Multiple Marker Mapping of Quantitative Trait Loci in a Cross Between Outbred Wild Boar and Large White Pigs

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ABSTRACT

A quantitative trait locus (QTL) analysis of growth and fatness data from a three generation pig experiment is presented. The population of 199 F_2 animals was derived from a cross between wild boar and Large White pigs. Animals were typed for 240 markers spanning 23 Morgans of 18 autosomes and the *X* chromosome. A series of analyses are presented within a least squares framework. First, these identify chromosomes containing loci controlling trait variation and subsequently attempt to map QTLs to locations within chromosomes. This population gives evidence for a large QTL affecting back fat and another for abdominal fat segregating on chromosome *4*. The best locations for these QTLs are within 4 cM of each other and, hence, this is likely to be a single QTL affecting both traits. The allele inherited from the wild boar causes an increase in fat deposition. A QTL for intestinal length was also located in the same region on chromosome *4* and could be the same QTL with pleiotropic effects. Significant effects, owing to multiple QTLs, for intestinal length were identified on chromosomes *3* and *5*. A single QTL affecting growth rate to 30 kg was located on chromosome *13* such that the Large White allele increased early growth rate, another QTL on chromosome *10* affected growth rate from 30 to 70 kg and another on chromosome *4* affected growth rate to 70 kg.

THE use of molecular genetic markers to dissect quantitative trait variation is well known. Initially, most of the analytical methods were developed for use with populations derived from inbred lines. Outbred populations, such as those found in livestock, pose additional problems, which, although theoretically possible to overcome, mean that large numbers of animals are required to characterize quantitative trait loci (QTLs). To make such analyses tractable, structured populations have been used, such as the large half-sub families found in dairy cattle (Georges *et al.* 1995). Alternatively, experimental populations can be created for some species, and a common example is the three generation pedigree, derived from a cross between breeds or lines that differ for one or more traits of interest.

An experiment was initiated in order to determine genes differing between the wild boar and the domesticated Large White pig. A preliminary analysis of these data has been reported (Andersson *et al.* 1994). Subsequently, the marker map has been extended (Marklund *et al.* 1996), and a more complete analysis will be presented here.

In the first stage of the analysis, the approach described by Haley *et al.* (1994) using multiple markers is used to obtain the probabilities of the alternative genotypes for each offspring at fixed locations through the genome. These probabilities are used to investigate the information content of the markers when used together and to check for regions where there is evidence of segregation distortion. Subsequently, the probabilities are used in a least squares framework to investigate the genetic model underlying the trait of interest.

Visscher and Haley (1996) propose a hierarchy of tests that test for the presence of genetic variation associated with a chromosome. If present, they test whether this variation can be explained by a small region of the chromosome (possibly one or two QTLs) or whether it is compatible with a polygenic model, where the QTLs are dispersed along the chromosome, so that all regions contribute the same genetic variance. Analyses to determine the contributions from different chromosomes, whether polygenic, oligogenic or monogenic, are presented.

Subsequently, more traditional genome scans looking for single or more QTLs are carried out. If two QTLs

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are located on the same chromosome, then fitting one at the best location while searching for a second may give biased results (Haley and Knott 1992); hence, two QTL models are fitted. For single QTL detected, confidence intervals are obtained by bootstrapping (Visscher *et al.* 1996).

The inclusion of cofactors has been suggested for the analysis of crosses between inbred lines (Jansen 1993; Zeng 1993). Both propose to fit markers in addition to those flanking the region of interest. This can remove biases due to QTLs linked to the region and take account of unlinked genetic variation, and hence potentially reduce the residual variance and increase power. Following the same principles, cofactors are included in all analyses in order to account for QTLs on other chromosomes. Multiple QTL models are fitted for QTLs on the same chromosome.

MATERIALS

Mapping population: Two European wild boars were crossed with eight Large White sows. From their offspring four F_1 boars were mated to 22 F_1 sows, producing 199 recorded F_2 offspring in 26 families. There were up to two parities per family. Within each parity were two feeding treatments.

Map construction: Linkage maps were constructed using Cri-map (Green *et al.* 1990). Full maps are reported in Marklund *et al.* (1996), including a sex-averaged map and sex-specific maps. The final map comprised 18 autosomes and the sex chromosome. The sex-averaged map spanned 2300 cM and contained 240 markers.

Traits: Pigs were weighed at birth, at two weekly intervals during the growth period and at slaughter. Growth rates were calculated using the recorded weights closest to 30 kg and 70 kg. Fat measurements and the length of the small intestine were taken at slaughter. The traits considered in this analysis, along with the number of F_2 individuals with records for each trait, are given in Table 1 [see Andersson *et al.* (1994) for further details].

STATISTICAL METHODS

An approach for the analysis of a three generation pedigree derived from a cross between outbred lines has been developed by Haley *et al.* (1994). The analysis can be considered in two stages. In the first stage, at locations throughout the genome, the probability of an F_2 offspring being each of four possible QTL genotypes (accounting for origin) is calculated conditionally on the marker genotypes. In the second stage, these probabilities are used in a least squares framework to investigate the genetic model underlying the trait of interest.

Genotyping offspring: Within one family, at any location in the genome, up to four alleles could be segregating in the offspring generation, one coming from each of the grandparents. Using multiple marker information as described in Haley *et al.* (1994) for a given location in the genome, we can calculate the probability of the two alleles in an offspring coming from any of the four possible pairs of grandparents.

If the alleles are located at a fully informative marker, the probabilities would depend on information from this marker only. Otherwise the probabilities are functions of the recombination rates between the location under consideration and the flanking informative markers. Assuming Haldane's mapping function for any given position in a linkage group, only two informative markers (one on either side of the position) are needed for each of the sire and the dam sides of the pedigree. Thus, up to four markers are needed for each individual progeny, although the four markers used will vary from progeny to progeny.

At a QTL we assume that the grandparental breeds are fixed for alternative alleles. Hence, only two alleles are segregating and these are the same across all families. Following Falconer and Mackay (1996) and denoting the effect of QQ as a, the effect of Qq as d and the effect of qq as $-a$, the expected value of an offspring can be written as a linear model in terms of the additive and the dominance contributions at a QTL,

$$y_i = \mu + c_{ai}a + c_{di}d, \qquad (1)$$

where μ is the mean, c_{ai} is the coefficient for the additive component for individual i at the given location that, denoting the probability of an individual being genotype XX as prob(XX), is equal to prob(QQ) − prob(qq), and c_{di} is the coefficient for the dominance component for individual i at the given location, which is equal to prob(Qq).

