Selection for increased litter size has been one of the main objectives in Danish pig breeding since 1992. This selection has led to an average increase of 0.30 piglets/litter per year for Landrace and Yorkshire sows, resulting in an average litter size of 15.3 piglets born alive in 2007, with an SD of 3.5 piglets. The objective of this study was to investigate differences in identity by state relationships and allele effects associated with litter size across 17 selected microsatellite marker positions on chromosomes 11, 13, and 15. For this purpose, 357 Danish Landrace sows with high and low EBV for litter size were genotyped. An assignment test showed that 91 and 90% of the sows could be assigned correctly to the group of sows representing high and low EBV, respectively, based on genotype information. Allele effects were estimated separately for each marker by using deregressed EBV and a linear model that include both a polygenic and an allele effect. The investigated region on chromosome 13 was found to have a greater average identity by state relationship compared with the other regions, indicating that selection has taken place in this region. This is supported by an increased average allele effect of microsatellite alleles in the region. In spite of the apparent increased historical selection pressure on chromosome 13, fairly large variation in allele effects was observed, indicating that the markers within the region may be used for marker-assisted selection. However, substantial variation in allele effects was observed for several markers on all 3 investigated chromosomes, indicating that selection should preferably be based on several markers.

**Key words:** estimated allele effect, identity by state relationship, litter size, pig, quantitative trait locus

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**INTRODUCTION**

Litter size is an important trait in the swine industry. It has been part of the selection objective for the 2 maternal breeds (Landrace and Yorkshire) in the Danish nucleus breeding program since 1992. Selection for litter size has led to an average improvement of 4.6 piglets/litter since 1992 for the purebred Landrace and Yorkshire sows, resulting in an average litter size of 15.3 piglets born alive, with an SD of 3.5 (unpublished 2008 data from Danish Pig Production, Copenhagen).

Several QTL affecting reproduction in pigs have been reported [e.g., a QTL affecting ovulation rate on SSC9, a QTL affecting number of stillborn piglets on SSC5 and SSC13, and QTL affecting number of fully formed piglets and number of piglets born alive on SSC11 have been described (Cassady et al., 2001; Holl et al., 2004)]. The strongest indication for QTL affecting litter size was found on SSC13 at position 101 cM (Cassady et al., 2001). Studies have also been performed in other species, for instance, in mice, in which QTL affecting reproduction have been identified in a region corresponding to part of SSC15 (Rocha et al., 2004).

This study is based on genotyping of 3 QTL regions on SSC11, SSC13, and SSC15, respectively, in 357 Danish Landrace sows with, respectively, high EBV (EBV-high) and low EBV (EBV-low) for litter size. The regions were chosen based on results from the QTL studies mentioned above. To test whether the 2 groups of sows differentiated by their EBV could be distinguished at the genotypic level, an assignment test was performed. An investigation of differences in identity by state (IBS) relationship was performed to assess historical selection for litter size in the 3 regions. Furthermore, allele effects associated with litter size across
marker positions were estimated to evaluate the future potential for marker-assisted selection in the 3 regions.

**MATERIALS AND METHODS**

The animals from which blood samples were collected for this study were kept according to Danish legislation for pig production.

**Animals**

Blood samples were collected from a total of 357 Danish Landrace sows born during the period from November 1998 to July 2007 by venipuncture of a jugular vein in 10-mL vacutainer tubes with 0.108 mL of EDTA. The sows were all past first parity and were selected from the Danish Landrace nucleus breeding herds, which consisted of an average of approximately 3,500 sows distributed across 17 herds in the period considered. The selected sows comprised 154 sows with official high EBV (4.2 < EBV < 5.0) and 203 sows with official low EBV (1.5 < EBV < 1.6) for litter size. The scale of these EBV corresponds to phenotypic observations (i.e., no standardizations).

The EBV were provided by the Danish Meat Association (Danish Pig Production, Copenhagen, Denmark). The EBV were calculated using a repeatability animal model that included the fixed effects of month, parity, breed of sire, age at first farrowing (first-parity litters only), farrowing interval (for later parities in first, second, and third order, and herd-year-type of fertilization). Random effects of permanent environmental, additive genetic values, and residuals were also included in the model. A genetic SD of 0.92 and a heritability of 0.08 were assumed for litter size. Litter data from 1986 and onward were included in predicting the EBV in this study. Litter size has been recorded in the Danish Landrace nucleus breeding herds of multiplier herds since the early 1970s, and from 1998, also in multiplier herds.

