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Candidate Reproductive Genes Do Not Explain Responses in Lines Selected for Ovulation Rate and Litter Size

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Summary and Implications

Molecular technologies have developed rapidly and provide methods to select directly for genes controlling economic traits. The swine genetic linkage map is the most highly developed of all livestock species. Positions on the chromosomes of several genes are known. Some of these genes have been shown to have direct effects on economic traits. Selection lines that differ from the control line by as much as 50% in ovulation rate and litter size exist at Nebraska. This experiment evaluated whether six specific genes that produce important proteins in reproductive processes explained responses in ovulation rate and litter size in two of these lines. The genes studied were follicle stimulating hormone (FSHβ), prostaglandin endoperoxide-synthase 2 (PTGS2), estrogen receptor (ESR), prolactin receptor (PRLR), retinol binding protein (RBP4), and epidermal growth factor (EGF). Distributions of genotypes for five of the six genes differed among lines. However, line differences in gene frequencies were not greater than what might have occurred due to random genetic drift associated with inbreeding. Furthermore, estimates of the effects of the genes on ovulation rate and litter size were not significant. Therefore, these genes did not have large effects on litter size in this population and did not explain the observed responses to selection. Either other genes with major effects were involved, or there are a large number of genes each with small effects that control expression of the traits. Additional work is being done to determine whether other genes were involved. However, until those genes are identified, swine breeders must rely on traditional breeding methods to improve reproductive traits.

Background

Ovulation rate sets the upper limit to litter size. It is heritable and responds to selection. However, in lines selected for increased ovulation rate, only 25% of each additional ova was realized as a pig at birth. Ovulation rate and number of embryos at 50 days of gestation are moderately correlated, but fetal losses after 50 days increased in the high ovulation line.

Uterine capacity is defined as the number of fetuses that a uterus can carry to term when ovulation rate is not limiting. Insufficient uterine capacity exists when number of potentially viable embryos, determined largely by ovulation rate, exceeds the number of fetuses the uterus can carry to parturition. The excess fetuses either die and are reabsorbed by the uterus or expelled as a mummified pig at birth, or survive to parturition but have very small birth weights and low survival rates.

At Nebraska, a selection strategy was used to select both for increased ovulation rate and increased uterine capacity. First gilts with increased ovulation rate were selected. Then, selection for increased litter size in females with high ovulation rate was practiced. The theory is that females first selected for ovulation rate have more potentially viable embryos than their uterus can carry to parturition. The number of pigs at birth is then a measure of the female's uterine capacity. Selection was practiced in two lines. One of these lines had increased ovulation rate and litter size due to previous selection; the other started from an unselected base. A randomly selected control line was maintained to monitor response in the selection lines.

The selection procedure used laparotomy to count corpora lutea as a measure of ovulation rate. This procedure still is not practical in most genetic selection programs. This surgical procedure could be avoided by selecting directly for genes controlling expression of the traits. Molecular technologies are developing rapidly and offer promise of being able to select directly for genes controlling economic traits. The swine genetic linkage map is the most highly developed of all livestock species. Positions on the chromosomes of several genes are known. Some of these genes have been shown to have direct effects on economic traits. Most of the genes mapped and with known effects control variation in growth and fatness traits. An example is the ryanodine receptor which

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reduces fatness, but also causes the PSS condition. A few genes with effects on reproductive traits have been identified. Certain other genes are prime candidates to have effects on reproduction because they produce products known to have physiological functions in the reproductive process.

Selection directly for genes controlling ovulation rate and litter size would enhance responses. Selection could be applied in both sexes instead of only in females. Selection accuracy would be increased. And surgical procedures to measure ovulation rate would not be necessary. The purpose of the experiment reported herein was to determine whether certain candidate genes explain a significant portion of the responses in the Nebraska lines selected for increased ovulation rate and uterine capacity.

The Lines

The pigs were from two selection lines designated IOL and COL and Line C, a randomly selected control. These lines originated from the Index selection and Control lines developed at the University of Nebraska. The Index and Control lines have a common base of the Large White and Landrace breeds. Beginning in 1981, the Index line was selected eight generations for increased ovulation rate and increased embryonic survival to 50 days of gestation. The control line was selected randomly. At generation 8, three new lines were formed, one from the Index line and two from the Control line. The line originating from the Index line is designated Line IOL, the lines originating from the Control line are designated as COL and C. Eight generations of two-stage selection for increased ovulation rate and increased litter size in lines IOL and COL were practiced. In the first stage, all gilts born to the 50% of the sows with the greatest litter size at birth were selected. Laparotomy about 10 days after their second estrous period (first estrus following their pubertal estrus) was performed to count number of corpora lutea. Stage two selection included the 50% of these gilts with the greatest ovulation rate. Each line had approximately 45 litters by 15 sires each generation. Replacement boars were from the 15 largest litters. Replacements in Line C were selected randomly.