Sex-different recombination rates: As suggested by Haley *et al.* (1994) it is relatively simple to accommodate differences in the male and female recombination rates. Using the sex-specific maps and looking at each interval in turn, for every centimorgan along the average distance between the two markers, the corresponding distance for the male and for the female map can be obtained. Subsequently, when calculating the probability of being a given genotype, given adjacent markers, the sex-specific distances are used and converted into recombination rates. The relevant probability can then be used depending on whether the allele is passed through the male or female parent. This model enables us to use the correct model for the Y chromosome, with no recombination

TABLE 1

Traits analyzed with their mean, standard deviation and the number of individuals with records

Trait		No. F_2	Mean	SD[a]
Birth weight (g)	BW	199	1384	195
Growth rate from birth to 30 kg (g/day)	GR30	196	236	34
Growth rate from birth to 70 kg (g/day)	GR70	193	367	36
Growth rate from 30 to 70 kg (g/day)	GROT	193	625	88
Length of small intestine (dm)	IL	191	175	16
Abdominal fat (%)	AF	191	2.40	0.55
Average depth of back fat (mm)	BF	191	26.4	3.7

[a] Residual standard deviation after fitting the basic fixed effects and covariates (see text).

except in the pseudoautosomal region, while allowing recombination between the *X* chromosomes in females.

For the autosomes and pseudoautosomal section of the sex chromosome, the same model as in Equation 1 can be fitted. For the sex chromosome, because all male grandparents were wild boar and all female grandparents were Large White pigs, in each sex only two genotypes will be present. For females, one *X* chromosome must have originated in a Large White pig, and locations on the other chromosome could be from either breed. For males, locations on the *X* chromosome could come from either breed. For each sex, therefore, only one effect can be fitted: the difference between the two possible genotypes. Because these effects are not necessarily the same for the two sexes, however, a separate effect is fitted for each sex.

Information content and segregation distortion: The four genotype probabilities calculated can be used to give an indication as to how informative the multiple markers are at any location. If the coefficient for an individual is 1 or -1 for *a*, or 1 for *d*, the genotype of the individual at that location is know, that is, there is complete information for that individual. Alternatively, as the coefficient of *a* tends to 0.0 and that of *d* tends to 0.5, there is no information available. The variance of these coefficients across the F_2 progeny gives a measure of information for *a* and *d*. When there is no information from markers, the variances are zero, and when the genotype of all individuals is known at the location being considered, the variance is expected to be 0.5 for the additive component and 0.25 for the dominance component. Figure 1 illustrates the benefits of including multiple marker information over using markers singly for chromosome *4*, using a combined measure of variance from the two components (variance of *a* plus twice the variance of *d*). It is possible for this measure to be greater than one because in the situation where there are more homozygous individuals than expected, the contribution from the additive component can be >0.5. The maximum is 1.125, obtained when the locus is fully informative, typed in all offspring and three-quarters of the offspring are homozygous, with equal numbers in each class.

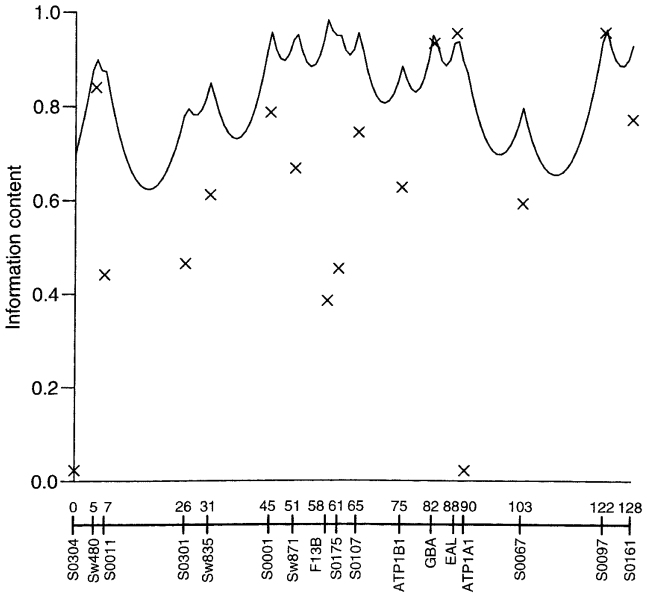

Figure 1.—Information content for pig chromosome 4 using markers singly (shown by a cross) and using all marker information simultaneously (the solid line). The marker map is the average of the male and female map, which is very similar to the sex averaged map (Marklund *et al.* 1996).

Additionally, the coefficients of *a* and *d* can be used to give an indication of any segregation distortion in the F_2. Two measures of distortion were considered. The first (DIS) considers whether there is an excess of alleles from either of the original lines and the second (HET) looks for an excess or lack of heterozygotes.

With equal contribution from the two lines, the mean additive coefficient over progeny (DIS) should be zero:

$$\text{DIS} = \frac{\sum_{i=1}^{N} c_{ai}}{N}$$

With an excess of one line DIS tends to 1, and with excess of the other line DIS tends to -1.

Under the two allele model being assumed for putative QTLs, the coefficient of *d* is expected, on average, to be 0.5,

$$\text{HET} = \frac{\sum_{i=1}^{N} 2(c_{di} - 0.5)}{N}$$

so that with no distortion HET $= 0$, with an excess of heterozygotes HET tends to 1 and with a lack of heterozygotes HET tends to -1.

Tests for segregation distortion were carried out every centimorgan throughout the genome. Four regions were significant at the 0.05 pointwise level for DIS and 11 for HET. From subsequent analyses a single scan through the genome was equivalent to ~250 independent tests, in which case the number of observed significant regions was no more than expected by chance. Adjusting the significance threshold to give a 0.05 genome-wide level gave no significant regions. Hence, in these data there was no evidence for segregation distortion.

Basic least-squares model fitted: As the subsequent analyses are carried out within a least-squares framework, it is simple to incorporate fixed effects and covariates into the models. Equation (1) can be extended to include fixed effects and covariates. For all traits the model fitted included the fixed effect of sex and parity and the random effect of family. In addition, the effect of a feed treatment was included for all traits except BW and GR30. The following covariates were also included in the basic model fitted: the litter size the individual is born into for BW, the exact weight, ~30 kg, used to obtain the growth rates for GR30 and GROT, the exact weight, ~70 kg, for GROT and GR70 and weight at slaughter for IL, AF, and BF.

