

# Effect of the Estrogen Receptor Locus on Reproduction and Production Traits in Four Commercial Pig Lines<sup>1</sup>

T. H. Short\*, M. F. Rothschild<sup>†</sup>, O. I. Southwood<sup>‡</sup>, D. G. McLaren\*, A. de Vries<sup>‡</sup>,  
H. van der Steen<sup>§</sup>, G. R. Eckardt\*, C. K. Tuggle<sup>†</sup>, J. Helm<sup>†</sup>, D. A. Vaske<sup>¶</sup>,  
A. J. Mileham<sup>§</sup>, and G. S. Plastow<sup>§</sup>

\*PIC USA, Franklin, KY 42135; <sup>†</sup>Department of Animal Science, Iowa State University, Ames 50011; <sup>‡</sup>PIC Europe, Fyfield Wick, Abingdon, Oxfordshire, UK; <sup>§</sup>PIC Group, Fyfield Wick, Abingdon, Oxfordshire, UK; and <sup>¶</sup>Marshfield Clinics, Marshfield, WI 54449

**ABSTRACT:** We investigated the effect of the estrogen receptor (ESR) gene on growth and reproductive traits in four Large White-based commercial pig lines. A total of 9,015 litter records from 4,262 sows genotyped at the ESR locus were analyzed to determine whether ESR influenced total number born (TNB) or number born alive (NBA). Teat number (TN), test ADG, ADFI, feed:gain ratio (F/G), and ultrasonic backfat (BF) were also analyzed to determine effects of ESR. The TNB and NBA were increased per favorable allele of ESR ( $P < .01$ ) with additive effects of .42 (.31) and .39 (.31) pigs/litter in the first parity (later parities), respectively.

Dominance effects were near zero in parity one, but they were .16 and .14 pigs for TNB and NBA, respectively, in later parities ( $P < .05$ ). A favorable additive pleiotropic effect was detected for BF ( $P < .001$ ;  $-.11$  mm per copy of the favorable litter size allele). There were no detectable effects on ADG or F/G ( $P > .10$ ), although ADF was reduced 18 g/d per copy of the favorable litter size allele ( $P < .05$ ). Average TN was 13.1 for pigs carrying the favorable litter size allele vs 13.2 for noncarriers ( $P < .05$ ). Marker-assisted selection using ESR is warranted to increase litter size in the Large White-based lines considered here and will be of considerable economic value to pork producers.

Key Words: Estrogen Receptors, Reproduction, Growth, Genetic Markers, Major Genes, Pleiotropy

J. Anim. Sci. 1997. 75:3138–3142

## Introduction

Efficiency of production of livestock is highly influenced by reproductive success, especially in litter-bearing species. Over the past 40 yr, litter size has improved due to management changes, use of superior dam lines, and through crossbreeding (reviewed by McLaren and Bovey, 1992; Rothschild, 1996). Selection for litter size has been reasonably successful in mice (reviewed by Nielsen, 1994), but the response in pigs has been extremely variable (Bolet et al., 1989). Successes include hyperprolific selection (Bichard and

David, 1985; Bidanel et al., 1994) and selection on an index of ovulation rate and embryo survival (Neal et al., 1989).

The development of genome maps offers the opportunity to identify individual genes controlling reproduction. Gene effects or associations with litter size have been reported for the Booroola gene in sheep (Montgomery et al., 1992) and major histocompatibility complex genes in pigs (Warner and Rothschild, 1991). More recently, associations with genetic variation at the pig estrogen receptor (ESR) locus have been discovered (Rothschild et al., 1991). In initial analyses involving limited numbers of pigs, estimates of the additive effect of the preferred allele varied from 1.25 pigs/litter in Meishan crosses to .50 pigs/litter in Large White crosses (Rothschild et al., 1994, 1996; Short et al., 1995). The objectives of this research were to evaluate the effect of the ESR locus on litter size in a large sample of pigs from four commercial lines and to measure pleiotropic effects on growth and carcass traits.

<sup>1</sup>The authors gratefully acknowledge the partial funding of the Biotechnology Research Development Corporation, Peoria, IL; Dalgety PLC, London, England; and the Iowa Agriculture and Home Economics Experiment Station. The authors also acknowledge Shoji Sasaki and Mike Rose for animal tissue collection and Amy Vincent, Darren Cuthbert-Heavens, and Ken Siggins for ESR genotyping. Journal paper no. J-17169 of the Agriculture and Home Economics Experiment Station, Ames, Iowa, Project 3215.