DNA Analyses

Ear tissue was collected from pigs in generations 7 and 8. Only selected gilts and boars of generation 8 were sampled. Tissue was collected from all generation-7 gilts in which laparotomy was performed. Genotypes of 190 animals of generation 7 and 334 of generation 8 were determined. DNA was extracted from the tissue and analyzed to determine the genotype of each pig for six genes. These genes were selected because of their known physiological function in reproductive processes or because they had been found in other studies to affect litter size.

Genes

Estrogen Receptor.

At least eight estrogens are secreted by the ovary, with estradiol being the primary one. These steroid hormones have a wide range of activities. They are important behavioral hormones and are involved in uterine growth and in maternal recognition of pregnancy. Estrogen receptor is a nuclear protein that binds steroid hormones and allows them to penetrate the plasma membrane to perform their function. Pigs with different genotypes for the estrogen receptor gene (ESR) were reported to differ in litter size. In some populations, females homozygous for the B allele had about .4 pigs more per litter than those homozygous for the A allele (Short et al., 1997; J. Anim Sci. 75:3138).

Prolactin Receptor.

Prolactin is important in mammary growth and in milk synthesis. It also affects the growth and function of ovaries and testes and the action of gonadotrophic hormones. It is necessary for maintenance of corpora lutea and affects production of the hormones progesterone and relaxin. Prolactin receptors (PRLR) are proteins that bind with prolactin in the corpora lutea. Females of Landrace, Large White, and Chinese Meishan breeds with the AA genotype had .66 pigs more than those with the BB genotype (Vincent et al., 1998; Proc. 6th World Cong. Applied to Livest. Prod. 15:18).

Follicle stimulating hormone β.

Follicle stimulating hormone is a protein produced by the anterior pituitary. It has two distinct subunits, α and β, coded for by two different genes. FSH acts predominantly on the cells of the follicles within the ovary. It is critical in the growth and selection of those that will mature and subsequently ovulate. FSHβ was chosen to study because in a report from the China Agriculture University in Beijing (Li et al., 1998; Proc. 6th World Cong. Applied to Livest. Prod. 15:183) it was reported to be a major gene affecting litter size in crosses of Chinese breeds with Duroc and Yorkshire.

Epidermal Growth Factor.

Epidermal growth factor (EGF) has many functions in adults, including proliferation and differentiation of the epidermis and in wound healing. It also is transcribed in early embryonic development by the conceptus and by the uterus of the sow. In embryos and neonates it stimulates pulmonary epithelia to grow and mature and it stimulates proliferation of skin epithelia. It was chosen as a candidate gene because Landrace, Large White, Pietrain, and Chinese breeds, which differ in litter size, also had quite different EGF genotypic frequencies (Mendez et al., 1999; J. Anim Sci. 77:492), although no direct relationship with litter size was reported.

Retinol Binding Protein 4.

Retinol binding protein 4 (RBP4) is secreted by the conceptus into the uterine lumen between 10 and 15 days

of gestation. It is a major secretory product during this period. It is thought to function in the transport of retinoids to the conceptus. This period is a dynamic time for mother and conceptus during which several physiological and biochemical interactions must occur for proper fetal development. This major protein enhances gene expression of a particular growth factor (Transforming Growth Factor β) via retinoic acid receptors. RBP4 was reported to have an additive effect on litter size of .52 ± .30 pigs in the French Hyperprolific Large White breed (Messer et al., 1996; Mammalian . Genome 7:396).

Prostaglandin-Endoperoxide Synthase 2.

Prostaglandin-Endoperoxide Synthase 2 (PTGS2) is the rate limiting enzyme in the formation of prostaglandins. Although it has not been shown to directly affect litter size, it was chosen as a candidate gene because mice homozygous for a "knockout" gene (a procedure to suppress expression of the gene) were infertile and had few ovulations. The uterus of mutant mice also did not support growth of normal embryos transplanted into them (Lim et al, 1997; Cell 91:197).