For all analyses the coefficients for the sex-different maps have been used. The pseudoautosomal region of the sex chromosome has been analyzed as an autosome, whereas the alternative, sex-specific model has been fitted for the rest of the sex chromosome. Hence, the genetic map has been treated as if there were 20 chromosomes, with chromosome *19* being the pseudoautosome and chromosome *20* being the sex chromosome.

Exploratory analyses

Genome substitution effects: The first analyses were carried out to look at the net effect of all chromosomes inherited from each breed and to consider evidence for heterosis. The mean coefficient of *a* and of *d* for each individual over all locations in the genome, excluding the sex chromosomes (*i.e.*, calculated from the coefficients obtained at each location, every centimorgan in this case), were obtained and the phenotype for each offspring regressed onto them. If all QTLs were linked in association in the grandparents, this would give the sum of the effect of those loci for the whole genome.

Cofactor selection: We fitted locations of markers (*i.e.*, using

the coefficients calculated using all markers at the location of a true marker) as cofactors. For each trait, first marker locations were selected from each chromosome independently. A marker location was eliminated if its omission did not significantly (at the 0.05 level) worsen the fit of the model. Second, all retained locations (across all chromosomes) were included in a single analysis and markers excluded by backward elimination with a 0.05 significance threshold. The remaining marker locations were used to account for variation caused by QTLs on chromosomes not currently being analyzed, and, hence, locations on the chromosome being searched were not included.

Comparison of alternative genetic models: The exploratory analyses proposed by Visscher and Haley (1996) can be extended to accommodate the three generation outbred pedigree currently under analysis. In the inbred line cross, information from single markers can be used, as all markers are completely informative. In our situation, however, markers were not completely informative, and the information available to genotype individuals at given locations can be increased by using all markers in the linkage group together. At sites of markers, information content tends to be higher (see Figure 1), and, hence, marker locations were selected to be evenly spaced throughout the chromosome (at between 10 and 20 cM) with high information.

Using models analogous to those proposed by Visscher and Haley (1996), we carried out the following sequence of analyses for each chromosome, in turn.

Multiple QTL model: Offspring phenotypes were regressed simultaneously onto the coefficients of *a* and of *d* for all marker locations selected from each chromosome. This is a test for the presence of genetic variation on the chromosome affecting the trait under consideration. The degrees of freedom for the genetic component of this model are twice the number of selected marker locations on the chromosome.

Single region: The coefficients of *a* and of *d* from the two selected marker locations flanking an interval were fitted. This tests for an effect associated with the flanked interval. The analysis was repeated once for each marker interval (*i.e.*, the number of selected markers minus one). The genetic component of this model has four degrees of freedom.

Polygenic model: This model tested whether the effect of each chromosome was explained by the proportion of each genotype present along the chromosome (*i.e.*, each equal length of a chromosome from one breed is assumed to have the same effect in the same direction). Note that if there are QTLs from a breed with opposite effects they may mask each other in this test. If all marker locations were used, this would be equivalent to calculating the mean coefficients for *a* and for *d* for the chromosome and regressing onto these. For the polygenic model to be nested within the multiple QTL model, however, only selected marker locations were used, and, hence, a weighted mean of the coefficients at the selected markers was required to account for the fact that markers were not equally spaced [Visscher 1996 and Knott *et al.* (1997) for a derivation of these weights for the three generation pedigree]. The genetic component of this model has two degrees of freedom.

For all three models the relevant fixed effects, random effect and covariates were fitted. Additionally, cofactors were fitted, except on the chromosome being analyzed. For a given trait these fixed and random effects, covariances and cofactors are the same for all models. The multiple QTL model was compared with a model with no genetic component on the chromosome of interest (*i.e.*, fitting the fixed and random effects, covariates and any cofactors on other chromosomes). If the multiple QTL model was significant, suggesting that there were genes involved in the expression of the trait segregating on this chromosome, the single region and polygenic models were compared with it to see if they provide an adequate description of the data. If there was one QTL or several linked together in a small region of a chromosome, then fitting coefficients from markers flanking this region would explain most of the genetic variance associated with the chromosome, and, hence, the multiple QTL model would not be a significant improvement over the single region model. Alternatively, if there were many QTLs linked in association in the grandparents, the polygenic model would provide an adequate description of the data and would not be rejected in favor of the multiple QTL model.

Repeating this procedure for each chromosome and for each trait gives an indication as to the important chromosomes and suggests possible underlying genetic models.

QTL analyses

Single QTL: Initially, the mapped genome was searched to identify regions where the markers explain a large proportion of the phenotypic variance. Every cM the offspring phenotypes were regressed onto the coefficients of *a* and of *d*. A simple F ratio was calculated to compare a model with a QTL at this location vs. a model without the QTL. Estimates were obtained for the additive and dominance effect of the putative QTL at this location in the F_2 population. The best estimate for the position of the QTL was taken to be the location giving the highest F ratio. Cofactors were fitted on chromosomes not currently being searched to account for variation caused by unlinked QTLs.

Single QTL with interaction: To investigate whether the effect of the putative QTL was different in male vs. female offspring, additive and dominance effects can be estimated for each sex separately. Comparing this model with one with no QTL gives a test with four degrees of freedom for the interaction of QTL effect and sex. If significant, this was tested against the best model with sex-equal effects for that chromosome.

Imprinting: As originally presented, the expected performance of an offspring was written in terms of the additive and dominance contributions at the postulated QTL. However, when accounting for the grandparental origin of the alleles, there are four possible genotypes in the offspring generation, and it is possible to fit three effects. The additional degree of freedom allows us to test whether there is any difference between the two classes of heterozygotes at the putative QTL. The difference in these two classes is in the origin of the two alleles; in one, the wild boar allele has been inherited through the F_1 mother and in the other through the father, and the reverse for the Large White alleles. For the autosomes, therefore, the difference between these heterozygotes should indicate whether imprinting is an important effect (*i.e.*, passaging through the male is different from through the female). (Note that this analysis is only possible because we are analyzing an outbred cross in which up to four alleles segregate. It would not be possible in the analysis of an intercross between inbred lines because the two types of heterozygotes are indistinguishable.)