Received December 16, 1996.

Accepted August 12, 1997.

Table 1. Numbers of observations, estrogen receptor B allele frequencies, means, and standard deviations (SD) for litter size and performance test traits

Trait <sup>a</sup>	n	freq (B)	Mean	SD
First parity				
TNB	4,262	.51	10.2	3.4
NBA	4,262	.51	9.2	3.4
Later parities				
TNB	4,753	.57	11.3	3.5
NBA	4,753	.57	10.3	3.3
Backfat, mm	27,073	.47	11.9	2.8
ADG, g/d	26,220	.48	835	111
ADF, kg/d	3,922	.51	2.00	.27
F/G	3,922	.51	2.30	.28
TN	22,379	.46	13.1	1.7

<sup>a</sup>TNB = total number born in litter; NBA = number born alive/litter; backfat = ultrasonically measured P<sub>2</sub> backfat; ADG = test average daily gain; ADF = test average daily feed consumed (boars only); F/G = feed:gain ratio (boars only); TN = teat number.

## Materials and Methods

### Data

A total of 9,015 litter records from 4,262 sows were included in the litter size analyses. Traits included were total number born (**TNB**) and number born alive (**NBA**) from four Pig Improvement Company (**PIC**) lines. Three of the lines were of Large White origin and the fourth was a 1/4 Large White synthetic. Two of the Large White-based lines were formed through a hyperprolific selection program (Bichard and David, 1985) in the United States and United Kingdom in the late 1970s and early 1980s and have been closed to outside introductions since that time. The other Large White origin line was developed in the United Kingdom in the 1960s and in 1983 underwent hyperprolific selection to form the existing line. The 1/4 Large White Synthetic line was developed by crossing Large White line females with boars from a Duroc origin line and backcrossing the F<sub>1</sub> to the Duroc line. This synthetic was formed in the late 1980s and early 1990s and has remained closed since its formation. These lines were all housed in genetic nucleus farms owned by PIC U. K. or PIC U. S. and were raised in accordance with approved farm management practices. Average daily gain, ADFI (boars only), and feed:gain ratio (**F/G**, boars only) were measured during a 13-wk performance test. Real-time ultrasonic backfat at the P<sub>2</sub> location (**BF**) and total number of teats (**TN**) were recorded at the completion of the 13-wk test period. Age at the beginning and completion of the 13-wk test period was approximately 84 and 175 d, respectively. Performance test data were from 15,614 female and 11,913 male pigs. Different numbers of observations were available for the performance traits (Table 1). Pigs with unknown ESR genotypes were genotyped at Dalgety Food Technology Center (Cambridge, U.K.),

PIC's Genetic Diagnostic Laboratory (Franklin, KY), or Iowa State University. Progeny from homozygous parents were assigned genotypes and were not individually tested.

### DNA Preparation

At birth, DNA was extracted from blood or tail tissue. The procedure described here pertains to blood cell preparations. Other tissues can be processed similarly by directly suspending material in potassium (K) buffer and proceeding from the same stage as the blood procedure. Blood was collected in 50 mM EDTA at pH 8.0 to prevent coagulation. A sample of blood (50  $\mu$ L) was dispensed into a small microcentrifuge tube (.5 mL Eppendorf or equivalent). A total of 450  $\mu$ L of Tris EDTA (**TE**) buffer was added to lyse the red blood cells, and this mixture was vortexed for 2 s. The intact white and residual red blood cells were then centrifuged for 12 s at 13,000  $\times g$  in a microcentrifuge. The supernatant was removed by gentle aspiration using a low pressure vacuum pump. An additional 450  $\mu$ L of TE buffer was then added to lyse the remaining red blood cells, and the white blood cells were collected by centrifugation. If any redness remained in the pellet, this process was repeated until the pellet was white. After removal of the supernatant from the pelleted white blood cells, 100  $\mu$ L of K buffer containing about 200  $\mu$ g/mL of proteinase K was added and the mixture was incubated at 55°C for 2 h. The mixture was then heated to 95 to 100°C for 8 min, and the DNA lysates were stored at -20°C. Polymerase chain reaction was used to amplify DNA in .5-mL Eppendorf tubes. Reactions included 1.3  $\mu$ L 10 $\times$  PCR buffer (Applied Biosystems, Foster City, CA), 1.3  $\mu$ L 15 mM MgCl<sub>2</sub>, 1.3  $\mu$ L 2 mM dNTP, .5  $\mu$ L forward (*F*) primer (5  $\mu$ M), .5  $\mu$ L reverse (*R*) primer (5  $\mu$ M), .1  $\mu$ L *Taq* DNA polymerase (.5 U), 7.6  $\mu$ L water, and 1.0  $\mu$ L DNA lysate. The ESR primers were as follows:

ESRF 5'-CCTGTTTTTACAGTGACTTTTTACAGAG-3',  
and  
ESRR 5'-CACTTCGAGGGTTCAGTCCAATTAG-3'.