Statistical Analyses

Gene and genotypic frequencies within each line were calculated. If a gene affects ovulation rate or litter size, then we expect both genotypic and gene frequencies to differ among lines, with the frequency of the favorable allele and the favorable genotype being greater in the selection lines than in the control line. Chi-square analyses were used to test whether genotypic distributions among lines were different. When lines are separated by several generations, both selection and the random changes associated with inbreeding can cause them to have different genotypic and gene frequencies. To determine whether changes in gene frequencies were greater than what might have occurred by chance, variances of gene frequency changes were adjusted for genetic drift before gene frequency differences among lines were tested statistically.

Favorable alleles of each gene were defined as the ones that had been increased in frequency in the selection lines compared to the control line. The effect of this gene on each trait was estimated by analyzing the data with analysis of variance procedures, calculating the average phenotypic value for each genotypic class and making contrasts among these means to estimate additive and dominance effects of the genes. The additive effect, (a) was calculated as the mean phenotype of females homozygous for the favorable allele minus the mean for those homozygous for the unfavorable allele. The dominance effect, (d) was calculated as the mean of animals with heterozygous genotypes minus the average of those with the two homozygous genotypes. For example, for favorable allele A, a = AA - BB, and d = AB - .5(AA + BB). Estimates of a and d were tested to determine whether they differed from zero. Values different from zero are interpreted to mean that the gene affected the trait being analyzed. All tests of a and d effects were performed with procedures that corrected for differences in genetic value due to inbreeding and to effects of other genes not included in the model.

Table 1. Phenotypic means[a] for generations 7 and 8.

Line	N_{OR}	N_{FF}	OR	FF	NBA	SB	M
			Generation 7				
IOL	90	43	19.0	13.4	11.1	2.3	.5
COL	90	45	15.1	11.8	10.8	1.1	.5
C	51	35	12.9	9.6	9.0	.6	.2
			Generation 8				
IOL		42		12.4	10.9	1.8	1.2
COL		40		10.2	9.9	.6	1.2
C		32		7.4	7.2	.6	1.0

[a]OR = ovulation rate, FF = number of fully formed pigs, NBA = number born alive, SB = number of stillborn pigs, and M = number of mummified pigs per litter. N_{OR} = number of ovulation rate records, and N_{FF} = number of litter size records.

Results

Phenotypic means of the traits studied are in Table 1. Line IOL and C are separated by 16 generations of selection and Lines COL and C are separated by 8 generations. Lines differ significantly for all traits studied. The genetic differences between Lines IOL and C at generation 8 were estimated to be 6.1 ova and 4.7 fully formed pigs at birth; whereas, Lines COL and C differ by 2.2 ova and 2.9 fully formed pigs. Total responses between Lines IOL and C are approximately 50% in both number of ova and fully formed pigs per litter. Differences in number born live are less because increased numbers of stillborn and mummified pigs accompanied the genetic increases in ovulation rate and fully formed pigs. However, these differences provide substantial genetic variation to determine whether specific genes were being selected for.

Distributions of genotypes of FSHβ, PTGS2, ESR, PRLR, and RPB4 differed significantly among lines (Table 2). For all but ESR genotypes, one genotype was most frequent in the selection lines compared to the control, as if selection had acted on these genes. For example, most animals had FSHβ genotype BB in both Lines IOL and COL, whereas the frequency of that genotype was less in Line C. Similar results occurred for PTGS2 and PRLR. There was a high frequency of ESR AA genotype in all lines and the distributions of ESR genotypes are such that the Chi-square statistic is biased. Chi-square tests are biased upward when fewer than five observations occur in some cells; therefore we cannot infer that distributions of ESR genotypes differ among lines.

The fact that lines differ in genotypic distributions does not mean that

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the difference was caused by selection acting on the genes. Other events such as nonrandom mating of parents, different distributions of genotypes in selected parents and random gene frequency changes over generations due to inbreeding along with selection can cause different genotypic frequencies.

Selection operates directly to increase frequencies of genes controlling the selected trait. But random drift associated with inbreeding also causes closed lines to have different gene frequencies. Gene frequencies of each line were calculated and contrasts of frequencies in selection and control lines were made (Table 3). Inbreeding in the lines was .14 in Lines C and COL and .19 in Line IOL. With this amount of inbreeding, considerable random drift in gene frequency might have occurred since lines were closed. To determine whether the gene frequency differences between selection and control lines were greater than what might have occurred by chance, standard errors of changes in gene frequency adjusted for inbreeding were calculated. When differences among lines were tested without adjusting standard errors for inbreeding, several of the differences were significant. However, after adjusting for inbreeding none of the changes in gene frequency were significantly different from zero. Therefore, genotypic distributions of the genes were different because the lines had different gene frequencies, but these differences likely were not caused by selection.

