Detection of quantitative trait loci for skatole and indole levels in Meishan x Large White F2 pigs

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EPISTASIS IS A FUNDAMENTAL COMPONENT OF THE GENETIC ARCHITECTURE OF PROLIFICACY IN PIGS

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INTRODUCTION

Prolificacy is the most important trait in the genetic improvement of the reproductive efficiency in pig production systems. Prolificacy is a complex trait, which genetic improvement by selective breeding has proved to be difficult because it displays a low heritability and it is expressed only in females. In this type of traits, the identification of genes influencing the trait, and the characterisation of the nature of their interactions, is of particular interest in order to use molecular information in selection schemes. In the last years, much effort has been invested in the mapping and identification of both QTL and causative genes influencing prolificacy in pigs (Bidanel and Rothschild, 2002). Most of these studies have just dissected the additive and dominance components of number of piglets born, thus neglecting the analysis of epistatic interactions that, paradoxically, are expected to explain a substantial portion of genetic variation of reproductive traits (Carlborg and Haley, 2004). In the current work, we have performed a one-dimensional QTL mapping and a bi-dimensional genome-wide epistasis scan, in a F_2 intercross between the *Iberian* and *Meishan* breeds, with the aim of elucidating if epistasis makes a major contribution in shaping the phenotypic variability of prolificacy in pigs.

MATERIAL AND METHODS

Experimental design and traits analyzed. For QTL analyses of reproductive traits a three-generation F_2 intercross was generated between *Iberian* and *Meishan* pigs breeds that differ substantially for prolificacy (over seven piglets born alive per parity, in average). Three *Iberian* boars (*Guadyerbas* line) were mated with 18 *Meishan* sows. Eight F_1 boars and 97 F_1 sows were parents of F_2 progeny born in the Nova Genètica S. A. experimental farm (Lleida, Spain). A total of 881 parities from 255 F_2 sows were recorded for number of piglets born alive (NBA).

Markers. DNA was extracted from frozen blood or tail tissue using commercial protocols (Gentra Systems, Minneapolis). Purebred grandparents, F_1 reproducers and the 255 F_2 sows were genotyped for 117 markers: 109 microsatellites and eight single nucleotide polymorphisms (SNP). Microsatellite loci were chosen based on their ease of scoring, genomic location and informativeness. PCRs were carried out in a MJ Research Thermal Cycler. The microsatellite PCR products were analyzed with the Genescan 3.7 software (Applied Biosystem, Warrington, UK) on capillary electrophoresis equipment with fluorescent detection (ABI PRISM 310 Genetic Analyzer). We used the linkage map reported in Rodriguez *et al*. (2005) for the same *Iberian* x *Meishan* population.

Statistical analyses. Two statistical models were used to analyze the experimental data. The trait NBA was considered to be same for all parities. The first model was a one-dimensional QTL mapping performed using a regression model (Haley *et al*., 1994), through the following animal model with repeated measures:

$$y_{ijk} = H_i + u_j + p_j + c_a a + c_d d + e_{ijk} \quad (1)$$

where y_{ijk} was the *ijk* observation for NBA, H_i was the i^{th} year-season effect, u_j was the polygenic effect of the j^{th} individual, p_j was the permanent environmental effects for the j^{th} individual, *a* was the additive effect, *d* was the dominant effect and e_{ijk} was the random residual term; $c_a = pr(QQ) - pr(qq)$ and $c_d = pr(Qq)$ where *pr(QQ)* was the probability of being homozygous of *Iberian* origin, *pr(qq)* was the probability of being homozygous of *Meishan* origin and *pr(Qq)* was the probability of being heterozygous.

The analysis was performed at every centimorgan for each of the 18 autosomes, by means of an F-test comparing the models with and without the QTL coefficients. For computational reasons, heritability and percentage of permanent environmental effects were assumed to be known. They were obtained from the posterior mode of a previous analysis. Genome-wide levels of significance were calculated using a Bonferoni correction and assuming independence between statistical tests every 30 cM.

Furthermore, a two-QTL analysis was performed with two different models. The first model included the effects of both QTL from two different locations but it did not allow for interaction between them. The statistical model was:

$$y_{ijk} = H_i + u_j + p_j + c_{a1}a_1 + c_{d1}d_1 + c_{a2}a_2 + c_{d2}d_2 + e_{ijk} \quad (2)$$

Where a_1 and a_2 were the additive effects and d_1 and d_2 were the dominance effects for QTL 1 and 2, respectively. The coefficients c_{a1}, c_{d1}, c_{a2} and c_{d2} were calculated as before for locations 1 and 2.

The second model allowed for epistasis:

$$\begin{aligned} y_{ijk} &= H_i + u_j + p_j + c_{a1}a_1 + c_{d1}d_1 + c_{a2}a_2 + c_{d2}d_2 + \\ &+ c_{axa}I_{axa} + c_{axd}I_{axd} + c_{dxa}I_{dxa} + c_{dxd}I_{dxd} + e_{ijk} \end{aligned} \quad (3)$$

where I_{axa}, I_{axd}, I_{dxa} and I_{dxd} were the additive x additive, additive x dominance, dominance x additive and dominance x dominance epistatic interaction effects, respectively. Moreover, c_{axa}, c_{axd}, c_{dxa} and c_{dxd} were the regression coefficients calculated following Cockerham (1954) and Varona *et al*. (2002). As before, the heritability and percentage of permanent environmental effects were assumed to be known. The statistical contrast for evidence of epistasis was carried out using an F test with 4 degrees of freedom in the numerator. Bi-dimensional genome-wide levels of significance were calculated using a Bonferoni correction assuming statistical independence every 30 cM.

