DISCRIMINATION BETWEEN LINKED AND PLEIOTROPIC QTL ON PIG CHROMOSOME 6 BY MULTITRAIT LEAST SQUARES ANALYSIS

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INTRODUCTION

Diverse quantitative trait loci (QTL) affecting carcass and meat quality traits had been detected on chromosome 6 (SSC6) in an F_2 Iberian x Landrace intercross (Ovilo *et al*., 2000; Clop, 2001). The associations QTL/trait observed in these previous works affect different traits and are located close together in two separate regions of the chromosome. Owing to the low precision of the mapping it is dificult to decide for each region whether there is only one QTL with pleiotropic effects or several linked QTL.

Diverse authors tried to solve the problem of discriminating between linked and pleiotropic QTL with approaches based on single trait analyses and maximum-likelihood methods (Cheverud *et al*., 1997) or bootstrap techniques (Lebreton *et al*., 1998). Recently, Knott and Haley (2000) have described the implementation of a straighforward multitrait least-squares analysis for QTL detection and location, including models for pleiotropic and linked QTL and alternative testing procedures. Here we present an application of this method to discriminate linkage and pleiotropy of these QTL, taking advantage of a finer mapping of SSC6 based on more genotyped markers and F_2 individuals of the experiment.

MATERIAL AND METHODS

Data. A comprehensive report of the experimental design and results of the quoted F_2 Iberian x Landrace intercross can be found in Ovilo *et al*. (2000). The pedigree was composed by 3 Iberian boars, 30 Landrace sows, 6 F_1 boars, 63 F_1 sows and 369 F_2 individuals, from 63 full sib families. Among all the traits recorded, the following traits were analyzed in the present paper: intramuscular, fat content in muscle *longissimus lumborum* (IMF), backfat thickness between 3rd and 4th last ribs (BF), eye muscle area (MA) measured on *longissimus thoracis* at the same level as BF, and percentages of palmitic (C16:0) and palmitoleic (C16:1) acids measured in backfat samples. Significant QTL in the SSC6 were detected for the selected traits in the quoted preliminary works using single trait regression procedures (Haley *et al*., 1994). IMF and BF presented maximum F values at locations 97 and 98 cM and MA at location 113 cM (Ovilo *et al*., 2000). Percentages of C16:1 and C16:0 presented maximum F values at locations 0 and 44 cM respectively (Clop, 2001).

Markers. For these new analysis, all individuals were genotyped for six additional microsatellites mapped on SSC6 chosen according to their position and informativity: *Sw1329*, *Sw1376*, *Sw71*, *DG32*, *Sw1328* and *Sw607*. PCR-amplified microsatellites were analyzed on a

capillar electrophoresis equipment with fluorescent detection (ABI PRISM 310 Genetic Analyzer). Linkage analysis of a total number of 13 microsatellites was performed using CRIMAP (Green et al., 1990). The correspondent sex-averaged map of SSC6 in cM was built as follows: *S0035* (0.0) – *Sw1329* (14.9) – *Sw1057* (45.7) – *S0087* (59.0) – *Sw1376* (75.8) – *Sw316* (85.7) – *Sw71* (93.8) – *S0228* (101.1) – *DG32* (104.0) – *Sw1881* (114.3) – *Sw1328* (146.4) – *Sw2419* (153.6) – *Sw607* (157.9). The informative content of these genetic markers in F_2 animals was, respectively: 0.654, 0.654, 0.963, 1.000, 0.994, 0.896, 0.862, 0.477, 1.000, 0.821, 0.933, 0.960 and 0.798.

Statistical methods. Separate bivariate least-squares analyses were performed for the different pairs of traits, following the method proposed by Knott and Haley (2000), that is an extension of the single trait regression analysis for detection of QTL (Haley *et al*, 1994). The basic model is $\mathbf{Y} = \mathbf{X}\beta + \mathbf{E}$, where: **Y** is a matrix relating the *n* F_2 individuals with records and the 2 analyzed traits; **X** is a design matrix relating *n* individuals to 68 explanatory variables (2 levels of sex, 63 families and the covariates: carcass weight and 2 functions of the genotype probabilities to estimate additive and dominance effects for the location being considered); β is a 68 *x* 2 matrix containing the estimates for each trait of the quoted effects and the matrix **E** contains the error values.

Diverse statistics (approximate likelihood-ratio tests) based on the determinants of the residual SS matrices, obtained fitting QTL affecting one trait, both traits or no QTL, were calculated to test for the presence of QTL or to discriminate two linked QTL *vs.* one pleiotropic QTL. For each location, these statistics are approximately distributed as chi-square values with four degrees of freedom, under the null hypothesis of no QTL, or with one degree of freedom, under the hypothesis of one pleiotropic QTL. In the last case, nonparametric bootstrap was used to distinguish between the models of linkage and pleiotropy (Lebreton *et al.,* 1998).