A model fitting imprinting in addition to the additive and dominance effects of a putative QTL was compared with a model with no QTL (with three degrees of freedom). If significant, this imprinting model was compared with the best QTL model without imprinting to see whether the imprinting effect was significant.

Two QTLs: A two-dimensional search was carried out, fitting the coefficients for two locations simultaneously. The F ratio obtained from fitting the two QTLs compared with no QTLs (with four degrees of freedom) was considered. If this was

significant, the improvement of the model, including the second QTL over that of the best single QTL, was considered.

Confidence intervals: Confidence intervals for the location parameter were obtained by bootstrapping (Visscher *et al.* 1996). Five hundred resamples were used. The 0.95 confidence interval was taken to be the region such that, in both directions, 0.025 of estimated positions were more extreme.

Significance thresholds

Following Lander and Kruglyak (1995) suggestive and significant linkages will be presented. The suggestive level (where, by chance, we expect to obtain one significant result per genome analysis) was obtained by considering that we were analyzing 19 (independent) chromosomes, each with a probability P of having a significant result. Assuming the number of significant chromosomes to follow a binomial distribution, we wish to set the required threshold, P, such that the expected number of significant chromosomes, $19p$, is equal to one. Therefore, P is $\sim$0.05, indicating that we can use the 0.05 level for each chromosome to give the threshold for the suggestive level.

The threshold for the significant level (where, by chance, we expect 0.05 significant results per genome analysis) was obtained using the Bonferoni correction; assuming 19 chromosomes are being analyzed (*i.e.*, there are 19 independent tests), the chromosomal test significance level would be 0.0027 to give the genome-wide 0.05 level ($(1 - 0.0027)^{19} = 1 - 0.05$).

For the various tests we need to find the F ratio corresponding to these significance thresholds. For the exploratory analyses, where one test was initially being carried out per chromosome, the F values were obtained from a standard F distribution. For the QTL searches, a large number of correlated tests was being performed, and, hence, the standard F distribution cannot be used to obtain the threshold F values. The correct null hypothesis distribution to obtain suitable thresholds was therefore obtained empirically by a permutation test (Churchill and Dorerge 1994). In our approach, all the genetic information is obtained within the calculated coefficients; hence, these can be calculated once and then permuted with respect to the phenotypes. For each permutation, the entire genome is searched, and the highest F ratio for each chromosome noted. A genome-wide 0.05 level was obtained by picking the highest F ratio each permutation for the whole genome. The suggestive level thresholds were from the 0.05 level obtained for each chromosome separately. One thousand permutations were studied for each trait. The relevant fixed effects, random effect and covariates were fitted. Cofactors were fitted in the same way as for the QTL analyses, that is, by omitting them from the chromosome being searched, and were permuted with the phenotype, such that prior to fitting the QTL the residual variance was constant across replicates.

For the alternative single QTL analyses, that is, interaction with sex or with imprinting, an approximate significance threshold can be obtained by converting the threshold F ratio obtained from the null hypothesis simulations described above into a probability of the F ratio under a standard F distribution with two degrees of freedom for the numerator. It is then possible to obtain the F ratio that would give this probability under a distribution with three, four or eight degrees of freedom as required for the relevant test.

For one chromosome (chromosome *4*) and one trait (BF), a permutation test was carried out in order to obtain an indication of the relevant significant level when two QTLs were being fitted.

Direct effect of the halothane locus

The pedigree was typed for the calcium release channel mutation (located on chromosome 6 at 81 cM), which identifies the "halothane" genotype (Fujii *et al.* 1991). One of the founder boars was heterozygous at this marker; all other grandparents were homozygous normal. The halothane mutation is known to be associated with leanness. The effect of the locus in our data was estimated in an analysis fitting the halothane locus. Additionally, the QTL analyses described above were repeated for the fat traits fitting the genotype at the halothane locus as an additional fixed effect.

RESULTS

Exploratory analyses: *Genome substitution effects:* Table 2 gives the net additive and dominance effects for the whole genome. This provides an estimate of half the breed difference and any effects of heterosis for the traits being considered. A negative estimate indicates that the net effect of alleles inherited from the wild boar causes a decrease in the phenotype. Hence, the wild boar has, on average, slightly lighter piglets that grow more slowly than those of the white pig, and the adult pig is more fat and has a shorter intestinal length. For all traits except birth weight, there is a significant difference between breeds. The standard error associated with the estimate of the effect of dominance is large, but there is an indication of nearly complete dominance for back fat, growth rate on test and birth weight. Table 2 also gives the percentage of the residual mean square after fitting the fixed and random effects and covariates explained by the additive and dominance substitution effects and the joint significance of them, assuming an F distribution with two degrees of freedom in the numerator.

Cofactor selection: Table 3 gives the number of marker locations selected for each trait after the two rounds of backward elimination. The percentage of the residual mean square explained after fitting the fixed effects, random effect and covariates, and the total joint effect of the cofactors is also given. The direction of effect is generally in agreement with the estimates obtained for the whole genome effect when fitting a polygenic model (see Table 2), but for all traits the estimated cofactor additive effects were both positive and negative in direction (not shown). The cofactors explain a greater proportion of the variance than the genome substitution effects.

Comparison of alternative genetic models: The results from the hierarchy of exploratory tests are given in Table 4, which includes all sets of tests where fitting all selected marker locations simultaneously (the multiple QTL model) is significant at the suggestive level ($P \ll 0.05$ chromosome test). The results from the single region explaining the highest proportion of the variance have been presented, and additional regions may also show significant effects. For only three traits (AF, BF, and IL) was the multiple QTL model significantly better (*i.e.*, at

TABLE 2

Estimated genome substitution effects

Trait	Additive[a]	Dominance[a]	Variance explained[b]	Probability[c]
BW (g)	−242 (144)	−284 (247)	1	0.11
GR30 (g/day)	−87.1 (24.9)	47.3 (42.3)	6	0.002
GR70 (g/day)	−117.2 (26.2)	3.6 (44.2)	10	0.000
GROT (g/day)	−148.7 (67.5)	−145.5 (113.6)	3	0.04
IL (dm)	−36.9 (13.2)	−22.1 (20.5)	4	0.01
AF (%)	1.06 (0.46)	0.40 (0.71)	2	0.06
BF (mm)	8.69 (3.05)	7.22 (4.76)	5	0.006

