Discovery of a Major Gene Associated with Litter Size in the Pig

M.F. Rothschild¹, D.A. Vaske¹, C.K. Tuggle¹, D.G. McLaren², T.H. Short², G.R. Eckardt², A.J. Mileham³ and G.S. Plastow and in collaboration with O.I. Southwood⁴ and H.A.M. van der Steen⁴

¹ Department of Animal Science, Iowa State University, Ames, IA 50011 USA,  
² PIC USA, PO Box 348, Franklin, KY 42135-0348 USA,  
³ Dalgety FTC, Station Road, Cambridge CB1 2JN, UK.  
⁴ PIC UK, Fyfield Wick, Abingdon, Oxon, OX13 5NA, UK

SUMMARY

Identification of individual major genes affecting quantitative traits in agricultural livestock species has been limited to date. Using a candidate approach, we investigated the possibility that the Estrogen Receptor (ESR) is a major gene for litter size in the pig. Initially, synthetics involving females from a 50% Meishan/50% U.S. line and 50% Meishan/50% European line were used. A total of 180 first parity and 140 later parity records were evaluated. Both total number born (TNB) and number born alive (NBA) were 2.4 pigs more for BB females than for AA females in first parity litters (P<.01). For later parities, BB females had 1.0 more pigs for TNB than AA females. The B allele also occurs in American Yorkshire and European Large White breeds. Analysis of PIC USA commercial lines derived from Large White revealed that the B allele had an additive effect of approximately 0.4-0.5 pigs per litter (P<.01) for first parity litters. Dominance effects on litter size were detected in later parities in the populations tested. The only negative pleiotropic effect detected was small and was for backfat in the Large White lines. The favorable allele is being incorporated into various PIC lines using marker assisted introgression and being fixed in hyperprolific lines using marker assisted selection.

INTRODUCTION

Animal geneticists have made considerable genetic improvement for many performance traits (Smith, 1984; McLaren, 1992) but have made only limited genetic improvement in reproductive and health traits (Haley et al., 1988; Rothschild, 1989; McLaren and Bovey, 1992). Molecular marker-assisted selection (Soller and Beckmann, 1983) may provide the opportunity to make significant genetic gains in the improvement of such traits. Implementation of marker-assisted selection programs will first require identification of candidate genes or anonymous genetic markers associated with the traits of interest. In pigs, examples of such genes or anonymous gene markers have to date been limited (Jung et al., 1989; LeRoy et al., 1989; Rothschild et al., 1990; Fujii et al., 1991; Vogeli et al., 1992; Andersson et al., 1994).

The relative economic importance of reproductive traits in pigs is high when compared to growth and carcass traits (NSIF, 1987). However, selection for improved litter size in pigs has been limited and has produced variable results (Ollivier and Bolet, 1981) with notable successes being hyperprolific selection (Legault, 1985) and selection on an index of ovulation rate and embryo survival (Lamberson et al., 1991). Research in France and the U.K. has confirmed litter size of
the Chinese Meishan breed to be three or four piglets greater than litter size of the European Large White (Haley and Lee, 1993). Identification of a genetic polymorphism associated with improved litter size in Meishan pigs would be of major importance and could be used in marker-assisted selection or introgression programs. Several candidate genes involved in reproduction have been cloned in some species. Among these possible candidate genes are those which code for steroid hormones or steroid hormone receptors.

Beginning in 1991 our laboratory began investigating the role of the Estrogen Receptor (ESR or ER) locus as a major gene for litter size in the pig. Initial research (Rothschild et al., 1991) had identified that genetic variability existed for the estrogen receptor gene both between and within several U.S., European and Chinese breeds of pig. One of the primary goals of the research was to investigate and determine the underlying genetic reasons for the increased prolificacy seen in the Chinese breeds of swine. A cooperative research project involving Iowa State University and PIC was begun in 1991 to determine if a major gene existed for litter size. The purpose of this joint research project was to determine if the ESR gene was genetically linked to a major gene(s) controlling increased litter size in the pig. The initial report (Rothschild et al., 1994) suggested that the ESR locus was associated with a significant effect in the PIC US Meishan synthetic line. Further research has confirmed this effect is in additional Meishan synthetics from PIC USA and PIC UK and Large White synthetics from PIC USA research herds (Rothschild et al., 1995). The purpose of this report is to update geneticists and the swine industry on recent research results involving the ESR locus.

MATERIALS and METHODS

Animals. All pigs used in these experiments were bred and raised at genetic nucleus herds owned and operated by either PIC USA or PIC UK. Females from a 50% U.S./50% Meishan synthetic line and females from a 50% European/50% Meishan synthetic line were used for the initial analysis. Since it was important to reduce the effect of background genes and to clearly examine the effect of the ESR locus, data involving only families were collected. These data consisted of litter records from groups of sisters in which at least two ESR genotypes existed. Planned matings, involving sires and dams that had been previously genotyped, were made to speed the process of collection of data from families of sisters with different ESR genotypes. In the Meishan synthetic analysis, the total number of females from the two lines was 180 from 65 families. For the Large White (LW) synthetic line analysis, over 1,750 females of PIC USA lines with Large White ancestry had this ER polymorphism and were then used.