**Isolation of DNA**

Deoxyribonucleic acid was isolated from blood samples using the MasterPure DNA Purification kit according to the instructions of the manufacturer (Epicentre Biotechnologies, Madison, WI). The protocol provided by Epicentre Biotechnologies was used for DNA purification. The concentration and purity of the DNA were determined using a spectrophotometer (GE Healthcare, Pittsburgh, PA). Samples of DNA were diluted to a concentration of 25 ng/μL.

**Genotyping**

A total of 17 microsatellite markers were selected from QTL regions on SSC11 (45 to 85 cM), SSC13 (80 to 120 cM), and SSC15 (85 to 110 cM; Cassady et al., 2001; Holl et al., 2004; Rocha et al., 2004) according to the USDA swine linkage map (US Meat Animal Research Center, 2007). Genotyping was performed on DNA collected from a total of 357 animals. The primer sequences were obtained from the USDA database and each of the forward primers was labeled with a fluorescent dye (Table 1). The PCR was performed in a total volume of 5 μL using 1× TEMPase Buffer, 0.125 U of TEMPase Hot Start DNA polymerase (both from Ampliqon, Skovlund, Denmark), 2.5 mM MgCl₂, 0.4 μM each primer, 0.2 mM deoxynucleotide 5'-triphosphate, and 12.5 ng of DNA. The PCR program was a touchdown program with an initial denaturation at 95°C for 15 min, and then 10 cycles with denaturation at 95°C for 20 s, annealing for 30 s, with the temperature decreasing 1°C in each cycle from 60 to 51°C and extension at 72°C for 45 s, followed by 25 cycles with denaturation for 20 s, annealing temperature at 51°C for 30 s, and extension at 72°C for 45 s. The program was concluded by an extension at 72°C for 10 min. Amplification was carried out on an MJ PTC200 thermocycler (Bio-Rad, Hercules, CA). An ABI Prism 3130XL Genetic Analyzer combined with the ABI Genemapper genotyping software (Applied Biosystems, Foster City, CA) was used for genotyping.

**Statistical Methods**

**Assignment of the Genotyped Sows.** An assignment test was performed using the program Doh, designed by Brzustowski (2002) and described by Paetkau et al. (1995). The program calculates genotype frequencies for each marker in all subpopulations. All individuals are then merged together and assigned one by one regardless of their population of origin. An individual is assigned to the population with the greatest probability for the genotype of this individual. The output is a population-level assignment matrix, giving, for each pair of populations, the number of individuals sampled in the first population but assigned to the second population and vice versa.

**Genetic Relationships Between Animals.** Additive genetic relationships (Wright, 1922) were calculated based on the full pedigree (6,014 pigs representing ancestors traced as far back as possible). These relationships represent the expected proportion of genes in common between 2 animals, based on the known pedigree only (0 < relationship < 1) and are therefore equal across all chromosomal regions.

The IBS relationship was determined among the genotyped sows and is based solely on the genotypes. The IBS relationship between 2 animals is therefore specific for a given marker position, unlike the additive genetic relationship. The IBS relationship between 2 animals is equal to the number of alleles shared by the 2 animals at the given marker position (i.e., IBS relationship ∈ {0, 1, 2}).

**Estimation of Allele Effects.** First, a subset of the EBV provided from the Danish Meat Association (i.e., for the 357 sows with known genotypes) was der-
egressed (Sigurdsson and Banos, 1995) using the following statistical model:

\[ \mathbf{d} = \mathbf{Z}_s \mathbf{a}_s + \mathbf{\varepsilon}, \]  

where \( \mathbf{d} \) is a vector of unknown deregressed EBV for the 357 sows, \( \mathbf{a}_s \) is a vector of known EBV for the 357 genotyped sows, \( \mathbf{Z}_s \) is a design matrix that connects EBV with deregressed EBV (simply an incidence matrix here), and \( \mathbf{\varepsilon} \) is a vector with random residual effects. The EBV and residuals were assumed independent, \( \sigma^2_a \) is the additive genetic variance, and \( \mathbf{R} \) is a diagonal matrix with diagonals equal to \( \sigma^2_e / w_i \), where \( \sigma^2_e \) is the residual variance and \( w_i \) is the reliability of \( a_{s(i)} \) attributable to own and progeny information only (here, \( w_i \) was assumed equal to unity for all sows, and it was assumed that \( \mathbf{R} = \mathbf{I}_s \sigma^2_e \), where \( \mathbf{I}_s \) is an identity matrix). Hence, the deregressed EBV can be computed as follows:

\[
\begin{align*}
\mathbf{\hat{d}} &= \left( \mathbf{Z}_s' \mathbf{Z}_s + \mathbf{A}_s^{-1} \sigma^2_e / \sigma^2_a \right)^{-1} \mathbf{Z}_s' \mathbf{a}_s \\
&= \left( \mathbf{Z}_s' \mathbf{Z}_s + \mathbf{A}_s^{-1} \sigma^2_e / \sigma^2_a \right)^{-1} \mathbf{Z}_s' \mathbf{Z}_s \mathbf{d} \\
&= \left( \mathbf{Z}_s' \mathbf{Z}_s + \mathbf{A}_s^{-1} \sigma^2_e / \sigma^2_a \right)^{-1} \mathbf{Z}_s' \mathbf{a}_s.
\end{align*}
\]