[a] With the standard error of the estimate in parentheses.
[b] Joint significance of additive and dominance effects.
[c] Given as the percentage of the residual mean square after fitting the fixed and random effects and covariates.

the genome-wide level) than a nongenetical model for any chromosome ($P \ll 0.0027$ chromosome test). For both AF and BF an effect on chromosome *4* was detected. For AF the multiple QTL model was not, however, a significant improvement over either the best single region model or the polygenic model, suggesting that the data can be explained by either one or a few QTLs in a small region of the chromosome or by many dispersed throughout the chromosome. The single region model, however, explains more of the residual variance. For BF, the polygenic model would be rejected in favor of the multiple QTL model, whereas the single region model would not be rejected, leading to the conclusion that a small region of the genome is important. For IL there are significant effects when all marker locations on chromosome *3* or on chromosome *5* are fitted together. In both cases, this model provides a significantly better explanation of the data than either the single region or the polygenic model, suggesting that several QTLs with both positive and negative effects are segregating on these chromosomes. The sex chromosome has a significant genetic effect on AF. The multiple QTL is significantly better than the polygenic and the single region model, again suggesting that several QTLs are responsible.

QTL analyses: *Null hypothesis:* The genome-wide 0.05 significance thresholds obtained using the sex different map are given in Table 5. Thresholds do not differ much across traits. These F values are equivalent to a probability of between 0.00018 and 0.00034 under the standard F distribution with two degrees of freedom in the numerator or about 250 independent tests. The chromosomal suggestive thresholds, except for the pseudoautosomal region of X/Y, range from ~4.2 to 6.0, depending on the trait and chromosome being analyzed. The pseudoautosomal region of X/Y has a suggestive threshold of ~3.5 for all traits.

Single QTL: Table 6 presents the location giving the highest test statistic and the parameter estimates obtained at this location for chromosomes with a QTL significant at the suggestive level. Location estimates are relative to the sex-specific maps given by Marklund *et al.* (1996). In addition, all chromosomes previously identified as being significant at the suggestive level when fitting the multiple QTL model (Table 4) have been included. For five of the seven traits, GR30, GROT,

TABLE 3

Selected cofactors

Trait	No. markers	Variance explained[a]	Net effect[b]	
			Additive	Dominance
BW (g)	6	19	−41	−62
GR30 (g/day)	6	20	−44.8	9.66
GR70 (g/day)	11	36	−33.6	−24.7
GROT (g/day)	12	36	28.0	−21.1
IL (dm)	19	56	−8.1	−7.8
AF (%)	9	44	0.26	0.10
BF (mm)	11	48	4.43	−0.05

[a] Given as the percentage of the residual mean square after fitting the other fixed and random effects and covariates.
[b] The sum of the additive and dominance coefficients for the selected cofactors.

TABLE 4

Comparison of models

Trait	Chromosome	Probability of F[a]		
		M	M *vs.* Q	M *vs.* P
BW	*1*	2.4	28.4	4.3
GR30	*7*	2.9	15.4	4.3
	13	0.5	12.1	5.1
GR70	*4*	1.8	33.1	21.4
	15	4.9	77.9	7.5
GROT	*4*	3.0	56.1	4.3
	10	0.7	64.6	9.1
IL	*2*	3.0	11.1	6.0
	3	0.0	0.1	0.0
	5	0.2	3.5	3.2
	12	3.5	72.6	3.6
	16	3.2	36.8	7.1
	17	4.2	48.9	3.2
AF	*1*	2.1	6.3	3.1
	4	0.2	70.5	24.2
	15	1.0	27.6	3.0
	X	0.2	2.7	0.6
BF	*4*	0.0	71.0	1.3
	X	1.2	4.4	0.6

[a] The probability (×100) of the F ratio obtained for various tests: M, the multiple QTL model against the null hypothesis of no genetic effect; M *vs.* Q, multiple QTL against the null hypothesis of the "best" single region model; M *vs.* P, multiple QTL against the null hypothesis of the polygenic model.

IL, AF and BF, one region of the genome was significant and for GR70 one region approached significance. The QTLs affecting the fat traits and IL are in a similar location on chromosome *4*. The QTL for GR70 is also on chromosome *4* with the best location 20 to 30 cM away from the fatness QTL. A significant QTL for GROT is found on chromosome *10* and for GR30 on chromosome *13*. Suggestive QTLs for BW and GR70 are also located in this region on chromosome *13*. Figure 2 gives the F ratio profile for BF on chromosome *4*.

The significant QTLs are essentially additive in their action, except for the QTL on chromosome *10* affecting GROT where the allele from the wild boar is completely dominant. A positive additive estimate means that the wild boar alleles are causing an increase in the trait value. That is, the individuals homozygous for the wild boar alleles at the fat QTLs on chromosome *4* have, on average, more abdominal fat (0.6%) and thicker backfat (4.0 mm) than those homozygous for the Large White alleles. In contrast, the wild boar QTL alleles decrease growth rate compared with the Large White pig and cause shorter intestinal length.

TABLE 5

Threshold F values for QTL analyses

Trait	Significance threshold		
	10%	5%	1%
BW	7.8	8.7	10.0
GR30	7.8	8.4	10.6
GR70	7.9	8.8	11.0
GROT	7.9	8.7	10.8
IL	8.2	9.1	11.1
AF	8.0	9.0	11.6
BF	7.9	8.6	10.8

Confidence intervals (0.95) for the location parameter are given in Table 6 for those QTLs attaining genome-wide 0.05 significance. For the two fat traits a region of about 40 cM (averaged over the the male and female map) is spanned by this 0.95 confidence interval, and for growth rate on test ~50 cM is covered. For growth rate to 30 kg, growth rate to 70 kg and intestinal length, the confidence interval included 90 cM or more (on the average map), which is a large proportion of the total chromosome length (171 cM, 128 cM and 128 cM, respectively.) Figure 2 gives the frequency distribution of the bootstrap results for the best location for the QTL for BF on chromosome *4* using the average of the male and female map. This distribution is a more extreme version of the F ratio profile, with most of the replicates giving a maximum test statistic ~66 cM, but another, much smaller peak being observed ~48 cM.

