic variance											
Chr_Mb	**lbw**	**albw**	**lpbw**	**alpbw**	**tnf**	**tb**	**nba**	**nwbs**	**nwfs**	**wwtbs**	**wwtfs**	**awwtfs**
1_21												0.30
6_81										0.38	0.31	0.38
6_154	0.95	0.96	1.09	0.75								
8_86										6.65	1.29	0.53
8_125	0.44	0.40	0.53	0.47								
8_128	1.30	0.54	1.29	0.58								
9_8	0.58	0.34	0.37	0.35								
9_9	0.91	0.58	0.67	0.72								
12_10	0.25	0.95	0.19	0.74								
12_11	0.29	0.64	0.23	0.56								
12_58					0.38	0.47	1.37					
13_29								0.32	0.30			
13_197	0.66	0.87	1.62	1.19								
16_79					0.37	0.42	0.59		1.04			

Table 5. Important QTL regions and genes for reproductive traits on first parity.

Chr_Mb	Previously reported Pig QTL database	QTL current search	Gene	Function
6_81	Teat number, nonfunctional nipples, total born	AWWTFS, WWTFS, WWTBS, ALBW, LPBW, ALPBW	PUM1- Pumilio homolog 1 LAPTM5 – Lysosomal protein trans membrane 5 SDC3 – Syndecan 3	Uterine support for embryo Follicles growth and maturation Role in obesity, feeding behavior and body weight, regulation whole body energy metabolisms
6_154	teat number	LBW, ALBW, LPBW, ALPBW	KLF17-Kluppel like transcription factor	Regulates fat metabolism normal reproduction essential role during embryonic development
8_86	Nonfunctional nipples, teat number, corpus luteum number, age at puberty	WWTBS, WWTFS, AWWTFS	PRMT10 – Protein arginine methyltransferase 10 EDNRA – Endothelin -1 receptor	Role in estrogen signaling Role in milking ability in sheep – milk flow level
8_125	Uterine capacity, corpus luteum number, teat number, tnb, nba, age at puberty	LBW, ALBW, LPBW, ALPBW, NWFS	CXXC-type Zink finger protein4-like	Act as regulator of Wnt signaling pathway - role in embryogenesis
8_128	Uterine capacity, corpus luteum number, teat number, tnb, nba, age at puberty	LBW, ALBW, LPBW, ALPBW, NWFS, TNF, TB, NBA, WWTFS	PPP3CA - protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform, CALN, CALNA, CNA1	Modulates two of most potent angiogenic factors important for placental blood flow – fetal growth, survival and neonatal weight
9_8		LBW, ALBW, LPBW, ALPBW	ARHGEF-rho guanine nucleotide exchange factor 17-like	Implantation related gene, between angiogenesis genes
9_9	ADG, body weight	LBW, ALBW, LPBW, ALPBW	UCP2/3 – Mitochondrial uncoupling protein MRPL48 – Mitochondrial ribosomal protein L48	Obesity – energy metabolism regulation, performance and longevity dairy cows energy metabolism
12_10	Teat number	LBW, ALBW, LPBW, ALPBW	GLO1 – Glyoxalase 1	Obesity gene
12_11	Teat number, removal parity, birth weight,	LBW, ALBW, LPBW, ALPBW	MKK6 (MAP2K6) – Mitogen-activated protein kinase 6	Obesity gene preimplantation Structural soundness traits in pigs
12_58	Teat number	TNF, TB, NBA, NWFS	SCO1 - protein SCO1 homolog, mitochondrial-like	Functions in mitochondrial cytochrome oxidase cooper delivery. Role in cooper homeostasis and embryo development
13_29	Nonfunctional nipples, corpus luteum number, age at puberty	NWBS, NWFS	ABHD5 - alpha/beta-hydrolase domain-containing protein 5	Fat mobilization regulation, body energy homeostasis
13_197	Nonfunctional nipples	LBW, ALBW, LPBW, ALPBW	RNU5A-RNU,U5A small nuclear	Maternal-fetus interface vascular development
16_79	Teat number ADG, body weight	TNF, TB, NBA, NWFS, NWBS	SEMA5A – Semaphorin 5A	Embryonic development Innate immunity mastitis in cattle

Reproduction traits second parity

We found 13 important informative QTL regions and the genes within these QTL associated with several reproductive traits in second parity (Table 6). There was a balance between the number of established important QTL regions associated with litter weight at birth (4 regions) and litter size at birth traits (6 regions) (Table 7).

Respectively three regions were associated with litter weight traits at weaning and one region with litter size at weaning. Most of these regions are new for the specific traits studied but some could be explained, connected or confirmed with previously found QTL in the regions.

The QTL region on chromosome 15 was previously found as a QTL for one litter size trait, TNB, was established to be connected with several litter size traits at birth: TNF, TNF, NBA. This region contains a gene that belongs to the TPC-1 cheperonin family, which is important for embryonic development. The genomic regions containing genes important for progesterone regulated implantation on chromosome 9 (e.g.ADAMTS8), ovarian development, still births and low birth weight on chromosome 1 (e.g. ZCCHC7), and preimplantation (e.g. RG9MTD3), embryo competence, implantation and early embryonic development on chromosome 5 (e.g. TIGD1, HSPD1), were associated with litter size traits at birth: TNF, TB and NBA. Litter weight traits at birth in second parity were associated with genomic regions containing genes with a role in successful pregnancy on chromosome 1 (e.g. IFNE) and on chromosome 6 (e.g. PLCG2) and with a role in maternal stress, hemolytic anemia abortions and low birth weight on chromosome 18, respectively AVL9 and NT5C3, and with placental functions and fetal growth on chromosome 6 (e.g. FTL). Genomic regions containing genes with a role in maternal care and nursing behavior on chromosome 9 (e.g. NGF1A) and with ovary and placenta development on chromosome 15 (e.g. LYPD6), were associated with litter weight traits at weaning.

