

MAPPING QUANTITATIVE TRAIT LOCI FOR EAR TRAITS IN PIGS

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INTRODUCTION

Line crosses utilising the Chinese Meishan (MS) have been used extensively in pigs to map quantitative trait loci (QTLs) for traits of economic and biological interest, including growth, meat quality, fat and prolificacy (Bidanel and Rothschild 2002). Limited attention has been given so far to appearance and morphology, despite striking differences between the MS (e.g. wrinkled skin, and very large flop ears) and European breeds such as the Large White (LW). The large, flop ears are one of the breed characteristics of the MS and have been selected together with other conformation traits (e.g. coat color) over many years. It is therefore interesting to understand the genetic architecture of these traits that represent a response to the pressures of domestication imposed by humans and to determine the nature of the genes and their interactions that control these extreme breed differences. Consequently, an F₂ line cross between the LW, with small, upright ears, and the MS was chosen to characterize QTLs affecting the ear size and ear appearance or aspect traits in this study.

MATERIAL AND METHODS

Animals, genotyping, and map construction. Eight unrelated founder animals (2 boars and 2 sows from the LW and MS breed respectively) were reciprocally crossed. From the F₁ offspring 7 boars were mated to 25 sows from a different grandparental pairing producing in total 34 full-sib F₂ families. A total of 461 F₂ individuals across 29 full-sib F₂ families, and the F₁ parents and F₀ grandparents were genotyped with microsatellite and SNP markers spanning the genome. The genotype data were analysed using CriMap (Green *et al.* 1990) to construct linkage maps with reference to the published consensus maps. Distances were estimated using the Kosambi mapping function within CriMap.

Ear trait measurement. Animals were evaluated subjectively at approximately 6 months of age based on recorded video images. Based on the evaluation, their ear sizes were recorded as either Small (LW like), Large (MS like) or Medium (intermediate in size), which were recoded as 1, 3 and 2, respectively. Similarly, the aspect of their ears were recorded as Down (flop eared), Up (prick eared) or Intermediate (part of the ear being flopped down) and recoded as 1, 3 and 2, respectively. All individuals with a Large ear size and three quarters of those with a Medium ear size had a down ear aspect indicating a strong correlation between the two traits.

QTL analyses. QTL analyses were performed using the Haley-Knott least squares methods (Haley *et al.* 1994) implemented in a web-based software QTLEXPRESS (Seaton *et al.* 2002). These analyses assume that any QTLs were fixed for alternative alleles in the founder lines. Two alternative genetic models were explored per trait: the Mendelian model and the parent-of-origin (potentially imprinting) model. The Mendelian model has a form $y_i = \mu + c_{ai}a + c_{di}d + X\beta + e_i$ where μ is the population mean; a and d are the estimated additive and dominance effects respectively; for the i^{th} F₂ individual and a putative QTL at a given location, y_i is the trait value; c_{ai} , and c_{di} are the coefficients for the additive and dominance components respectively; X is an incidence matrix for the fixed factors of family, litter and sex; β is a vector of estimators of the fixed factors; e_i is the residual error. The parent-of-origin model adds an extra c_{ii} term into the Mendelian model, where c_{ii} and i stand for the coefficient for the parent-of-origin component and parent-of-origin effect respectively.

Genome scans were performed using the forward selection approach (Carlborg *et al.* 2005) at one centiMorgan (cM) intervals iteratively for each trait to detect multiple QTLs. When no new QTLs could be found, the positions of each QTL identified were re-estimated by scanning only the chromosome where the concurrent QTL was located while fitting the remaining identified QTLs as cofactors until the QTL positions became stable. The significance thresholds and the 95% confidence interval (CI) for QTLs detected were then determined empirically by permutations ($n = 10000$) and bootstrapping ($n = 1000$), respectively (de Koning *et al.* 2001).