Single QTL with interaction with sex: Converting the 0.05 significance thresholds to be suitable for the test of a single QTL interacting with sex *vs.* no QTL gives F values of between 5.5 and 5.9 for the genome-wide level for the seven traits. One region of the genome for each of three of the traits (IL, AF and BF) gave a test statistic greater than this threshold. For AF and BF the QTLs detected are at the same location on chromosome *4* as when an interaction was not fitted (see Table 6), and fitting the interaction with sex does not provide a significantly better explanation of the data. The highest F ratio on chromosome *8* now reaches significance for IL when an effect for each sex was fitted. This location gave a suggestive result when the interaction with sex was omitted, and the improvement in fit obtained by fitting the interaction is significant (with an F ratio statistic of 6.4 with two and 124 degrees of freedom). The model with the same effect fitted in both sexes gave a completely dominant model with the heterozygote having the same effect as the wild boar (Table 6). When a separate effect is estimated in the two sexes, the QTL has an additive effect in males with increased effect ($a = -5.7 \pm 1.7$ dm, $d = -0.7 \pm 2.5$ dm), whereas in females the additive effect was much smaller ($a = -2.0 \pm 2.0$ dm) but heterozygotes were estimated to have an intestinal length shorter than the midhomozygote effect ($d = -12.6 \pm 2.9$ dm).

The regions significant for IL (chromosome *4*), GR30 (chromosome *13*) and GROT (chromosome *10*), when fitting the same effect for each sex, fell below the genome-wide significance threshold, although they

TABLE 6

Results from fitting a single QTL

Trait	Chromosome	F ratio[a]	Location (cM)[b]		Estimates (SE)	
			Male	Female	Additive	Dominance
BW (g)	*1*	6.4*	11	31	−59.5 (22.1)	74.1 (32.8)
	12	5.4*	0	0	−36.6 (37.1)	−303.3 (94.6)
	13	6.5*	104	80	75.4 (21.1)	11.8 (28.5)
GR30 (g/day)	*7*	4.8	77	171	−11.1 (3.6)	−1.2 (5.4)
	13	8.6**	132 (0–145)	104 (0–123)	−16.4 (4.0)	5.0 (6.6)
GR70 (g/day)	*4*	8.8**	86 (22–102)	94 (32–130)	−14.6 (4.0)	−7.8 (5.1)
	13	5.9*	122	90	−11.1 (3.6)	7.8 (5.2)
	15	3.5	50	78	10.7 (4.1)	−5.5 (5.4)
GROT (g/day)	*2*	5.2*	112	164	7.3 (12.8)	−62.1 (20.5)
	4	3.3	62	60	−15.3 (8.7)	23.5 (12.1)
	10	9.8**	34 (22–55)	74 (32–95)	−32.8 (8.6)	−31.4 (13.0)
IL (dm)	*2*	4.9	52	48	−2.52 (1.32)	5.79 (2.09)
	3	3.5	94	118	4.11 (1.67)	−2.61 (2.37)
	4	10.2**	66 (14–101)	76 (16–129)	−6.36 (1.48)	2.53 (2.14)
	5	6.4*	20	52	−4.65 (1.46)	−2.31 (2.11)
	8	7.5*	53	69	−3.97 (1.33)	−5.29 (1.96)
	12	6.4*	33	9	−0.68 (1.84)	−11.7 (3.29)
	14	5.3*	94	122	−1.86 (1.75)	−8.81 (2.71)
	16	6.9*	3	5	−5.70 (1.62)	3.09 (2.44)
	17	6.9*	0	0	5.45 (1.48)	0.77 (2.35)
AF (%)	*1*	6.4*	109	91	−0.129 (0.054)	0.220 (0.079)
	4	15.8**	62 (41–77)	64 (49–91)	0.284 (0.051)	0.011 (0.072)
	6	6.3*	46	78	−0.101 (0.059)	−0.267 (0.083)
	8	5.7*	8	8	0.171 (0.054)	−0.086 (0.075)
	14	5.6*	81	105	0.181 (0.057)	0.181 (0.094)
	15	8.4*	5	11	0.204 (0.056)	−0.190 (0.081)
	X	6.3*	—	106	0.080 (0.043)	−0.144 (0.046)
BF (mm)	*1*	8.4*	2	6	1.69 (0.41)	0.03 (0.66)
	2	5.6*	0	0	1.00 (0.30)	0.36 (0.46)
	3	6.4*	113	169	−1.05 (0.39)	−1.39 (0.61)
	4	18.6**	64 (39–73)	68 (47–89)	2.03 (0.34)	0.43 (0.48)
	5	7.8*	26	76	0.83 (0.36)	1.53 (0.58)
	15	5.8*	7	15	1.27 (0.37)	−0.30 (0.53)
	X	4.8	—	106	0.82 (0.27)	−0.01 (0.30)

[a] For test of 1 *vs.* 0 QTL. * indicates significance at the suggestive level and ** at the genome 0.05 level.
[b] With 95% confidence interval in brackets.

were still suggestive. For IL and GROT the location explaining the greatest variance was the same, whereas for GR30 (where the test statistic has dropped more) it has shifted 13 cM along the chromosome.

Testing for imprinting: The genome-wide 0.05 significance thresholds converted from the single QTL level were between 6.5 and 6.9 for the different traits. For AF, BF and IL the region of chromosome *4* previously found to contain a QTL was significant when fitting an imprinting effect, in addition to the additive and dominance model. Likewise, the region on chromosome *10* for GROT was still significant, whereas for GR30 the previously significant region on chromosome *13* dropped below this level. Only for AF on chromosome *4* did the addition of the imprinting effect cause a significant improvement ($F = 4.20$). The estimates for this location were an additive effect of $0.286 \pm 0.050\%$, a dominance effect of $0.016 \pm 0.072\%$ and an estimated difference in the effect of the heterozygotes of $0.115 \pm 0.054\%$, with the heterozygote with wild boar allele inherited through the male parent having the higher percentage of abdominal fat.

Two QTLs: The permutation test for chromosome *4* using BF gave a threshold for the suggestive level of significance of 4.5 for the test of two *vs.* no QTLs. In Table 7 chromosomes are presented where both the test of two *vs.* no QTLs is suggestive (based on the BF level) and where the test of two QTLs is significantly better than one QTL using the suggestive levels obtained for the test of one *vs.* no QTLs. None of the test statistics reach the genome-wide 0.05 level.