Table 6. Percentage of genetic variance - important QTL regions(>0.30% genetic variance) for reproductive traits on second parity

	Percentage of genetic variance											
Chr_Mb	**lbw**	**albw**	**lpbw**	**alpbw**	**tnf**	**tb**	**nba**	**nwbs**	**nwfs**	**wwtbs**	**wwtfs**	**awwtfs**
1_224		0.40										
1_266					0.41	0.38	0.45					
2_51												0.36
5_25					2.91	1.88	0.48					
5_27					0.38	0.36						
5_28					1.99	1.55						
6_6	0.76		0.80									
6_7	0.36		0.41									
9_48												0.31
9_63					0.30							
15_2												0.47
15_17					0.45	0.46						
18_44		0.44		0.35								

Table 7. Important QTL regions and genes for reproductive traits on second parity

Chr_Mb	Pig QTL database	QTL current search	Gene	Function
1_224	Teat number, nonfunctional nipples, body weight at birth	ALBW, LBW, ALPBW, LPBW	IFNE – Interferon epsilon	Protective role on reproduction tissue, important for successful pregnancy
1_266	teat number, gestation length, age at puberty	TNF, TB, NBA, NWFS, LBW, AWWTFS	ZCCHC7 – zink finger CCHC domain containing protein 7 like	Zink finger proteins – nuclear receptors for steroids, ovarian development, still births, low birth weight
			RG9MTD3 - RNA (guanine-9-) methyltransferase domain containing	Between the important genes (markers) during pre-implantation in bovine embryo, developmental competence of early embryo
2_51		AWWTFS, WWTFS, WWTBS, LBW, ALBW, ALPBW	FTL – Ferritin light polypeptide	Early embryonic lethality, Placentation functions
5_25	Uterine horn length	TNF, TB, NBA, NWFS	LRIG3 – Leucine rich repeats and immunoglobulin-like domain 3	Role in implantation – in optimal development of the endometrium as essential prerequisite for successful blastocyst implantation
5_27	Uterine horn length, number of stillborn	TNF, TB, NBA	TIGD1 – Tigger transposable element derived 1	Role in oocyte and embryo competence
			HSPD1 – Heat shock 60kDa protein1	Implantation and early embryonic development
5_28	Uterine horn length	TNF, TB, NBA, NWFS	TIGD1 – tigger transposable element derived 1	Oocyte and embryo competence
6_6		LBW, LPBW, WWTBS, ALBW, ALPBW	FTL ferritin light polypeptide	Early embryonic lethality in one of ferritin genes knockout in mice Role in placental functions in humans
6_7	Gestation length	LBW, ALBW, LPBW, ALPBW	PLCG2 – Phospholipase C, gamma 2	Role in uterine-embryo interaction – normal or pathological pregnancy
9_48	Nonfunctional nipples, corpus luteum number	AWWTFS,WWTFS, WWTBS, LBW, ALBW, LPBW, ALPBW	NGF1A – NGF1 –A-binding protein	Role in maternal condition, maternal care – grooming and nursing behavior in rats
9_63	corpus luteum number	TNF, TB	ADAMTS8 – Desintegrin and metaloprotease with trombospondin motif 8-like	Role in obesity and progesterone regulated implantation gene
15_2		AWWTFS, WWTBS, TNF,TB	LYPD6/LYPD6B – PLAUR domain containing 6	Ovary development, placenta, differentially expressed in cattle with high/low residual feed intake
15_17	Total number born	TNF, TB, NBA, NWFS	One uncharacterized protein – belongs to TCP – 1 chaperonin family	Heat shock genes and proteins among first proteins produced during embryonic development and are crucial for cell function. Involved in diverse essential cellular functions including fertility
18_44	Nonfunctional nipples	ALBW, ALPBW	NT5C3 – 5-nucleotide cytosolic 3	Hemolytic anemia in humans – abortion, low birth weight
			AVL9 – AVL 9 homolog	Connected with long term stress in pigs

Sow Feed Efficiency Traits

Parity wise LS means, descriptive statistics and overall heritability estimates for the sow lactation feed intake and efficiency associated traits are in table 8. All traits studied, followed an approximate normal distribution.

Table 8. Summary statistics and marker-based heritability (h^2) for the lactation feed intake and efficiency related traits

Group	Trait	Parity 1			Parity 2		
		n	LSmean ±SE	h^2	n	LSmean ±SE	h^2
1 Traits measured at Farrowing	Body weight at Farrowing	479	179.8 ± 1.1	0.17	320	198.8 ± 1.3	0.32
	Fat mass at farrowing	423	47.9 ± 0.7	0.37	274	48.5 ± 0.8	0.28
	Protein mass at farrowing	423	25.8 ± 0.2	0.16	274	29.7 ± 0.2	0.40
	Back fat at farrowing	481	21.5 ± 0.3	0.39	298	20.6 ± 0.3	0.31
	Litter weight at farrowing	512	13.2 ± 0.2	0.07	350	16.0 ± 0.2	0.32
2 Traits measured during / end of lactation	Body weight loss	445	12.3 ± 0.8	0.12	307	4.1 ± 0.9	0.12
	Fat mass loss	409	7.5 ± 0.6	0.17	248	7.2 ± 0.5	0.09
	Protein loss	411	1.5 ± 0.2	0.03	248	-0.3 ± 0.2	0.13
	Back fat loss	460	3.1 ± 0.2	0.09	277	3.4 ± 0.3	0.06
	Litter weight at weaning	509	51.9 ± 0.5	0.17	337	57.5 ± 0.7	0.19
3 Lactation efficiency traits	Feed intake - total	479	112.5 ± 1.7	0.36	346	135.3 ± 2.0	0.22
	Feed intake - per day	479	4.8 ± 0.06		346	5.8 ± 0.08	
	Energy Input	411	59.8 ± 1.2	0.10	248	66.3 ± 0.9	0.20
	Energy Output	500	27.7 ± 0.2	0.08	336	30.3 ± 0.3	0.09
	Energy balance	411	-15.0 ± 1.1	0.17	248	-12.1 ± 0.9	0.13
	Lactation efficiency	411	51.9 ± 2.9	0.13	248	46.8 ± 2.5	0.28
	Sow RFI	411	3.5 ± 1.5	0.13	248	5.4 ± 1.2	0.16

The LSmeans in table 8 shows that on average, the sows performed well in the second parity, evident from the higher lactation efficiency and lower net energy balance. Loss of body weight, fat mass and protein mass were less in second parity, whereas back fat loss was slightly higher for parity 2.

In addition to sow lactation feed intake, five other traits, viz. Energy input, Energy output, Energy balance, Lactation efficiency, and Sow RFI were calculated to assess the feed efficiency of sows during lactation. The traits belonging to groups one and two are the component traits required for the calculation of feed efficiency during lactation. The group one traits are those traits which are relatively free of noise due to litter size and lactation length, whereas the traits belonging to group two and three depend on the lactation length and number of piglets nursed by the sow. The effect of this noise can be found in the heritability estimates of these traits. The heritability estimates of group one traits were moderate to high (ranged from 0.43 to 0.62), whereas the group two and three traits were lowly heritable (0.08 to 0.37).