RESULTS AND DISCUSSION

Linkage maps, information content and segregation distortion. The sex-averaged linkage maps for the 18 pig autosomal chromosomes (SSC) were constructed and can be made available on request. In total, 152 markers span 2109 cM of the whole autosomal genome giving an average distance between markers of 13.9 cM. Before QTL analyses, marker information content and segregation distortion were checked using QTLEXPRESS. There was no evidence of segregation distortion in the population and the information content for markers used was generally satisfactory.

Ear size. Six single QTLs were detected using the Mendelian model (Table 1). The QTLs on SSC5 and SSC7 had high F ratios as well as large additive effects, and were genome-wide significant ($P < 0.01$). The 95% CI for the QTL on SSC5 was only 4 cM whereas the CI for SSC7 was 19 cM (Table 1). The remaining QTLs were genome-wide suggestive. Most QTLs detected in the Mendelian model had negative additive effects, with no significant dominance, indicating that LW allelic effects reduced ear sizes. The sum of the additive effects under the Mendelian model when jointly estimated in the full model was 1.19. Thus the difference expected between individuals homozygous for all MS alleles and those homozygous for all LW alleles is 2.39, i.e. slightly greater than the full range on the scale from large to small ears. When using the parent-of-origin model, in addition to four QTLs that had only significant additive effects found on SSC5, SSC7, SSC9 and SSC12, two suggestive QTLs with significant parent of origin effects ($P < 0.01$) but non-significant additive and dominance effects were found on SSC8 (0.136 ± 0.042) and SSC15 (0.179 ± 0.050) (Table 1).

Table 1. QTL mapping results for ear size

Model	SSC	Flanking markers (position, cM)	F ratio	CI (cM) (start – end)	Additive ^A	Dominance ^A
Mendelian	1	S0122 – S0082 (67)	6.64	32.0 – 107.0	-0.146 (0.040)	-0.005 (0.061)
	5	SWR453 – GDF11 (51)	62.3**	49.0 - 53.0	-0.427 (0.038)	-0.003 (0.055)
	6	SW122 – SW316 (99)	6.61	38.0 – 170.0	0.007 (0.039)	-0.219 (0.060)
	7	SN5430 - SN5718 (70)	41.3**	59.0 - 78.0	-0.352 (0.039)	0.014 (0.054)
	9	APOA1 - SW1677 (60)	6.69	50.0 – 124.0	-0.140 (0.038)	-0.028 (0.055)
	12	SW2490 – S0143 (0)	6.41	0.0 - 50.0	-0.135 (0.039)	0.046 (0.054)
Parent of Origin ^B	8	S0225-2 - SW61 (101)	4.37	0.0 - 126.0	-0.008 (0.037)	-0.086 (0.054)
	15	SW936 – SW1119 (86)	5.37	18.5 – 101.0	0.055 (0.048)	-0.105 (0.090)

** significant at the 1% genome-wide level; ^A standard error values in brackets; ^B SSC5, 7, 9 and 12 were fitted as Mendelian cofactors; either SSC8 or SSC15 was fitted as a parent-of-origin cofactor in the analyses as appropriate

Ear aspect. Using the Mendelian model, five QTLs were found affecting ear aspect (Table 2). The QTLs on SSC1, SSC7 and SSC5 were genome-wide significant, each of which had a positive additive effect and a negative dominance effect, indicating that the LW allele

increased erectness of ears, with the MS allele for flop ears being dominant. This is in line with the observation that F₁ animals tend to have flop like ears. The sum of the additive effects when jointly estimated in the full model was 0.85. Thus the difference expected between individuals homozygous for all MS alleles and those homozygous for all LW alleles is 1.71, slightly less than the full scale range from flop to fully erect ears. Two suggestive QTLs on SSC9 and SSC12 had smaller effects. No parent of origin effects were found in the QTLs for ear aspect.