RESULTS AND DISCUSSION

Results of single-QTL analyses for number of piglets born alive are summarized in Table 1. Two genome-wide highly significant QTL (P<0.01) were located on *SSC13* and *SSC17*. In *SSC13*, the QTL mapped at position 50cM (between markers *SW398* and *SW2440*). The most

probable position for the QTL on *SSC17* was at 22cM (between markers *SW2142* and *SW1920*). For both QTL highly significant additive and dominance effects were detected acting in opposite directions for both additive and dominance effects: the QTL on *SSC13* increased additively by 0.71 (± 0.18) piglets per copy of the *Meishan* allele, while the QTL on *SSC17* increased by 0.73 (± 0.19) piglets with each copy of the allele associated with the *Iberian* breed. We found also evidence for a genome-wide significant (P<0.05) QTL on *SSC12* (between markers *SW1956* and *SO106*). The additive effects was estimated as a highly significant increase per allele of the *Iberian* breed (0.82 piglets). A significant dominant effect was also detected (0.73 piglets). These three QTL for number of piglets born alive had not been previously reported in other pig populations.

Table 1. Significant single quantitative trait loci (QTL) for NBA

Chromosome	Position cM	F	Genome-wide significant level (P-value)	a (se)	d (se)
13	50	11.94	<0.01	-0.71 (0.18)	0.69 (0.25)
17	22	10.92	<0.01	0.73 (0.19)	-0.82 (0.29)
12	78	7.71	<0.05	0.82 (0.22)	0.73 (0.39)

F, F value; a, additive effect; d, dominance effect

Results from the bidimensional genome-wide scan are shown in Table 2. Six highly significant (P<0.01) and three significant (P<0.05) epistatic interactions were found, involving 13 of the 18 pig autosomes (1, 2, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15 and 18). To the best of our knowledge, these highly significant epistatic interactions for litter size are the first ones reported in pigs. It has to be noticed that the single QTL found on SSC12 and SSC13 have highly significant epistatic interactions with others regions across the genome, but not in the case of the single QTL on chromosome 17. Several chromosomes exhibited more than one interacting region. This is the case of chromosome 12, which had two non-overlapping regions with epistatic interactions effects between them. It is interesting to mention that three regions of *SSC10* had epistatic interactions with regions of *SSC2*, *SSC8*, *SSC12* and *SSC15*. The four types of epistasis considered (a x a, a x d, d x a and d x d) were found among the significant epistatic pairs.

CONCLUSION

In this paper, we demonstrate that the genetic architecture of prolificacy in pigs is mostly built as a complex network of interacting genes rather than by the sum of the additive effects of a yet to be defined number of loci. This complexity will make more difficult both the identification of causative genes from the detected chromosomal regions and their practical application in pig selection. These results give a new insight about the role of epistasis in modulating the phenotypic variation of complex traits of economic interest in farm species.

Table 2. Results of bi-dimensional genome-wide significant epistatic effects for NBA

Epistasis				F	Bi-Genomewide Significant level (P-value)	Epistasis Type	Genotypic value
SSC_1	$Position_1$ (cM)	SSC_2	$Position_2$ (cM)				
6	20	14	29	7.87	< 0.01	a x d d x d	1.43(0.4) 2.67(0.7)
1	76	7	107	7.84	< 0.01	a x d d x d	2.59(0.6) 3.34(0.9)
2	95	10	101	7.50	< 0.01	a x a d x a d x d	-1.22(0.3) -1.68(0.5) -1.96(0.7)
10	34	12	11	7.77	< 0.01	d x a d x d	-1.27(0.4) -2.43(0.6)
9	3	13	72	7.63	< 0.01	a x a d x d	0.66(0.3) -2.66(0.6)
5	113	18	11	7.31	< 0.01	a x a a x d	-0.96(0.3) 1.85(0.5)
10	65	15	8	7.08	< 0.05	a x a a x d d x a	0.85(0.3) 1.14(0.4) -1.05(0.4)
8	91	10	73	6.94	< 0.05	a x d d x d	-1.45(0.4) -2.57(0.7)
12	15	12	71	6.86	< 0.05	a x a a x d	-1.08(0.2) 0.74(0.4)

SSC_1 and $Position_1$: Chromosome and position, in centimorgans, of the first location. SSC_2 and $Position_2$: Chromosome and position, in centimorgans, of the second location. F, value of the epistasis model *versus* the no epistasis model. Epistasis type: a x a: additive by additive effect; a x d: additive by dominance effect; d x a: dominance by additive effect: d x d, dominance by dominance effect.

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The objective of this study is to investigate the genetic basis of prolificacy in pigs. For QTL analyses an F_2 intercross was generated between *Iberian* and *Meishan* breeds. A total of 881 parities from 255 F_2 sows were recorded for number of piglets born alive. A one-dimensional QTL mapping and a bi-dimensional genome-wide epistasis scan were performed with 117 markers. Genome-wide highly significant QTL ($P<0.01$) were located on *SSC13* and *SSC17*, and a QTL on *SSC12* ($P<0.05$). Six highly significant ($P<0.01$) and three significant ($P<0.05$) epistatic interactions were found, involving 13 of the 18 pig autosomes. These results give a new insight about the role of epistasis in modulating the phenotypic variation of prolificacy.