In Table 8 is described the overall proportion of phenotypic variance explained by the markers. In line with the trend followed by pedigree based heritability estimates, the proportion of variance explained by markers for group one traits were higher than that of group 2 and 3 traits, as they are less influenced by lactation length and piglets nursed. A direct comparison of heritability for these traits with the proportion of variance explained by the SNP markers because of the difference in the method of analysis (separate analysis for parity 1 and 2 compared to a joint pedigree based analysis) and also due to some differences in covariates used in both methods. As the number of genotypes available for second parity sows was less than for parity 1, the parity 2 results might be more error prone, hence they should be taken with caution. The proportion of variance explained by markers for group 1 traits were less than the pedigree based heritability estimated by Young et al. (2012). For traits such as litter birth weight, the results were comparable with that reported by Ehlers et al. (2005), and for body weight at farrowing, the results from parity 1 was comparable with Gilbert et al. (2012) but were higher for parity two sows. But in general the variance proportions of the efficiency traits (group 3 traits) were almost similar to heritability figures reported by Bergsma et al. (2008) and Young et al (2012).

In order to identify regions or windows having a substantial effect on the trait, for each trait, the variance explained by each 1 Mb non overlapping windows was calculated and from each trait selected all windows

which explained at least 0.5% of the phenotypic variance or if there weren't any window which explained 0.5%, the top three windows were selected. Accordingly, the top 147 windows for all 16 traits are plotted in figure 1.

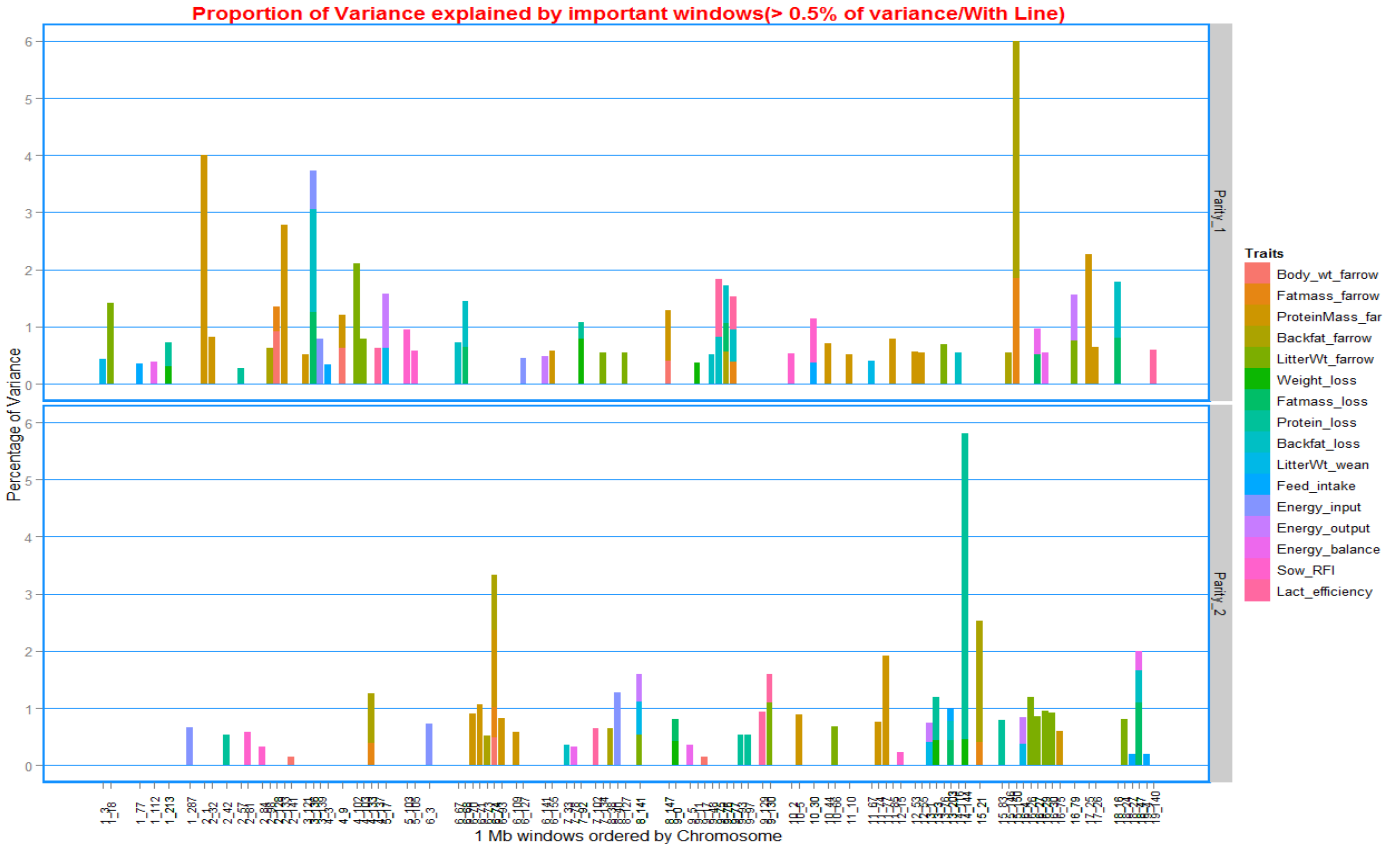

Figure 1. Windows of 1 Mb that explain at least 0.5% of genetic variance or that are in the top 2 for a given trait.

For figure 1, the x axis represents the 147 windows and the y axis represents the percentage of variance. Each color represents a trait and the traits are ordered per group, with body weight at time of farrowing as the first and lactation efficiency as the last trait. The proportion of variance accounted by these windows range from 0.14 (chromosome 9, position 17 Mb) for sow body weight at farrowing, to 5.36% (chromosome 14, position 144 Mb) for protein mass loss during lactation. The traits that explained the highest proportion of variance belonged to either group one or two, whereas the proportion of variance explained by these windows were small for the efficiency traits (group 3). Figure 1 also shows that some of those windows are associated with more than one trait, seven windows (Chr 6 pos 74, Chr 8 pos 141, Chr 9 pos 76 and 77, Chr 8 pos 141, Chr 12 pos 32 and Chr 13 Pos 203) were associated with three traits and three windows (chr 3 Pos 136, chr 6 Pos 74 and chr 9 Pos 0) with four traits. The candidate genes in these regions were looked into and are explained later.
Table 9 shows the trait wise distribution of these top 147 windows, i.e. the total proportion of variance explained by the top windows for each trait and for each parity separately. Protein mass at farrowing had the highest number of windows (14) selected and also the highest percentage of variance explained by all these windows (16.2%). Most of the traits in group one and two showed a higher proportion of variance explained by the markers when compared to the group three traits.