Genetic parameters were as assumed in the EBV estimation performed by the Danish Meat Association (i.e., \( \sigma^2_e / \sigma^2_a = 11.5 \), or equivalent \( h^2 = 0.08 \)).

The deregressed EBV (\( \mathbf{d} \)) is independent for all the effects considered in model [2], whereas effects that were considered in the EBV estimation performed by the Danish Meat Association are not considered in model [2] (e.g., relationships among animals not genotyped), but are still regressed. Finally, the effect of genetic markers was estimated using the following statistical model (only one marker was considered per analysis):

\[ \mathbf{d} = \mathbf{M} \mathbf{m} + \mathbf{Z}_s \mathbf{u} + \mathbf{\varepsilon}_z, \]

where \( \mathbf{d} \) is a vector of deregressed EBV from model [1], \( \mathbf{m} \) is a vector of fixed allele effects, \( \mathbf{M} \) and \( \mathbf{Z}_s \) are design
matrices (elements of $M \in \{0, 1, 2\}$ refer to the number of alleles carried by each animal), $u$ is a vector of random polygenetic effects $[u \sim N(0, A_s \sigma_n^2)]$, and $e$ is a vector with random residual effects $[e \sim N(0, 1\sigma_e^2)]$. Hence, additive allele effects ($m$) can be solved from the following mixed model equations:

$$
\begin{bmatrix}
\hat{m} \\
\hat{u}
\end{bmatrix} = 
\begin{bmatrix}
M'M & M'Z_s \\
Z_s'M & Z_s'Z_s + A_s \sigma_n^2
\end{bmatrix}^{-1}
\begin{bmatrix}
M'd' \\
Z'd'
\end{bmatrix}.
$$

For each marker locus, the average ($\mu_m$) and the SD ($\sigma_m$) of the effect of the $2 \times 357$ individual alleles were calculated to summarize the allele effects:

$$
\mu_m = \frac{\sum_{i=1}^{x} (\hat{m}_i n_i)}{\sum_{i=1}^{x} n_i}, \text{ and }
\sigma_m = \sqrt{\frac{\sum_{i=1}^{n_x} (\hat{m}_i - \mu_m)^2}{n_x - 1}},
$$

where $x$ is the number of unique alleles at a given locus, $n_i$ is the number of copies of the $i$th allele observed in the investigated Landrace population, and $m_i$ is the additive genetic effect of the $i$th unique allele on litter size.

Hence, alleles with low frequency and imprecise estimates influence results relatively little compared with frequent alleles. The average $\mu_m$ can also be interpreted as a weighted (by frequency of allele) average of allele effects at a given locus.

**RESULTS AND DISCUSSION**

**Assignment of the Genotyped Sows**

Based on the genotypes generated within the 17 microsatellites analyzed, 140 of the 154 sows belonging to the EBV-high group (91%) were correctly assigned to EBV-high, whereas 183 of the 203 (90%) sows were correctly assigned to EBV-low. Thus, the distribution of variation was sufficiently different between the 2 groups to distinguish between them; the 2 groups were clearly distinguishable ($P < 0.001$) at the genotypic level within the QTL regions on SSC11, SSC13, and SSC15.

**Genetic Relationships Between Animals**

An increased IBS relationship is expected when selection pressure is high for the given marker in a specific locus compared with other loci. However, an increased IBS relationship may also be caused by a reduced mutation rate, assortive mating directed toward traits associated with the given marker, or by chance (Falconer and Mackay, 1996). However, in Danish pig breeding, the main criterion used for deciding how to mate selected animals is avoidance of pedigree-based inbreeding, which is expected to act equally across the genome.