Several performance traits were recorded. Litter traits (records) included total number born (TNB), which was calculated as live births plus stillborn animals (excluding mummified fetuses), and number born alive (NBA). Growth performance traits were measured on nearly all PIC USA females. These performance records included average daily gain (ADG) over a test period, ultrasonically measured backfat thickness (BF) and number of functional nipples (FN).

Molecular Biology. Blood or other tissue samples were collected by PIC personnel under approved animal care procedures and shipped either to Iowa State University or a PIC laboratory for analysis. Initial analyses consisted of isolation of genomic DNA, digestion with PvuII
endonuclease, electrophoresis, blotting and hybridization with a 1.3 kb cDNA insert of the human ESR gene probe, as previously described by Rothschild et al. (1994). Fragment sizes were estimated using molecular weight standards. In order to genotype a larger number of animals a second method of detection of the ESR polymorphism was developed. A polymerase chain reaction (PCR) test was developed by obtaining sequence data from the genomic regions surrounding the polymorphic PvuII site. This test is now in use to determine all ESR genotypes.

**Statistical Analyses.** Data from the Meishan and LW synthetics were analyzed separately since they represented synthetic lines with very different genetic backgrounds. A mixed model with full relationships was used for each analysis. In the past both animal models and sire model have been used and produced the same results. For simplicity the assumed statistical models used to analyze the litter size traits TNB and NBA included the fixed effects of contemporary group farrowed, and ESR genotype effect and the random effects included sire and random residual effects. Heritabilities were estimated from the data for both TNB and NBA and were 0.09 for the Meishan synthetic data, and were 0.12 and 0.17 for TNB and NBA, respectively for LW synthetic data analysis. For the performance traits, the assumed model in analysis 1 included the fixed effects of contemporary group tested and ESR genotype effect and the random effects for sire and error. Heritabilities were assumed to be 0.25 (ADG), 0.30 (BF), and 0.09 (FN). For the LW synthetic data analysis, the model was similar but a fixed effect for synthetic line was included and an interaction of line by ESR was included since there are some genetic differences between the lines. For this analysis, the assumed heritabilities were 0.46 (ADG), 0.48 (BF), and 0.17 (FN). Additive effects and their significance were estimated using linear regression techniques, regressing the litter trait on number of copies of the B allele of ESR. The dominance deviation was calculated as the deviation of the heterozygotes from the mean of the homozygotes, using the least squares means. Significance was tested as the lack of fit to the additive model.

**RESULTS**

Results for the litter traits for the Meishan synthetics are given in Table 1. Differences between ESR genotypes appears to be essentially additive for first parities with the two homozygotes differing by 2.4 pigs for TNB and NBA. The estimate of the additive effect for the B allele is about 1.25 pigs (P<.01). For later parity records, differences were not significant though the effect is about .6 pigs for TNB. In later parities the effects appear to be dominant though not enough records exist to sufficiently test the magnitude of the effects.

Results for the LW synthetics are found in Table 2. Just as with the Meishan synthetics, in the LW synthetics the effect of the ESR B allele is significant. In first parity litters the additive effect of the B allele is .40 pigs for TNB and .39 pigs for NBA (P<.01). In later parities the effect is .50 pigs for TNB and .41 pigs for NBA (P<.01). Again in later litters the effect appears to be dominant, especially for TNB (P<.05).

A comparison of the effect of the ESR gene differences on other important traits is given in Table 3. For the Meishan synthetics no significant negative pleiotropic effects of the ESR gene were seen. For the LW synthetics there was a small but significant negative effect of the desired allele

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**This** PCR test for ESR genotype is performed by PIC under a license with Roche Molecular Systems, Inc.
DISCUSSION

Genetic improvement of litter size has been limited in the past because of the low heritability of reproductive rate. Identification of a specific candidate gene or an anonymous genetic marker for litter size traits could have a major impact on the improvement of reproductive performance by increasing accuracy of selection. The research reported here was directed at confirming that the ESR gene, a steroid binding hormone receptor gene, was associated with increased litter size. Our results continue to demonstrate that genetic differences for the ESR locus exist and that one ESR allele, initially found in the Meishan, is significantly associated with higher litter size. In addition, this allele had no significant negative pleiotropic effects on growth and backfat. In first parity litters it acts in an additive manner and in later parities it appears to act in a dominant fashion in regards to litter size. The purpose of using the Meishan breed in synthetic line development is to improve prolificacy. However the Meishan's slow growth and excessive fat (Young, 1992) could make it difficult to develop both a prolific and lean synthetic line involving the Meishan breed. Discovery of the significant effect of the ESR locus on litter size makes it now possible to use marker-assisted selection to improve prolificacy, growth and backfat simultaneously in this synthetic population. Additionally, the favorable allele may be incorporated into conventional dam lines using marker-assisted introgression.