Table 9. Total proportion variance explained by the top selected windows for each trait for two different parities.

Group	Traits	Parity 1					Parity 2				
		# of windows	# of SNPs	Min variance	Max variance	Total variance	# of windows	# of SNPs	Min variance	Max variance	Total variance
1	Body weight at Farrowing	3	61	0.41	0.91	1.95	3	111	0.14	0.48	0.77
	Fat mass at farrowing	3	68	0.38	1.85	2.67	3	99	0.39	0.54	1.33
	Protein mass at farrowing	14	253	0.51	4	16.19	9	247	0.58	1.91	8.87
	Back fat at farrowing	4	84	0.54	4.15	5.88	5	139	0.51	2.13	5.12
	Litter weight at farrowing	7	159	0.55	2.11	6.87	8	177	0.53	1.2	7.06
2	Body weight loss	3	86	0.31	0.79	1.47	3	90	0.42	0.45	1.31
	Fat mass loss	5	115	0.5	1.25	3.71	3	82	0.39	1.1	1.92
	Protein loss	3	65	0.28	0.41	0.98	6	134	0.53	5.36	8.51
	Back fat loss	9	203	0.51	1.8	7.42	3	83	0.34	0.56	1.26
	Litter weight at weaning	3	81	0.4	0.63	1.46	3	78	0.38	0.58	1.37
3	Total feed intake	3	62	0.34	0.37	1.06	3	78	0.2	0.22	0.62
	Energy Input	3	61	0.45	0.79	1.92	3	63	0.66	1.28	2.66
	Energy Output	3	85	0.48	0.94	2.22	3	78	0.34	0.49	1.29
	Energy balance	3	56	0.38	0.55	1.38	3	70	0.32	0.36	1.02
	Lactation efficiency	4	79	0.58	1.02	2.83	3	55	0.49	0.94	2.07
	Sow RFI	4	110	0.53	0.95	2.84	3	59	0.23	0.59	1.15

From these 147 windows, those that explained at least 1% of the variance, either for a single trait or for multiple traits considered together, were selected for detailed examination for candidate genes. A total of 47 unique windows distributed across all 18 autosomes belonged to this category. The details of these 47 regions distributed among the four rounds of analysis are given in tables 10 and 11.

Table 10. Windows explaining more than 1% of genetic variance (either for a single trait or for multiple traits) for the analysis of Parity 1 sows.

Chr	Position (Mb)	# of SNPs	Total % variance explained	# traits associated	Traits associated	Trait group
1	18	22	1.42	1	Litter weight at farrow	1
2	1	9	4	1	Protein mass at farrowing	1
2	128	21	1.35	2	Body weight at farrow, Fat mass at farrow	1
2	133	29	2.78	1	Protein mass at farrowing	1
3	136	23	3.73	3	Back fat loss, Energy input and Fat mass loss	2,3
4	9	23	1.2	2	Body weight at farrow, Protein mass at farrow	1
4	102	23	2.11	1	Litter weight at farrow	1
5	17	25	1.57	2	Energy output, Litter weight at weaning	2,3
6	68	34	1.44	2	Back fat loss, Fat mass loss	2
7	92	36	1.08	2	Protein loss, Weight loss	2
8	147	17	1.29	2	Body weight at farrow, Protein mass at farrow	1
9	75	21	1.84	2	Back fat loss, Lactation efficiency	2,3
9	76	18	1.72	3	Back fat at farrow, Back fat loss, Fat mass loss	1,2
9	77	17	1.53	3	Back fat loss, Fat mass at farrow, Lactation efficiency	1,2,3
10	30	23	1.15	2	Feed intake, Sow RFI	3
15	150	30	6	2	Back fat at farrow, Fat mass at farrow	1
16	79	31	1.56	2	Energy output, Litter weight at farrow	1,3
17	25	13	2.27	1	Protein mass at farrow	1
18	16	18	1.79	2	Back fat loss, Fat mass loss	2

Table 11. Windows explaining more than 1% of genetic variance (either for a single trait or for multiple traits) for the analysis of Parity 2 sows.

Chr	Position (Mb)	# of SNPs	Total % variance explained	# traits associated	Traits associated	Trait group
4	133	32	1.25	2	Back fat at farrow, Fat mass at farrow	1
6	71	28	1.06	1	Protein mass at farrow	1
6	74	45	3.34	4	Back fat at farrow, Fat mass at farrow, Protein mass at farrow and Body weight at farrow	1
8	40	17	1.28	1	Energy Input	3
8	141	31	1.6	3	Energy output, Litter weight at farrow, Litter weight at weaning	1,2,3
9	130	16	1.59	2	Lactation efficiency and Litter weight at farrow	1,3
11	77	28	1.91	1	Protein mass at farrow	1
13	3	26	1.19	2	Protein loss, Weight loss	2
14	144	29	5.81	2	Protein loss, Weight loss	2
15	21	22	2.53	2	Back fat at farrow, Fat mass at farrow	1
16	26	29	1.2	1	Litter weight at farrow	1
18	47	21	2	3	Back fat loss, Fat mass loss and Energy balance	2,3

Candidate genes and QTL

The 47 windows which explained more than 1% of the genetic variance were studied in detail for the candidate genes and previously reported QTL. These windows, along with their adjacent regions (± 1 Mb) were scanned for genes / QTL responsible for traits related to growth, feed intake, metabolism, milk production etc. The details are summarized in table 12.