The reactions were loaded onto a Perkin Elmer 480 thermal cycler under the following conditions: 1 cycle at 94°C for 4 min, 55°C for 1 min, and 70°C for 1 min; 31 cycles of 94°C, 55°C for 1 min, and 70°C for 1 min; 1 cycle at 72°C for 8 min; hold at 4°C. After PCR, 1  $\mu$ L of a mixture of React 6 buffer (Life Technologies, Grand Island, NY) and *Pvu*II (5  $\mu$ L) was added to each sample, and the reaction was incubated at 37°C for 2 h. Using gel electrophoresis, DNA samples were separated with 4% agarose and .5 $\times$  Tris-borate EDTA and visualized by Polaroid (Cambridge, MA) photography under UV light after staining with ethidium bromide. Two alleles (A and B) were identified. In the original RFLP test, a 4.3-kb fragment was denoted as the A allele and a 3.7-kb fragment was denoted as the

B allele (Rothschild et al., 1996). The new PCR test is seen in Figure 1.

### Statistical Analysis

Litter size traits were analyzed separately by first parity and later parities. Total number born and NBA were analyzed with an animal model that included fixed effects of farm, line nested within farm, month of farrowing, service type (natural or AI), ESR genotype, random effects of animal, and residual. A fixed parity effect was also included in the analysis of later parity litter records. Relationships were traced back to base animals for all females with records. Heritability of .10 was assumed for TNB and NBA (Short et al., 1994). Pairwise *t*-tests were used to test differences among ESR genotypes. Allele substitution effects were estimated by substituting for ESR genotype a covariate that included the number of B alleles present (0, 1, or 2). Dominance effects were estimated as the deviation of heterozygotes from the mean of the homozygous genotypes and tested as a quadratic effect.

Due to the large number of records, growth and carcass traits were analyzed with a mixed sire model that included fixed effects of farm, line nested with farm, week off test, sex, ESR genotype of the pig, random effects of sire, and residual. Assumed heritabilities were .60 for BF, .34 for ADG, .16 for TN, and .20 for ADF and F/G (T. H. Short, unpublished data). Allele substitution effects were estimated the same way for litter traits by substituting for ESR genotype a covariate for the number of B alleles present for each animal. Dominance effects were estimated as deviations of heterozygotes from the average of homozygote means.

### Results

Allele frequencies in the females included in the litter size analysis averaged .49 for the A allele and .51 for the B allele in first-parity females but increased to .57 for the B allele in later parities (Table 1). Frequencies of the B allele among all pigs with an ESR genotype (including males) with performance data ranged from .46 to .53, depending on the sample of pigs measured for the trait considered. In females included in the litter analysis, frequency of the B allele was similar in the three Large White lines (range .64 to .74) but was considerably less in the 3/4 Duroc line (.17). Means for litter size and performance test traits are also shown in Table 1.

Least squares means and allele substitution effects for litter size are shown in Table 2. In first-parity females, all three genotypes differed for TNB and NBA ( $P < .01$ ), and in second and later parities the homozygous AA pigs differed from either heterozygote or homozygous BB genotypes ( $P < .01$ ). The favorable