Further analysis in PIC USA lines involving the LW synthetics suggests that ESR is also associated with increased litter size. The size of the effect is less than that seen in the Meishan synthetics and there appears to be a small negative pleiotropic effect on backfat. Further investigation of the effect of the ESR allele in other LW populations is underway. Analysis of PIC UK LW lines (Southwood et al., 1995) has shown that the effect of the ESR allele is either diminished or in the opposite direction. These results coupled with those from the PIC USA lines suggests that the ESR mutation is only a marker for the real mutation influencing litter size. Analysis is underway to determine DNA sequence differences for the two ESR alleles and to determine if differences exist in the RNA transcripts for the two alleles. It is possible that ESR is just a marker for the litter size gene but mapping efforts around ESR and preliminary analysis of the effects of the linked genes would suggest that if ESR is not the gene it must be closely linked to it.

Our ESR results also demonstrate the usefulness of the candidate gene approach to discover genes with significant effects on economic traits in pigs. Future genetic improvement using marker-assisted selection and genes with significant effects like the ESR locus might have a major impact on the efficiency of production of high quality pork for the consumer.
ACKNOWLEDGEMENTS

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REFERENCES


Table 1. Effect of the ESR genotypes on reproductive traits in Meishan synthetic line females

<table>
<thead>
<tr>
<th>ESR Genotype</th>
<th>First Parity</th>
<th>Later Parities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>TNB</td>
</tr>
<tr>
<td>AA</td>
<td>71</td>
<td>11.1a</td>
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<tr>
<td>AB</td>
<td>82</td>
<td>12.4b</td>
</tr>
<tr>
<td>BB</td>
<td>27</td>
<td>13.5b</td>
</tr>
</tbody>
</table>

Effect†
Additive
1.26** 1.24**
Dominance 0.10 0.05

* N = total number of records, TNB = total number born, NBA = number born alive.
† Additive effect was estimated as the linear regression of litter size on ESR. **P<.01. Dominance effect was estimated from the Least Squares means.
\textsuperscript{a,b} Means in the same column with different superscripts significantly differ P<.01.

Table 2. Effect of the ESR genotypes on reproductive traits in Large White lines*

<table>
<thead>
<tr>
<th>ESR Genotype</th>
<th>First Parity</th>
<th>Later Parities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>TNB</td>
</tr>
<tr>
<td>AA</td>
<td>674</td>
<td>9.7c</td>
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<tr>
<td>AB</td>
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<td>10.1b</td>
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<tr>
<td>BB</td>
<td>425</td>
<td>10.4b</td>
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</tbody>
</table>

Effect†
Additive 0.40** 0.39**
Dominance 0.05 0.00

* N = total number of records, TNB = total number born, NBA = number born alive.
† Additive effect was estimated as the linear regression of litter size on ESR. **P<.01,*P<.05. Dominance effect was estimated from the Least Squares means.
\textsuperscript{a,b} Means in the same column with different superscripts significantly differ P<.05.
\textsuperscript{c,d} Means in the same column with different superscripts significantly differ P<.10.
\textsuperscript{e,f} Means in the same column with different superscripts significantly differ P<.01.
<table>
<thead>
<tr>
<th>ESR</th>
<th>Meishan synthetic females</th>
<th></th>
<th>Large White synthetic females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADG (g/d)</td>
<td>BF (mm)</td>
<td>FN</td>
<td>ADG (g/d)</td>
</tr>
<tr>
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<tr>
<td>AA</td>
<td>71</td>
<td>777</td>
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<td>789</td>
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<tr>
<td>BB</td>
<td>27</td>
<td>792</td>
<td>18.7</td>
<td>14.2</td>
</tr>
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</table>

* N = Number of records, ADG = average daily gain, BF = backfat, and FN = number of functional nipples.
<sup>a</sup><sup>b</sup> Means in the same column with different superscripts significantly differ P<.05.
Comment: D. Rhoads

It seems that you are assuming that the B allele arose only once and that the polymorphism you detect is the cause of litter size.

Response: M. Rothschild

No, I have said that all B alleles appear to be the same in the 2 breeds that we see them in. We have shown that this is a marker for a mutation or may be the mutation which affects litter size. We can't be sure yet though we suspect ESR is the major gene.

Question: C. Haley

Selection on litter size in the first parity would tend to reduce the gene affect in later parities. Could this be happening in your data?

Response: M. Rothschild

That is an interesting question. Certainly the effect is less but it is quite significant biologically and economically. I think we would need to consider parity effects but I don't see a real problem.