Table 12. Candidate genes and previously reported QTL in the important windows

Chr	Position (Mb)	Candidate genes	Previously reported QTL
1	18	AKAP12	
2	1	CPT1A, CHID1, OSBPL5, PNPLA2, TALDO1	Daily gain, FCR, Body weight
	40	PRMT3	Body weight, Average daily gain
	81 83	CLK4, MXD3, RUFY1, FGFR4, CDHR2, B4GALT7	Body weight, Average daily gain
	128 133	CSNK1G3, HSD17B4	Daily gain, FCR, Body weight
3	136 139		Body weight at end of test
4	9		Average daily gain, Body weight, Loin eye area
	99 102	ATP1A2, ARHGEF2, BGLAP, CRABP2, NCSTN	Average daily gain, Body weight
	133	ABCD3, CNN3	Average daily gain, Body weight
5	17	DIP2B, CELA1, GPD1, IGFBP6	Average daily gain
6	0 3	CDK10, DPEP1, GALNS, MAP1LC3B,	Average daily gain
	36		Average daily gain, Body weight
	67 68 71	ATP13A2, EPHB2, PINK1, CELA2A, CDA, PLA2G2F,	FCR, Body weight, Average daily gain
	74 133	CTH, SLC35D1	Average daily gain, Body weight
7	92	ST8SIA2	Average daily gain, Body weight
8	40	ATP10D, CWH43, LNX1, TEC, USP46	Average daily gain, Lipid accretion
	141 147	AGPAT9, CDS1, ENOPH1, HPSE, NUDT9, SCD5	None
9	0 48	APOA4, APOA5, APOC3, BACE1, PCSK7, SIK3	Average daily gain, FCR Feed intake, Average daily gain
	75 76 77	ADAM22, CDK14, C4BPA,	Average daily gain, Protein accretion rate
	130	PAPPA2, RFWD2	None
10	5		Average daily gain, Protein accretion rate, Body weight
	30	ADIPOR1, CYB5R1, FBP1, FBP2, KLHL12	Average daily gain
	66		None
11	77	PCCA	None
12	32	SCPEP1, TOM1L1	None
13	3	HACL1, CAPN7	None
14	144	ACADSB, ATE1, CPXM2,	None
15	21	MGAT5, SLC35F5	None
	150		None
16	26 27	OXCT1	Daily feed intake, Feed conversion ratio
	79	MTRR, GPX3	Average daily gain
17	25		Body weight
18	16	AGBL3	Body weight, Loin eye area
	47	CREB5, PLEKHA8	Body weight, Feed conversion ratio

Objective 2

Genotypes of a total of 2,386 pigs from the two RFI lines that have been genotyped using the 60k SNP panel in various projects were compiled and used to verify the parentage of pigs in comparison to recorded pedigree. The full pedigree included 16,026 animals. Figure 2 shows how the genotyped animals were spread across generations and between the two lines.

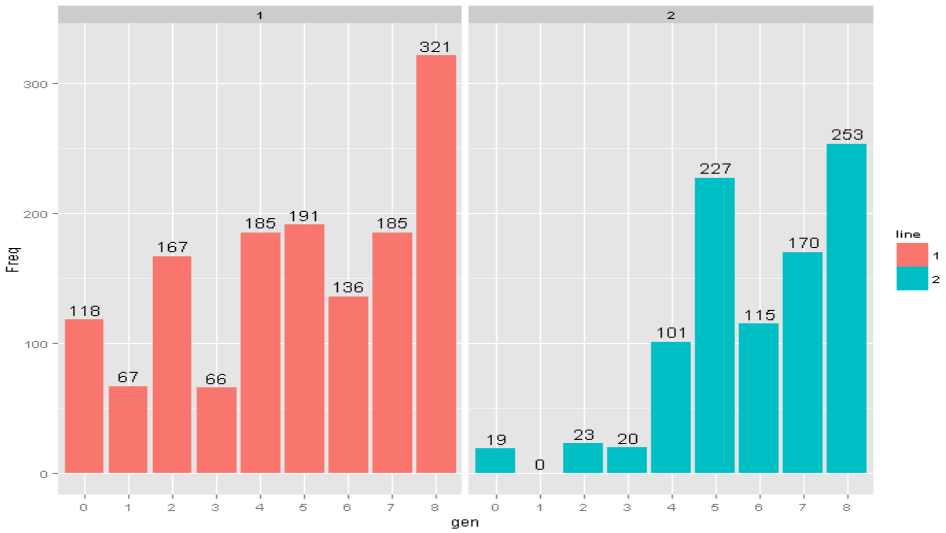

Figure 2. Distribution of the genotyped animals across generation and lines (line 1 = low RFI line; line 2 = high RFI line). Generation 0 is common to both lines.

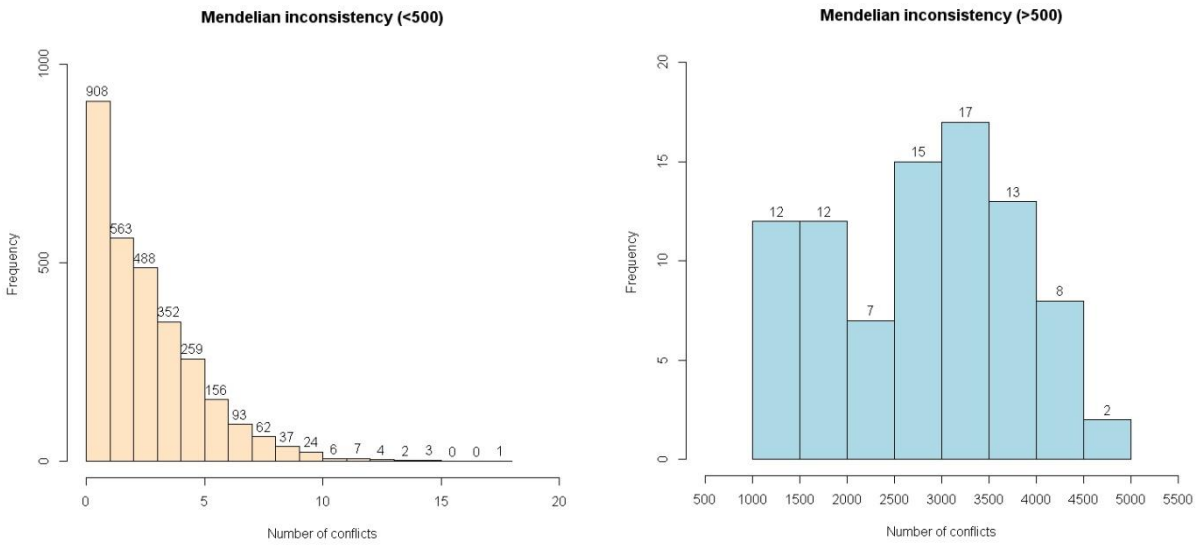

Figure 3. Frequency distribution of the number of SNPs for which a given progeny parent pair has opposite homozygotes, for pairs with <18 and pairs with >1000 inconsistent SNP genotypes.

Four of the genotyped animals were removed because they had >10% missing genotypes, likely because of poor DNA quality. A total of 2,914 SNPs were removed because they had missing genotypes for more than 10% of animals, 1,474 SNPs were removed because they were not in Hardy Weinberg Equilibrium($p<10^{-18}$), and 9,571 SNPs were removed because their minor allele frequency was less than 1%. This left a total of 2,382 genotyped animals and 48,156 SNPs.