The distributions of gene frequencies in Table 3 have a pattern consistent with what would have occurred if the genes controlled expression of the traits selected for in Lines IOL and COL. For example, Lines IOL and C are separated by 16 generations of selection and Lines COL and C are separated by 8 generations of selection. Compared to Line C, the frequency of the B allele of FSHβ increased by .15 in COL and .35 in IOL, as if the change was directly related to the selection applied. A similar pattern occurred for PTGS2 and PRLR. To further evaluate effects of these genes, the average performance of animals with each genotype was calculated and used to calculate a and d effects. Estimates of these effects along with their standard errors are in Table 4. No estimates differed significantly from zero. Furthermore, in some cases the sign on the estimate is

Table 2. Distributions of genotypes and Chi-Square (χ^2) statistics.

Item	IOL			COL			C			
	AA	AB	BB	AA	AB	BB	AA	AB	BB	χ^2
FSHβ	0	12	176	8	73	115	16	70	50	124.7**
PTGS2	181	8	0	162	33	2	94	36	7	47.2**
ESR	166	22	1	198	0	0	136	0	0	42.5**
PRLR	4	64	121	23	97	78	35	73	29	79.6**
RBP4	8	46	13	15	35	19	12	21	21	12.3*
EGF	0	8	58	0	13	55	0	11	45	2.7

*P < .05.
**P < .01.

Table 3. Frequency of each gene and contrasts of frequencies between selection lines and the control.

Gene	IOL	COL	C	IOL-C	SE	COL-C	SE
FSHβ, B	.97	.77	.62	.35	.20	.15	.24
PTGS2, A	.98	.90	.82	.16	.16	.08	.18
ESR, A	.94	1.00	1.00	.06	.12	0	.12
PRLR, B	.81	.64	.58	.33	.25	.16	.04
RBP4, B	.54	.53	.58	.04	.29	.05	.06
EGF, B	.94	.90	.90	.04	.06	0	.16

Table 4. Additive (a) and dominance (d) effects with an animal model.[a,b]

Candidate gene	Contrast		OR	s.e.	FF	s.e.	NBA	s.e.	Stillborn	s.e.	Mummies	s.e.
PRLR	BB-AA	a	-.287	.27	-.039	.38	-.007	.366	-.028	.184	.091	.103
		d	-.445	.32	-.229	.462	-.466	.44	.164	.219	.063	.126
PTGS2	AA-BB	a	.036	.64	.589	.833	.403	.795	.184	.399	.273	.226
		d	.448	.71	.354	.953	.076	.909	.278	.454	.741	.259
ESR	BB-AA	a	.108	1.3	1.74	1.6	.474	1.52	1.25	.761	.341	.437
		d	2.33	1.42	2.72	1.98	1.58	1.88	1.13	.933	.37	.54
FSHβ	BB-AA	a	-.04	.34	.163	.466	.12	.446	.045	.223	.246	.127
		d	-.039	.41	.979	.577	.759	.549	.222	.273	.0481	.157
RBP4	BB-AA	a	.284	.38	-.179	.457	-.526	.436	.346	.22	.026	.124
		d	.315	.49	.441	.627	.313	.595	.0936	.298	-.0479	.17

[a]EGF could not be estimated with contrasts because there was only two genotypes.
[b]Traits ovulation rate (OR), number of fully formed pigs (FF), number born alive (NBA), stillborn and mummies. Candidate genes used were follicle stimulating hormone (FSHβ), prostaglandin endoperoxide-synthase 2 (PTGS2), estrogen receptor (ESR), prolactin receptor (PRLR), retinol binding protein (RBP4), and epidermal growth factor (EGF). The additive contrast is given as the favorable genotype minus the less favorable genotype, as determined by the most frequent allele in the selection lines.

opposite of what was expected based on gene frequencies in the lines. For example, the PRLR B allele was increased in the selection lines, but it had a negative effect on both ovulation rate and number of pigs in the litter. This provides additional evidence that the genes studied did not affect the traits selected for in this experiment and that the changes in their frequency in the selection lines were due to random genetic drift.

Conclusion

Some of the genes studied had different gene frequencies in the selection lines compared to the control line. However, these differences were not greater than what might have occurred by chance due to inbreeding. Estimates of the effects of these genes on ovulation rate and litter size were not significant and in some cases signs of these effects were opposite of the changes in gene frequencies. Thus, we conclude that these genes did not contribute to the genetic changes in ovulation rate and litter size in the selection lines.

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[1]Regina Linville was a graduate student in Animal Science; Daniel Pomp is associate professor of animal science, and Rodger Johnson is professor of animal science.