For GR70, two QTLs on chromosome *15* provide a better explanation of the data than one or no QTLs. One of the two QTLs is at the same location as the

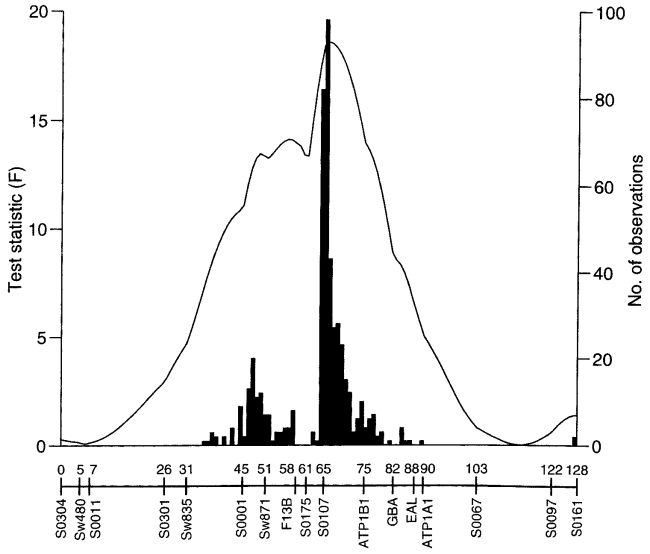

Figure 2.—The F ratio profile for backfat thickness on pig chromosome 4 when fitting a single QTL (the solid line). Additionally, the histogram of the best location estimates from the bootstrap analysis is given. The marker map is the average of the male and female map, which is very similar to the sex averaged map (Marklund *et al.* 1996).

best single QTL. There is evidence for two QTLs on chromosomes *3* and *13* for IL. On chromosome *3* both QTLs are located near the best location obtained from the single QTL analysis. An effect for IL on chromosome *13* has not been previously detected. Two chromosomes were also suggestive of two QTLs affecting AF; these were chromosome *5* and the *X* chromosome. Chromosome *5* has not been picked up previously in connection with AF, whereas in the exploratory analyses, a model with several QTLs had been postulated for the *X* chromosome.

Halothane mutation: The animals heterozygous for the halothane mutation were more lean than normal homozygotes, as expected from the known effect of this mutation (Fujii *et al.* 1991). The largest effect was observed for back fat, where the heterozygous individuals had, on average, 1.63 ± 0.74 mm less back fat than the normal homozygous individuals. For abdominal fat, heterozygous individuals had 0.16 ± 0.11% less fat. The QTL analyses were repeated fitting halothane genotype as a fixed effect. The addition of the halothane genotype, however, had little effect on the conclusions reached from these analyses. The best location for the QTL detected on chromosome *4* for BF was moved to 49 cM with the F ratio reduced to 10.2. The additive effect of the QTL was reduced to 1.4 ± 0.35 mm and the dominance effect increased to 1.0 ± 0.50 mm. Smaller changes in the highest F were observed for other chromosomes, but these changes only caused a difference in the chromosomes classified as suggestive (adding some chromosomes and losing others). The inclusion of the halothane genotype in the analysis of AF caused only small changes in the results.

DISCUSSION

We have analyzed the data from the wild boar × Large White F_2 population in several ways and have some convincing evidence for QTLs affecting fatness and growth traits. For chromosomes significant at the genome-wide level, the results are fairly consistent. The tests point to a single QTL affecting each of the two fat traits on chromosome *4*, with the best locations between markers S0175 and ATP1B1. There is evidence of genome imprinting at the chromosome *4* QTL affecting AF, as the effect of the heterozygous genotype is different, depending on whether the allele was passed through the male or female F_1 parent. The effect was not seen for BF, and we have to bear in mind that the result for AF could be a false positive result (Type I error) as we were carrying out a large number of tests. The results for GR30, GR70 and GROT suggesting single QTLs on chromosomes *13*, *4* and *10*, respectively, are also consistent across all tests. The results for IL are not

TABLE 7

Results from fitting two QTLs

		F ratio		Location (cM)		Estimates (SE)	
Trait	Chromosome	*vs.* 0 QTL	*vs.* 1 QTL	Male	Female	Additive	Dominance
GR70 (g/day)	*15*	4.9	6.0	24	56	−13.0 (5.1)	−14.9 (6.5)
				50	78	19.9 (5.2)	0.4 (5.9)
IL (dm)	*3*	5.1	6.4	97	131	29.83 (7.99)	−13.1 (8.23)
				99	141	−27.07 (7.72)	12.6 (7.61)
	13	4.8	6.6	118	86	1.34 (3.53)	−17.92 (4.22)
				126	94	0.03 (3.20)	15.51 (3.89)
AF (%)	*5*	5.0	6.9	5	27	−0.230 (0.069)	−0.159 (0.080)
				21	55	0.145 (0.070)	0.229 (0.086)
	X	5.3	4.2	—	24	−0.192 (0.073)	0.108 (0.079)
				—	92	0.199 (0.067)	−0.252 (0.071)

so clear, however. The single QTL detected on chromosome *4* was not picked up in the multiple QTL model, presumably because of the large number of degrees of freedom being used in this test. Chromosomes *3* and *5* suggest a multiple QTL model, which is consistent with no single effects being detected on these chromosomes. The two QTL results were not conclusive, although for chromosome *3* there is good evidence for at least a second QTL. Five different cofactor locations were on chromosome *3* for IL, which also supports a multiple QTL model. When fitting an interaction with sex, IL gave a significant test statistic on chromosome *8*. This chromosome was not picked up in the exploratory analyses, when the same effect was fitted in both sexes, but was suggestive when fitting a single QTL without interaction. The convincing QTLs are in accordance with the expected effect due to selection. That is, the white pig will have been selected for less fat and for a faster growth rate, causing fixation of the favorable alleles at some loci.

These results are consistent with the previous study of these data where a less complete marker map was used (Andersson *et al.* 1994). An additional QTL has been detected here for GROT on chromosome *10*; this may be because of the inclusion of cofactors, which bring this region up to significance. The QTLs for IL and GR70 on chromosome *4* and GR30 on chromosome *13* are found in the same marker intervals in both analyses. The best location for the QTLs for the fat traits, however, has moved into the adjacent interval, between S0175 and S0107 rather than between S0001 and S0175, as found previously. S0001 was the last marker on the chromosome *4* map for the previous analyses, whereas now the map has been substantially extended. The additional markers, presumably, are less compatible with the observations than further along the chromosome, moving the QTL location into the next interval. The QTLs affecting fat and growth rate on chromosome *4* have subsequently been confirmed in an analysis of a backcross population derived from two of the F_2 individuals included in this analysis (L. Marklund, unpublished results).