The genotypes of 3,051 genotyped progeny-parent pairs were evaluated for having opposite homozygous genotypes, which would indicate an incorrect pedigree (or genotyping error). Figure 3 shows the distribution of the number of SNPs for which a progeny-parent pair had opposite homozygotes. For most progeny-parent pairs, the number of inconsistencies was very small (<18 of over 48,000 SNPs), indicating the correct pedigree; only

86 (2.8%) of progeny-parent pairs were identified to be incorrect because they had many more inconsistencies (>1,000).

Of the 86 errors, 27 could be attributed to sample mix-ups, 35 to pedigree recording errors, and 16 were undetermined. For 56 of these 86, the correct parent could be found based on comparison of genotypes.

Inbreeding

Inbreeding within each line was computed based on pedigree and based on the genetic markers. Trends in pedigree-based inbreeding are in Figure 4.

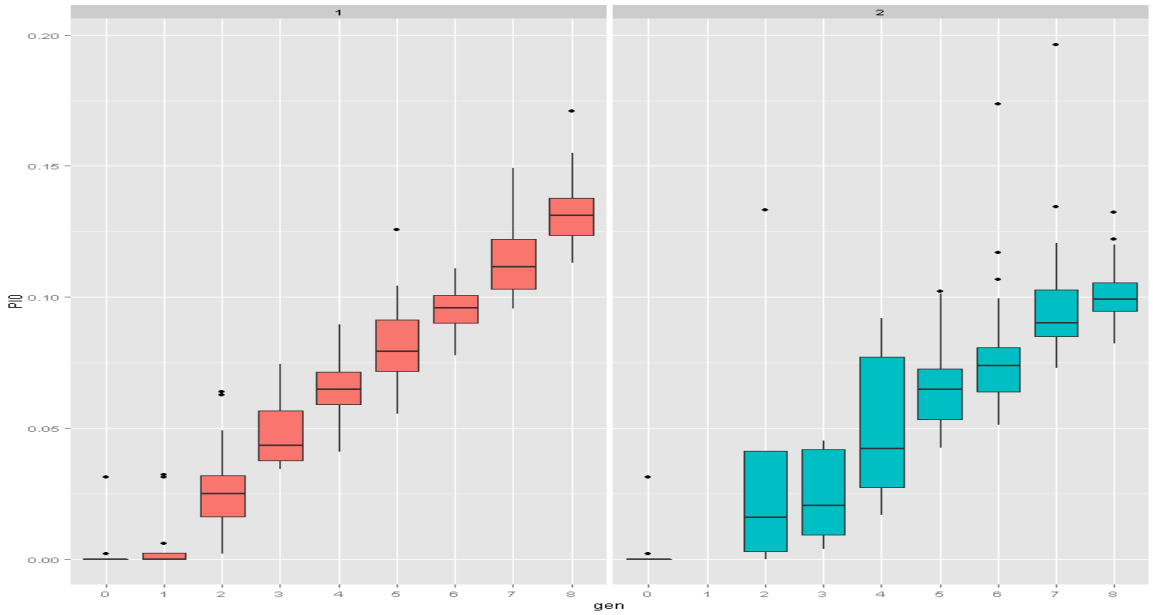

Figure 4. Pedigree-based inbreeding in the low RFI (left) and high RFI (right) lines.

Six approaches were used to compute genomic-based measures of inbreeding for each genotyped individual. This included 5 marker-by-marker based estimators:

VanRaden

$$GI_{VanRaden} = \frac{\sum_{i=1}^{N}(x_i - 2p_i)}{\sum_{i=1}^{N}(2p_iq_i)} - 1$$

Yang

$$GI_{Yang} = \frac{1}{N}\sum_{i=1}^{N}\frac{(x_i - 2p_i)}{2p_iq_i} - 1$$

VanRaden_0.5 $GI_{0.5}$ *Set p = 0.5 for all SNP*

Plink

$$GI_{Plink} = \frac{[O(\#\text{hom}) - E(\#\text{hom})]}{1 - E(\#\text{hom})} = \frac{1}{N}\sum_{i=1}^{N}\left(1 - \frac{x_i(2 - x_i)}{2p_iq_i}\right)$$

Yang_new

$$GI_{Yang\_new} = \sum_{i=1}^{N}\frac{x_i^2 - (1 + 2p_i)x_i + 2p_i^2}{2p_iq_i}$$

F_{Yang} weighted by sampling error

VanRaden 2008 J. Dairy Sci
Yang et al. 2010 Nat. Genet.
Purcell et al. 2007 AJHG

The 6th measure of genomic inbreeding was bases on runs of homozygosity (ROH), which is the sum of homozygous stretches in the genome, as implemented in PLINK. Results for genomic inbreeding are in Figure 5.