Average IBS relationships differed across marker positions (Figure 1), which may reflect different historical selection pressure for different markers. The range of average IBS was from 0.9 (in SW1983 on SSC15) to 1.6 (in S0289 on SSC13). In addition, the magnitude of the average IBS relationship between sows in the EBV-high and EBV-low groups varied relative to the average IBS relationship for all genotyped sows across marker positions (Figure 1). These variations may be explained by historical selection for litter size because the most prevalent distinction between the groups is EBV for litter size. This is supported by the fact that the average IBS relationships between the 2 groups were comparable with the average IBS relationships among all genotyped sows (results not shown) because the latter was a result of general selection in the population. As opposed to the average additive genetic relationship coefficient (i.e., 0.30, 0.23, and 0.24 in EBV-high, EBV-low, and all genotyped sows, respectively), there was no uniform trend pointing toward generally greater IBS relationships in EBV-high compared with EBV-low.

Greater average IBS relationships within than between the 2 groups were observed for 10 of the 17 investigated markers, which may indicate that historic selection has taken place with respect to litter size in the markers in question. These 10 markers were distributed over the 3 QTL regions, and the greatest differences were observed for SW1056 and SW38 on SSC13 (Figure 1).

Four of the 5 markers on SSC11, 5 of the 7 markers on SSC13, and 1 of the 5 markers on SSC15 had the greatest average IBS relationship in EBV-high, revealing that these markers had more alleles in common in EBV-high. In agreement with the above-mentioned observation, the markers SW1056 and SW38 had the greatest average IBS values among all calculated IBS values in EBV-high, thus providing support for greater historical selection in this region compared with the other investigated regions. However, because these were also the markers for which the average IBS relationships were greatest among all genotyped sows, it could be hypothesized that general selection also has had an impact on this region. The mucin 4 ($MUC4$) polymorphism, known to be associated with resistance or susceptibility to *Escherichia coli* F4 infection (Jørgensen et al., 2004), is located at 13q41. The fact that the $MUC4$ marker has been used for selection during the last 6 yr in Danish pig production could potentially influence IBS on SSC13. However, the SW1056 and SW38 markers are located more than 20 cM (65 Mb) distal to $MUC4$, and we do not see any indication of linkage.
disequilibrium between the 2 regions. Therefore, we do not consider that selection against susceptibility to *E. coli* F4 has any influence on the IBS observed in this study.

The average IBS relationship is comparable among EBV-high, EBV-low, and all genotyped sows. That is, the average IBS relationships across all markers were 1.29, 1.28, and 1.28 for EBV-high, EBV-low, and all genotyped sows, respectively. In contrast, these groups were different with respect to the average additive genetic relationship coefficients, which were 0.30, 0.23, and 0.24 among EBV-high, EBV-low, and all genotyped sows, respectively. Thus, on average, the sows in the EBV-high group were more closely related by descent than sows in the EBV-low group (*P* < 0.0001, calculated using the *F*-test; GLM procedure, SAS Inst. Inc., Cary, NC).

**Estimated Allele Effects**

To identify the marker or region with the greatest effect on litter size, the SD of the allele effect (*σ*ₘ) and average allele effects (*μ*ₘ) were calculated for each marker locus. Consider the results for marker SW2608 as an example. Here, 3 different alleles were identified, that is, alleles 113, 117, and 119, with counts of 464, 195, and 49, respectively. The estimated allele effects were 3.41, 1.22, and 0.91, respectively. This corresponds to *σ*ₘ = 0.63 and *μ*ₘ = 1.17 for SW2608.

A large *σ*ₘ indicates that individual differences between allele effects at a given marker locus are large, but it does not imply anything about *μ*ₘ. Thus, a large *σ*ₘ implies that future selection of favorable alleles in the given locus is expected to increase EBV for litter size. Selection for a given marker is expected to decrease *σ*ₘ for this marker, and a large *σ*ₘ may therefore indicate low historical selection pressure or divergent selection for the given marker. Apparent low historical selection pressure on a given marker, despite general selection for increased litter size in addition to other traits, may be due to either little effect of the given marker on litter size or antagonistic effects on other traits included in the selection index.

A large *μ*ₘ indicates that alleles in a given marker locus have a relatively positive effect on EBV compared with alleles at other loci. Hence, it may, to some extent, reflect a large historical selection pressure with respect to litter size on alleles at the given marker. The relative difference between the greatest and least *μ*ₘ was 10% (results not shown). In comparison, the *σ*ₘ varied more across the investigated markers (Figure 2).

The marker with the greatest *σ*ₘ (SW2440) contained 8 different alleles in the sample population. Four of these alleles were present in only 10 copies or fewer, whereas the most frequent SW2440 alleles (frequencies of 52 and 29%) had intermediate effects on litter size. Estimates of allele effects were 0.73 and 0.79 extra piglets for these alleles. For the infrequent alleles, estimates were more extreme, ranging from −4.6 to 9.4. However, these estimates were based on only 10 or fewer allele copies; hence, the results are considered unreliable.