Table 2. QTL mapping results for ear aspect

SSC	Flanking Markers (position cM)	F ratio	95% CI (cM) (start – end)	Additive ^A	Dominance ^A
1	S0122 - S0082 (53)	17.33**	49.5 – 110.0	0.180 (0.035)	-0.150 (0.049)
5	SWR453 - GDF11 (43)	15.01**	23.0 - 77.0	0.203 (0.038)	-0.060 (0.059)
7	SN5430 – SN5718 (70)	30.61**	64.0 - 80.0	0.264 (0.036)	-0.139 (0.050)
9	SW1677 - S0295 (83)	5.48	0.0 - 129.0	0.137 (0.043)	-0.030 (0.068)
12	SW874 - S0090 (59)	7.69	0.0 - 63.0	0.070 (0.040)	-0.217 (0.061)

** significant at the 1% genome-wide level; ^A standard error values in brackets;

The F ratio profiles for the genome-wide significant QTLs detected for both traits are displayed in Figure 1. Compared to the counterpart QTLs found on ear size, on ear aspect the QTL effect on SSC5 was less significant while the QTL effect on SSC1 was more significant. Only the relative position of QTL on SSC7 remained unchanged in both traits. Positional candidate genes are currently being identified for the QTLs with major effects via comparative mapping and will be further studied for sequence variation.

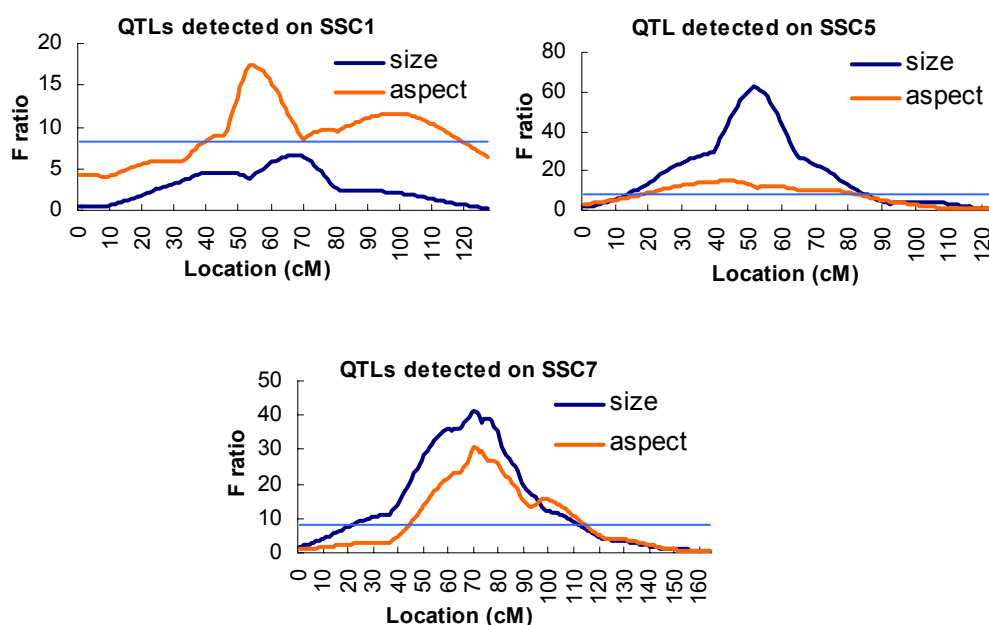


Figure 1. The F ratio profiles for genome-wide significant QTLs detected for ear size and ear aspect (the horizontal lines showing the 5% genome-wide thresholds)

CONCLUSION

The two traits shared a similar genetic architecture – phenotypic variation was controlled by two or three QTLs with major effects and several QTLs with small effects. The QTLs identified covered SSC1, 5, 7, 9 and 12 for both traits indicating pleiotropic effects in these QTLs. Three more suggestive QTLs were found responsible for ear size than ear aspect implying a more complex genetic architecture in ear size than ear aspect. Nonetheless, for both traits, the sum of the additive effects of the QTLs under the Mendelian model suggests that these analyses may have detected the majority of the between breed variation. However, we need to be cautious about this conclusion in the light of the simple three-class trait indices used and also the potential overestimation often associated with QTL effect estimates.

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