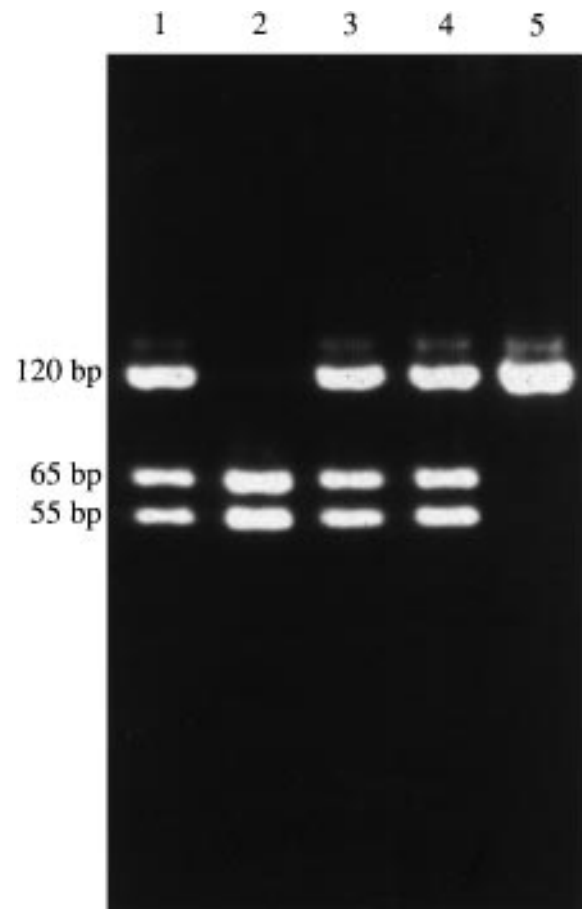


Figure 1. The PCR-RFLP test for the estrogen receptor polymorphism. Lane 2 has a homozygous animal for the favorable B allele. Lane 5 has an animal homozygous for the A allele and lanes 1, 3, and 4 contain heterozygous animals.

allelic substitution effects were .42 and .39, respectively, for TNB and NBA in first parity and .31 for TNB and NBA in later parities ( $P < .01$ ). Dominance effects were near zero in the first parity, but they were .16 and .14 pigs for TNB and NBA in later parities ( $P < .05$ ), respectively. Line  $\times$  ESR interaction was tested in a preliminary model ( $P > .40$ ). Allele substitution effects were similar for all lines.

As shown in Table 1, the ESR allele B frequency increased from .51 in the first parity to .57 in later parities. This increase was likely due to the effect of selection for the B ESR allele on litter size. Culling in first parity for small litter size may reduce the number of AA and AB genotypes in later parities, resulting in a higher frequency of the B allele in these parities.

In Table 3 are the least squares means by ESR genotype for the performance test traits. Favorable pleiotropic effects were detected for BF ( $P < .05$ ). The additive effect was  $-.11$  mm per copy of the B allele. There was no difference between genotypes for ADG or F/G ( $P > .10$ ); however, the average effect of the B allele was  $-18$  g/d on ADF ( $P < .05$ ). Number of teats

Table 2. Least squares means and allele substitution effects for total number born (TNB) and number born alive (NBA) in first and later parities

ESR genotype	First parity		Later parities	
	TNB	NBA	TNB	NBA
AA	10.14 <sup>a</sup>	9.42 <sup>a</sup>	11.36 <sup>a</sup>	10.03 <sup>a</sup>
AB	10.59 <sup>b</sup>	9.87 <sup>b</sup>	11.86 <sup>b</sup>	10.51 <sup>b</sup>
BB	10.97 <sup>c</sup>	10.22 <sup>c</sup>	12.04 <sup>b</sup>	10.71 <sup>b</sup>
Effect				
Additive	.42**	.39**	.31**	.31**
Dominance	.04	.05	.16*	.14*

<sup>a,b,c</sup>Means within a column without a common superscript differ ( $P < .01$ ).

\* $P < .05$ .

\*\* $P < .01$ .

and ADF were the only traits to display negative effects of ESR. Pigs with AB and BB genotypes had .1 fewer teats than AA animals ( $P < .05$ ). Number of teats was the only performance trait with a significant dominance effect ( $P < .05$ ).

### Discussion

The ESR B allele has been seen primarily in Chinese pigs and the Large White or Yorkshire breeds. The line of Duroc origin used in formation of the 1/4 Large White origin synthetic in this trial was monomorphic AA. Rothschild et al. (1996) speculated that the presence of the effect of the gene in Large White pigs may be the result of interbreeding of Chinese pigs with pigs in England before 1800 in what eventually became known as the Large White breed.