A similar picture was observed for marker SN287, which was located close to SW2440. This marker contained 5 alleles in the sample population, and the most frequent allele (79%) also had an intermediate estimated effect (i.e., 1.04) on litter size. Marker SW38, also located close to SW2440 and SN287, contained only 3 alleles in the sample population. The predominant allele in SW38 showed an intermediate effect (i.e., 1.51) on litter size.

Other markers with relatively large *σ*ₘ were SW936, SW1510, and KVL4775 on chromosomes 15, 15, and 1607.
The first 2 markers each contained an allele with very low frequency but with an unexpectedly large estimated effect on litter size. Each of these markers contained a frequent allele with a large estimated effect as well. The most frequent alleles in marker SW936 and in marker SW1510 had allele frequencies of 24 and 57% and estimated allele effects of 2.4 and 1.3, respectively. The 2 most frequent alleles in marker KVL4775, with allele frequencies of 43 and 37%, also had the greatest estimated effect on litter size, with 1.3 and 2.0 extra piglets.

Selection for alleles with large estimated effects is expected to lead to increased litter size. Predictions of effects based on alleles with a small number of copies in the sample population should be used with care because they are believed to be unreliable. In addition, even if the effects are real, there is a great risk that the low frequency, despite polygenic selection for litter size, means that the alleles have an antagonistic effect on other traits in the breeding goal. However, it would be feasible, using the information presented above, to select for alleles with intermediate effects to establish a constant litter size. This may be desirable because both overly large litters and overly small litters pose production economic problems.

The results indicate that litter size is controlled by several genes spread throughout the 3 investigated chromosomes, each having a different effect on the trait. Thus, several markers would be needed for efficient marker-assisted selection. Important genes may also be controlling litter size in regions other than those investigated here, although the regions were carefully selected based on previous findings. Genomic selection would ensure that all regions and markers throughout the genome influencing litter size are taken into account. This also provides the prospect of taking other traits into account so that balanced selection can be performed. However, most other traits in the breeding goal are sufficiently covered by traditional BLUP EBV.

Discussion of the Method Used to Estimate Allele Effects

The estimation of allele effects is likely biased. One reason for this is that official EBV provided by Danish Pig Production may not capture the entire effect of QTL. In addition, the selective genotyping of animals with extreme EBV made unbiased estimation of allele effects difficult (e.g., Lander and Botstein, 1989; Darvasi and Soller, 1992). However, this selection bias has been reduced because phenotypes from the whole population were used to predict official EBV, and therefore were also accounted for in the deregressed EBV. In the deregression step, it was assumed that the accuracy of EBV attributable to own and offspring performance was equal for all genotyped pigs because accuracies were not available. Here, all animals were sows with at least 1 litter (i.e., 1 own observation). Although a few sows had 2 litters, the assumption of equal accuracy is not expected to cause much bias in estimated genetic effects because the use of different weighting factors caused little bias in a more unbalanced case (Fikse and Banos, 2001). Although the estimation of allele effects
is likely biased, we expect that the relative bias is similar for all estimated allele effects. Hence, the comparison of scaled effects is appropriate for the purpose of this study.

Association Between IBS and Allele Effects

Because high historical selection pressure is expected to increase both IBS and $\mu_m$, it was hypothesized that a positive association existed between the 2 measures. The correlation between the 2 measures across all investigated markers was 0.25, which strengthened our hypothesis, but also illustrated that these measures were influenced by factors other than selection pressure, as discussed above. Likewise, IBS were hypothesized to be negatively associated with $\sigma_m$ because selection and mutations with extreme effects are expected to have opposite effects on the 2 measures. However, the correlation between the 2 measures was 0.025 across all markers, indicating that they were not associated or that other factors, such as selection for other traits or random chance, may have disrupted the results. Further research involving more markers would be needed to confirm our hypotheses regarding associations between the measures used in this study.

Conclusions

Marker-assisted selection, either for single QTL or genomic breeding values, is of great interest for litter size because records are available only from maternal ancestors at the time of primary selection for both sexes. This means that the accuracy of breeding values for litter size is low at the time of primary selection and that breeding values contain no information about the Mendelian sampling component for this important trait.

The results obtained in this study indicate that litter size is controlled by several genes spread throughout the 3 investigated chromosomal regions, each having a different effect on the trait. Thus, several markers would be needed for efficient marker-assisted selection.

As found in the IBS results, the greatest historical selection, according to litter size, was on SSC13 near the markers SW1056 and SW38. Alleles with effects on litter size were found for all markers, but with the greatest effect of 0.79 extra piglets for SW2440 on SSC13.

LITERATURE CITED