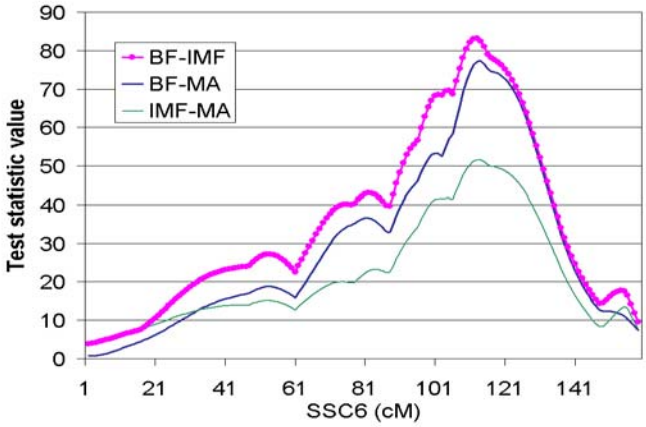

Figure 1. Profile of statistic values of the QTL scan for pig chromosme 6 to test the pleiotropic model *vs* the no QTL hypothesis from bivariate analysis for backfat (BF), intramuscular fat (IMF) and muscle area (MA)

RESULTS AND DISCUSSION

Evidence for a pleiotropic QTL affecting BF, IMF and MA. A chromosomal region flanked by the microsatellites *DG32* (104.0 cM) and *Sw1881* (114.3 cM) contains a QTL with effects on backfat thickness, intramuscular fat content and eye muscle area (Figure 1).
For each pair of traits, the values of test statistics at the position explaining the most variance in the two analyzed traits jointly (Model P) and at the best positions for each trait (Model L) are presented in Table 1.
Although specific permutation tests were not performed, all these values largely exceed the usual chromosomal significance thresholds.

According to the known differences between the parental breeds (Serra *et al*., 1998), the Iberian allele increased the backfat depth and the loin intramuscular fat content and decreased the eye muscle area (Table 1). All the analyses shown significant additive and dominant effects on the three analyzed traits. The percentage of phenotypic variance of the different traits explained by the QTL was 15% (BF), 14% (IMF) and 6% (MA). The best positions for the two QTL mapped using the linkage model are very close, and the corresponding values of the test statistics for comparison between L and P models are lower than the nominal values of significance thresholds ($\chi^2_{.05[1]}$ = 3.84). As a consequence, the null hypothesis of one pleiotropic QTL cannot be rejected.

Table 1. Value of statistic tests, position on SSC6 (cM), additive (*a*) and dominant (*d*) effects estimated for the traits BF, IMF and MA analyzed fitting pleiotropic (P) and linkage (L) QTL models

Traits			Test	QTL Trait 1			QTL Trait 2		
1	2	Model	Value	Position	*a* (s.e.)	*d* (s.e.)	Position	*a* (s.e.)	*d* (s.e.)
BF	IMF	P	83.1	112	4.5 (0.5)	-2.3 (0.7)		0.3 (0.1)	-0.2 (0.1)
		L	83.2	111	4.6 (0.5)	-2.3 (0.7)	112	0.3 (0.1)	-0.2 (0.1)
		L *vs* P	0.04						
BF	MA	P	77.3	113	4.6 (0.6)	-2.6 (0.8)		-1.8 (0.4)	1.7 (0.5)
		L	78.6	113	4.5 (0.6)	-2.6 (0.8)	119	-1.8 (0.4)	1.7 (0.5)
		L *vs* P	1.35						
IMF	MA	P	51.6	112	0.3 (0.1)	-0.2 (0.1)		-1.7 (0.4)	1.6 (0.5)
		L	51.84	113	0.3 (0.1)	-0.2 (0.1)	119	-1.8 (0.4)	1.6 (0.5)
		L *vs* P	0.25						

Evidence for two linked QTL affecting content of palmitic (C16:0) and palmitoleic (C16:1) acids. A chromosomal region flanked by the microsatellites *Sw1329* (14.9 cM) – *Sw1057* (45.7 cM) contains a QTL with effect on the percentage of palmitic acid determined on backfat samples. The chromosomal region around the location of microsatellite *S0035* (0.0 cM) contains a QTL with effect on the percentage of palmitoleic acid.

The values of the statistic tests indicate that the linkage model performs better in this case (Table 2). The following significance thresholds were established by nonparametric bootstrap with a total of 1000 replicate simulations and analyses of data: 5.36 (90%), 6.82 (95%) and

10.19 (99%). The value of the statistic test for linkage vs. pleiotropy (13.55) clearly exceeds these thresholds and the pleiotropic model can be rejected.

The Iberian alleles increased the percentage of the saturated C16:0 and decreased the percentage of the unsaturated C16:1 (Table 2). It agree with the differences for these fatty acids between the parental breeds found by Serra *et al*. (1998). The percentage of phenotypic variance explained by these QTL was 2.4% (C16:0), and 2.9% (C16:1). The results also shown both significant additive and dominant effects of the two QTL on the respective traits.

Table 2. Value of statistic tests, position on SSC6 (cM), additive (*a*) and dominant (*d*) effects estimated for the traits C16:0 and C16:1 analyzed fitting pleiotropic (P) and linkage (L) QTL models

	Test	QTL C16:0			QTL C16:1		
Model	Value	Position	*a* (s.e.)	*d* (s.e.)	Position	*a* (s.e.)	*d* (s.e.)
P	17.17	36	0.32 (0.11)	0.57 (0.19)		-0.02 (0.03)	0.02 (0.06)
L	30.72	39	0.31 (0.11)	0.52 (0.17)	1	-0.09 (0.03)	-0.15 (0.05)
L *vs* P	13.55						

CONCLUSION

The above results refine the map position of some QTL previously identified on SSC6, improves the estimation of their additive and dominance effects on some meat and fat quality traits of practical interest for pig breeders and particularly clarify their relation of linkage or pleiotropy. It can facilitate the choice and investigation of positional candidate genes, although the identification of the corresponding genes and causal mutations remains a difficult task.

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