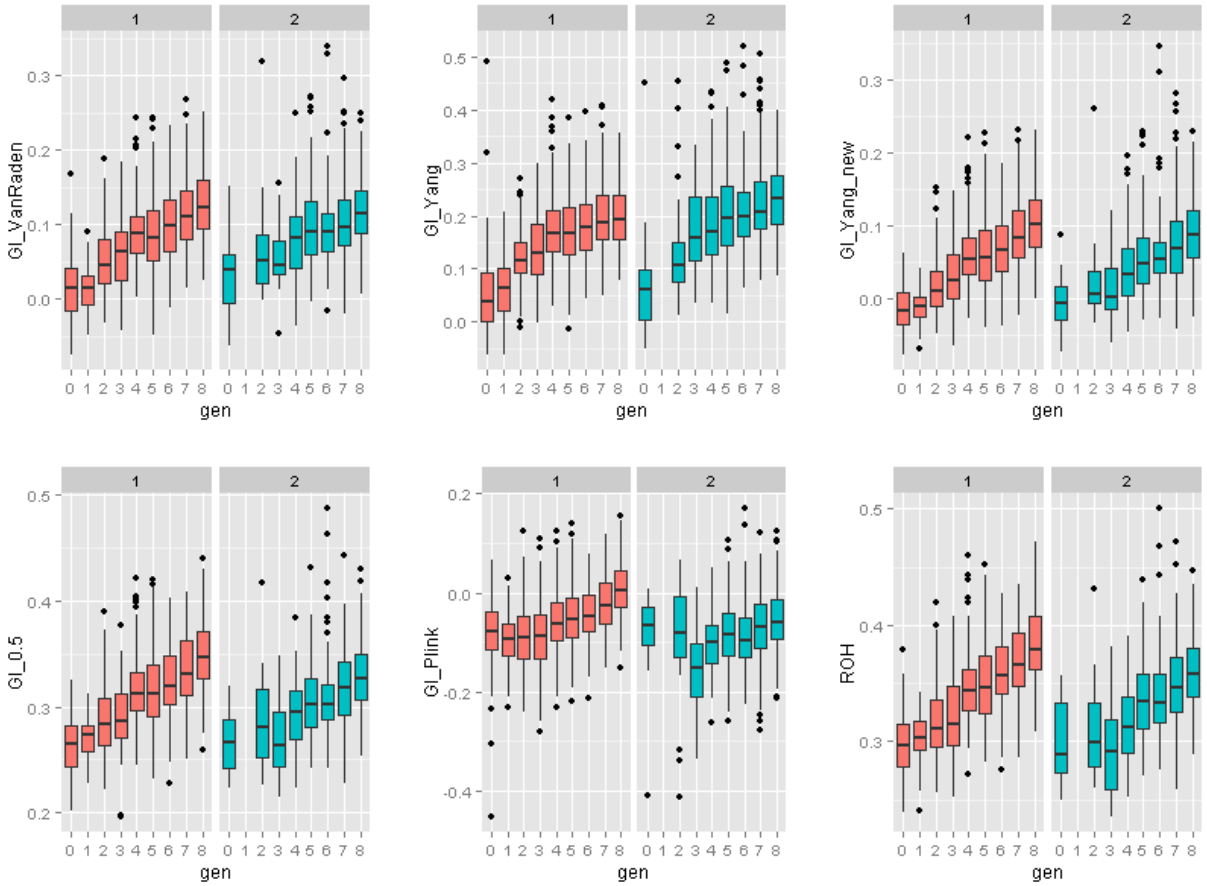

Figure 5. Genomic inbreeding in the low RFI (orange) and high RFI (blue) lines based on six measures.

Table 13 shows the correlations between the different measures of inbreeding. Results clearly show substantial differences between pedigree-based and genomic measures of inbreeding, as well as differences among genomic measures.

Table 13. Correlations between measures of inbreeding based on pedigree and 5 measures based on genomics.

	Pedigree	GI_VanRaden	GI_0.5	GI_Yang	GI_Plink	GI_Yang_new	ROH
Pedigree	**1.000**						
GI_VanRaden	**0.569**	**1.000**					
GI_0.5	**0.629**	**0.789**	**1.000**				
GI_Yang	**0.500**	**0.864**	**0.541**	**1.000**			
GI_Plink	**0.444**	**0.482**	**0.838**	**0.082**	**1.000**		
GI_Yang_new	**0.643**	**0.932**	**0.920**	**0.781**	**0.686**	**1.000**	
ROH	**0.650**	**0.755**	**0.951**	**0.526**	**0.797**	**0.883**	**1.000**

Inbreeding depression

One way to attempt to identify the most relevant measure of inbreeding is to identify the measure that shows the greatest relationship with phenotype, in terms of capturing inbreeding depression. Inbreeding depression is the expected decline in average phenotype of inbred animals. So a measure of inbreeding that shows the strongest relationship with average phenotype is expected to be the most appropriate or relevant measure of inbreeding. To determine this, both reproduction and growth performance traits were analyzed with an animal model with level of inbreeding of the animal as a cofactor. For the reproduction traits tb, nba, nwfs, albw, alpbw, padg, a total of 872 records on 532 sows were fitted with the following model:

$$y = \mu + generation \quad + parity \quad + \beta \cdot F + A + e$$

Where F is the individual's inbreeding coefficient, b the measure of inbreeding depression, and A the animal's additive genetic effect, modeled using the pedigree-based relationship matrix. For the growth and feed efficiency traits ADG, ADFI, Feed Efficiency, and RFI, the following model was fitted to data on 1084 animals:

$$y = \mu + generation \quad + parity \quad + sex \quad + line \quad + \beta \cdot F + A + e$$

In Figure 6 and 7 are shown the resulting p-values of the inbreeding depression effects. Although results differed substantially between traits, likely because of limited size of the data sets, the genomic measures of inbreeding tended to have stronger relationships with phenotype than the pedigree-based measure. Among the genomic measures, there was no clear winner.