The ESR locus has been shown here and in previous studies to have a significant influence on litter size in pigs. Most previous studies are in agreement with the ESR allelic effect ranging from .3 to 1.4 pigs per copy of the B allele. Earliest reports show the effect to be largest in lines of Meishan origin and intermediate in

Large White-based lines (Rothschild et al., 1996). Southwood et al. (1995) reported results from a 50% Meishan synthetic and four European Large White origin line sows. In the Meishan synthetic, the result was similar to that reported by Rothschild et al. (1994, 1996) with the additive effect being greater than one pig born per copy of the B allele in the sow. In three of the four European Large White lines, no relationship was detected between ESR genotype and litter size, although in the fourth line the effect was similar to that found in the present study. However, the data in Southwood et al. (1995) were limited in number of animals sampled in each of the lines. In France, Legault et al. (1996) investigated ESR gene effect for litter size in 59 sows from a hyperprolific Large White line and a control Large White line. Although ESR genotype was not significant in either line, the effect in the first parity was similar in magnitude to that reported by Rothschild et al. (1996) and to that reported here. In the initial stages of the current study reported here, nonsignificant results were found with limited data, but as more sows were genotyped the ESR effect was verified. Our results suggest that at least 1,000 litter records are

Table 3. Least squares means and allele substitution effects for performance test traits

ESR genotype	Trait <sup>a</sup>				
	BF	ADG	ADF	F/G	TN
AA	12.4 <sup>b</sup>	808	1.99 <sup>d</sup>	2.27	13.2 <sup>d</sup>
AB	12.2 <sup>c</sup>	807	1.98 <sup>d</sup>	2.26	13.1 <sup>e</sup>
BB	12.1 <sup>c</sup>	806	1.96 <sup>e</sup>	2.25	13.1 <sup>e</sup>
Effect					
Additive	-.11***	-1.7	-.018*	-.013	-.05*
Dominance	-.05	0	.006	0	-.05**

<sup>a</sup>Backfat = ultrasonically measured P<sub>2</sub> backfat, mm; ADG = test average daily gain, g/d; ADF = test average daily feed consumed, kg/d (boars only); F/G = feed:gain ratio (boars only); TN = teat number.

<sup>b,c</sup>Means within a column without common subscripts differ ( $P < .001$ ).

<sup>d,e</sup>Means within a column without common subscripts differ ( $P < .05$ ).

\* $P < .05$ .

\*\* $P < .01$ .

\*\*\* $P < .001$ .

needed for the estimate of the effect to be stable for a litter size effect of .4 pigs per litter.

Earlier research of Rothschild et al. (1996) had suggested the effect of the favorable B ESR allele might be antagonistic relative to BF. However, the present study with nearly 12 times as much data detected a favorable effect. Also of interest was the effect on TN. Rothschild et al. (1994) suggested the B allele was associated with increased TN in Meishan synthetic pigs. This result was not repeated in these four Large White lines, in which the B allele has a slight but significant negative effect on TN. Estrogen receptors are thought to interact with growth factors, and this may be the cause of the small association seen in this study.

The identification of single genes with large effects on quantitative traits provides the opportunity to improve accuracy of selection for litter size by marker-assisted selection (Rathje et al., 1996; Rothschild et al., 1996). Marker-assisted selection using ESR has been underway since 1994 at PIC in the United States and Europe, and use of a second litter size marker is currently being developed (Short et al., 1997).

The effect of the ESR B allele seems to be additive, with an effect of .4 pigs/litter in the first parity and .3 pigs/litter in later parities. There was no dominance effect in the first parity, but in later parities a dominance effect of half the magnitude of the additive effect was detected. If the total effects are examined in context of a commercial female, then the value of fixing the ESR B allele is considerable. Assuming an initial gene frequency of .25 in parent females, an additive effect of .4 pigs/litter per copy of the B allele, 2.3 litters-sow<sup>-1</sup>·yr<sup>-1</sup>, and a marginal economic value of \$30 per additional pig, then for a 1,000 sow farm the potential value of fixing the ESR B allele amounts to an additional \$20,700 per year (.75 × .4 × 2.3 × 30 × 1,000), or over \$20-sow<sup>-1</sup>·yr<sup>-1</sup>. This value well exceeds testing costs and offers an improvement over traditional selection based upon phenotypic performance (only) of relatives.

### Implications

The effect of the estrogen receptor (ESR) locus on litter size was demonstrated in a large sample of pigs from four commercial lines in which the B allele was present. Potential economic value from marker-assisted selection using the B ESR allele is considerable. In addition to the positive effects of the ESR B allele for litter size, a small favorable response was detected in backfat thickness associated with a small decrease in average daily feed intake but no effect on growth rate. Therefore, selection for prolificacy based in part on ESR genotype should not adversely affect growth and carcass traits. The only negative effect was a slight reduction in teat number. Because teat number is moderately heritable and included in maternal line indexes used to improve the lines studied, selection

emphasis on this trait will overcome the slight unfavorable relationship observed. The use of this genotype offers a clear example of economically useful marker-assisted selection in pigs.