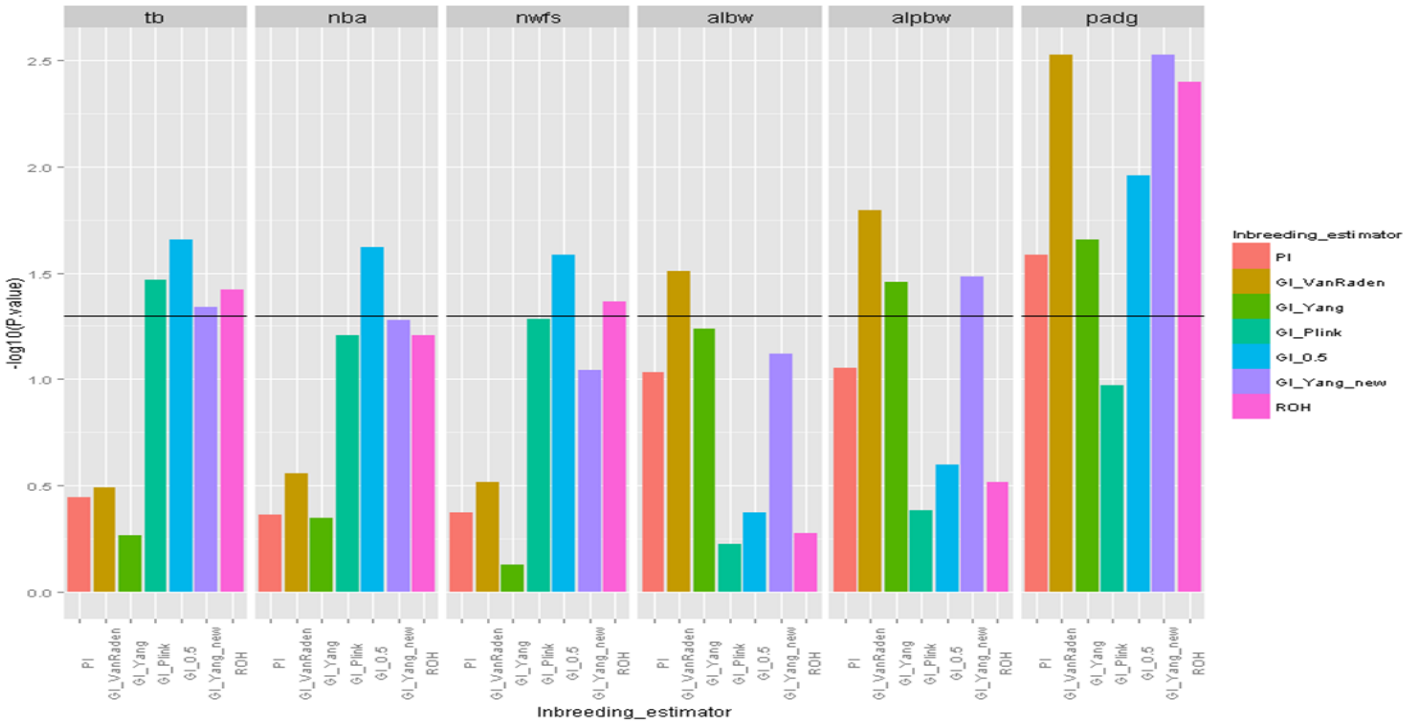

Figure 6. –log of the p-value of the significance of the regression of phenotype on inbreeding computed using pedigree (PI) and five genomic measures for reproductive traits.

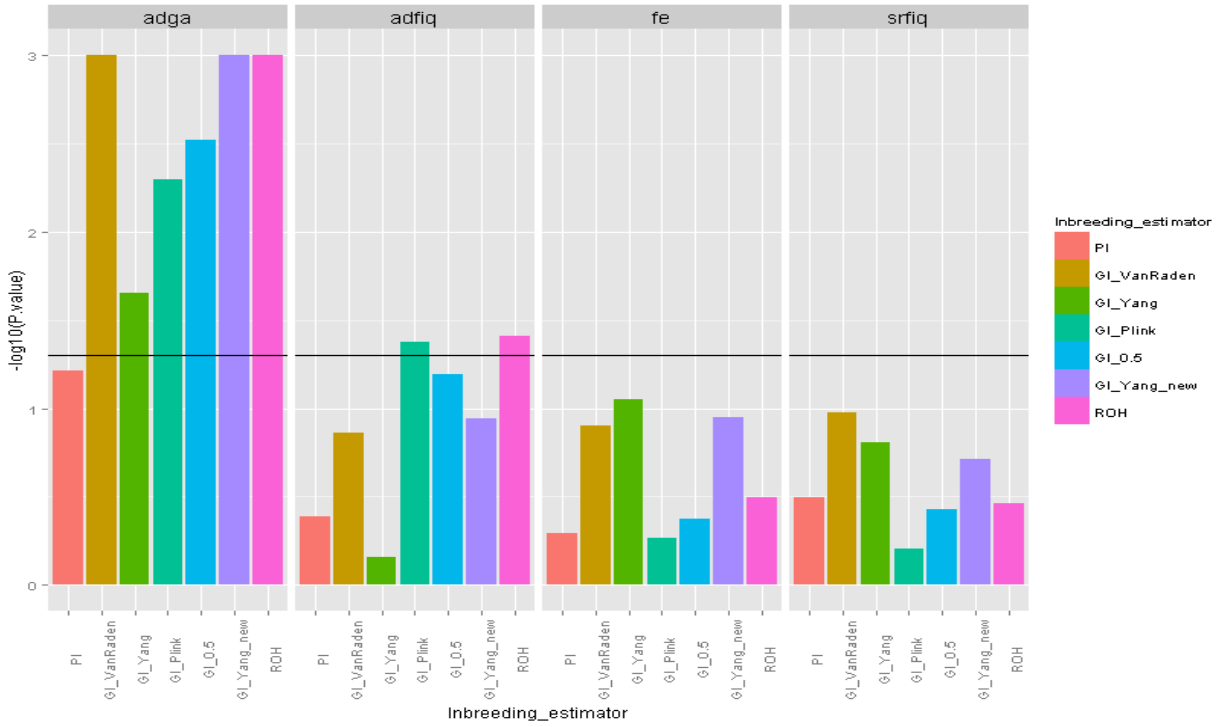

Figure 7. –log of the p-value of the significance of the regression of phenotype on inbreeding computed using pedigree (PI) and five genomic measures for growth performance traits.

In Table 14 is shown the estimates of inbreeding depression for one reproduction and one growth traits, as examples. Because the scales of the different measures of inbreeding are not comparable, inbreeding depression was also expressed per 10% increase in the mean inbreeding level for a given measure. Pedigree-based inbreeding showed an increase in number born alive with an increase in level of inbreeding, which was opposite to expectations. The genomic measures show the expected direction of estimates, i.e. lower phenotype with an increase in inbreeding.

Table 14. Mean and SD of alternate measures of inbreeding and estimates of inbreeding depression (b) for number born alive and average daily gain, expressed per 10% increase in the mean level of inbreeding based on a given measure.

Inbreeding estimator	mean	SD	Number born alive		Average daily gain (kg)	
			b	b*10%*mean	b	b*10%*mean
Pedigree	0.08	0.04	6.28	0.050	-0.39	-0.003
GI_VanRaden	0.09	0.06	-2.52	-0.023	-0.17**	-0.002
GI_Yang	0.18	0.08	-1.24	-0.022	-0.09*	-0.002
GI_0.5	0.31	0.04	7.59*	-0.235	-0.23**	-0.007
GI_Plink	-0.06	0.07	-3.18	-0.019	-0.11**	-0.001
GI_Yang_new	0.06	0.06	-4.82	-0.029	-0.18**	-0.001
ROH	0.35	0.04	3.25*	-0.114	-0.12**	-0.004

Discussion

This study provides additional information on regions of the genome that harbor genes that affect reproduction traits and represents first attempt to identify regions of the genome that control lactation efficiency traits. Multiple regions were found for most traits, with several regions affecting multiple traits. Data on parity 1 and parity 2 were analyzed separately; the amount of overlap between regions was limited, suggesting that different genes control reproduction and efficiency in first versus later parities. Several of the identified regions were consistent with regions that were found in previous research and for most regions important candidate genes that could harbor the causative mutations were identified. Although these results are promising and provide avenues for further research, these results need to be validated in other populations before they can be recommended as markers to be used for selection. To this end, we will be able to use data from a parallel GWAS project on sow feed efficiency in two commercial breeding lines that we are collaborating on with the University of Alberta and Genesus Inc.

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