### Literature Cited

- Bichard, M., and P. David. 1985. Effectiveness of genetic selection for prolificacy in pigs. *J. Reprod. Fertil. Suppl.* 33:127-138.
- Bidanel, J. P., J. Gruand, and C. Legault. 1994. An overview of twenty years of selection for litter size in pigs using "Hyperprolific" schemes. *Proc. 5th World Congr. Genet. Appl. Livest. Prod.* 17:512-515.
- Bolet, G., L. Ollivier, and P. Dando. 1989. Selection sur la prolificat chez le porc. I. Resultats d'une experience de selection sur onze generations. *Genet. Sel. Evol.* 21:93-106.
- Legault, C., J. Gruand, J. Lebest, H. Garreau, L. Ollivier, L. A. Messier, and M. F. Rothschild. 1996. Frequency and effect on prolificacy of the ESR gene in two French Large White lines. *J. Rech. Porcine (France)* 28:9-14.
- McLaren, D. G. and M. Bovey. 1992. Genetic influences on reproductive performance. *Vet. Clin. N. Am. Food Anim. Prac.* 8: 435-459.
- Montgomery, G. W., K. P. McNatty, and G. H. Davis. 1992. Physiology and molecular genetics of mutations that increase ovulation rate in sheep. *Endocr. Rev.* 13:309.
- Neal, S. M., R. K. Johnson, and R. J. Kittok. 1989. Index selection for components of litter size in swine: Response to five generations of selection. *J. Anim. Sci.* 67:1931.
- Nielsen, M. K. 1994. Selection experiments for reproductive rate in mice. *Proc. 5th World Congr. Genet. Appl. Livest. Prod.* 19: 219-225.
- Rathje, T.A., G. A. Rohrer, and R. K. Johnson. 1996. Quantitative trait loci affecting reproductive traits in swine. *J. Anim. Sci.* 74 (Suppl. 1):122 (Abstr.).
- Rothschild, M., C. Jacobson, D. Vaske, C. Tuggle, L. Wang, T. Short, G. Eckardt, S. Sasaki, A. Vincent, D. McLaren, O. Southwood, H. van der Steen, A. Mileham, and G. Plastow. 1996. The estrogen receptor locus is associated with a major gene influencing litter size in pigs. *Proc. Natl. Acad. Sci. USA* 93: 201-205.
- Rothschild, M. F. 1996. Genetics and reproduction in the pig. *Anim. Reprod. Sci.* 42:143.
- Rothschild, M. F., C. Jacobson, D. A. Vaske, C. K. Tuggle, T. H. Short, S. Sasaki, G. R. Eckardt, and D. G. McLaren. 1994. A major gene for litter size in pigs. *Proc. 5th World Congr. Genet. Appl. Livest. Prod.* 21:225-228.
- Rothschild, M. F., R. G. Larson, C. D. Jacobson, and P. Pearson. 1991. Pvu II polymorphisms at the porcine estrogen receptor locus (ESR). *Anim. Genet.* 22:448.
- Short, T. H., E. R. Wilson, and D. G. McLaren. 1994. Relationships between growth and litter traits in pig dam lines. *Proc. 5th World Congr. Genet. Appl. Livest. Prod.* 17:413.
- Short, T. H., G. R. Eckardt, S. Sasaki, M. Rose, A. Vincent, D. G. McLaren, A. Mileham, and G. S. Plastow. 1995. Marker assisted selection for litter size in pigs. *J. Anim. Sci.* 73 (Suppl. 1):109 (Abstr.).
- Short, T. H., O. I. Southwood, D. G. McLaren, A. DeVries, H. van der Steen, G. J. Evans, A. J. Mileham, and G. S. Plastow. 1997. Evidence of a new genetic marker for litter size in pigs. *J. Anim. Sci.* 75 (Suppl. 1):29 (Abstr.).
- Southwood, O. I., H. A. M. van der Steen, A. J. Mileham, and D. Cuthbert-Heavens. 1995. Evaluation of the oestrogen receptor (ESR) gene in Meishan synthetic and Large White pigs. *Eur. Assoc. Anim. Prod., Prague, Sept. 4-7.* (Abstr.).
- Warner, C. M., and M. F. Rothschild. 1991. The major histocompatibility complex of the pig. In: R. Srivastava, B. P. Ram, and P. Tyle (Ed.) *Immunogenetics of the MHC.* pp 368-397. VCH Publishers, New York.