A long-term goal in QTL mapping is to identify the causative genes at the molecular level. This is a very difficult task but positional candidate cloning is currently the most promising strategy to achieve this. However, because the transcript map is poorly developed in farm animals, animal geneticists need to utilize comparative map information from better-studied organisms, particularly humans and mice, to identify possible candidate genes, that is, comparative positional candidate cloning (Womack and Kata 1995). Such candidate genes will then be screened for genetic polymorphism and mapped in relation to the QTL. As regards the major QTL for fatness on chromosome *4*, ZOO-FISH analysis (Retenberger *et al.* 1995) has revealed that this pig chromosome shares homology with human chromosomes *1* and *8*. Unfortunately, the QTL region is close to the breakpoint of conserved synteny, complicating the identification of possible candidate genes. There are some candidate genes for human obesity, however, that are found in possibly homologous regions. These include the β-3-adrenegic receptor gene (ADRB3) on human chromosome *8q* (Clement *et al.* 1995) and ATP1B1 on human chromosome *1q* (Deriaz *et al.* 1994). ATP1B1 was in fact one of the markers included in this QTL study, and it maps very close to the QTL peak for fatness (Figure 2). None of the obesity genes so far cloned in the mouse (*e.g.*, *ob* and *db*, encoding leptin and the leptin-receptor, respectively) map to a region showing conserved synteny with the actual region of pig chromosome *4*. We are currently improving the map position of this fatness QTL by marker-assisted backcrossing, and we plan to use a recently developed pig radiation hybrid panel to make a high-resolution comparative map over the QTL region. The human homologue of the QTL for early growth on pig chromosome *13* is expected to be located on human chromosome *3*. The actual region harbors the PIT1 gene encoding a pituitary-specific transcription factor known to be important for normal growth. Interestingly, an association between a PIT1 polymorphism and early growth was detected using a cross between European and Chinese pigs (Yu *et al.* 1995). The region on pig chromosome *10* harboring a QTL for growth is expected to be homologous to some part of human chromosome *1*, but the precise region is not sufficiently well defined to make the search for candidate genes meaningful.

The results of the present study have several important implications from an animal breeding point of view. First, it is an interesting question whether the QTLs identified here, which explain an important part of the genetic difference between these two divergent pig populations, also control part of the genetic variation in these traits within commercial pig populations. We are currently collecting material to answer this important question. Second, QTLs may be exploited in animal breeding programs by marker-assisted selection (MAS). As regards all the QTLs reaching the genome-wide significance threshold in this study, the wild pig alleles were associated with a less-favored phenotype (in modern pig production), that is, with a more slow-growing and fat pig. But it is quite possible that some QTLs where the wild pig allele has a favorable effect could be identified in a more powerful QTL study (*e.g.*, larger F_2 generation), as has been reported in crosses between wild and cultivated rice (Xlao *et al.* 1996). Thus, it should be possible to use marker-assisted selection to facilitate the development of a synthetic line combining favorable QTL alleles from two divergent lines.

The substitution effects estimated for the whole genome (Table 2) are consistent with the observed differences between purebred Large White and the F_2 population reported by Andersson *et al.* (1994), despite the

fact that the underlying model in the analysis, which assumes that all regions of the genome contribute equally to the trait, is questionable.

Some of the two-QTL models gave very high estimates for the effects of the QTLs, but on closer inspection, the two locations are very close together, and the estimates are in opposite directions. Separating two closely linked QTLs is difficult (Haley and Knott 1992; Whittaker *et al.* 1996), and estimates at two positions close together will be highly confounded and hence unreliable. These results should be taken as indicating evidence for complex genetic control, with two or more QTLs, but the large estimates should be discounted.

The model of the analysis assumes that alleles at the QTLs are fixed in the original lines (wild boar and Large White). For traits of economic importance it is likely that alleles with a large favorable effect will have been fixed in the Large White population. If this assumption is violated and the alleles are still segregating in either of the lines at a QTL, the power for its detection will be greatly reduced, and its effect will be underestimated (Alfonso and Haley 1997).

Fitting cofactors unlinked to the chromosome currently being searched should reduce the residual variance by accounting for other genetic effects. This should increase the test statistics, possibly giving more significant chromosomes. The test statistic profile along the chromosome, however, should not change shape other than because of chance associations between the probabilities in the region being searched and those at cofactors. In fact, when analyzing the same data without cofactors we removed the significant effect on chromosome 10 for GROT, although the test statistic was still high, at 6.9. For GR70, the effect on chromosome *4* increased to give a significant test statistic when cofactors were omitted, suggesting that there is a correlation between the genotype probabilities in this region and one or more of the cofactors. The other locations that were significant when searching for a single QTL fitting cofactors remained significant when cofactors were omitted, with some traits giving an increase and some a decrease in the test statistic. Generally, there was a decrease in the number of suggestive locations when cofactors were omitted.

Analyzing the data using a genetic map that ignores sex-different recombination rates also changed the results very little. Allowing for recombination differences simply scales the distance between markers differently for the two sexes (results not shown).

As only single trait analyses have been performed, we cannot test the hypothesis that a single QTL on chromosome *4* had a pleiotropic effect on both fat traits, although this seems likely. To test the hypothesis of one or two QTLs a multitrait analysis would be required. Such analyses have been proposed for crosses between inbred lines using maximum likelihood (Jiang and Zeng 1995), and this is an obvious area for future work with outbred populations. Additionally, we could test the hypothesis that IL and GR70 are also affected by the same QTL. The growth rate traits all show a peak in test statistic in a similar region of chromosome *13*, although significance is not attained for all traits. A multitrait analysis would allow us to consider whether a single QTL was affecting these traits and may also improve the power of the test.

The 0.95 confidence intervals obtained by bootstrapping included a large proportion of the chromosome even for tests of the largest effects we found. An improvement to the method, to give improved estimation of the location of a QTL and possibly to increase the power, might be obtained by fitting cofactors on the chromosome currently being searched. These would account for additional QTLs linked to the one currently being fitted. For the largest effects on fatness traits, however, there is little evidence for other QTLs on chromosome *4*, and hence, the addition of cofactors on the same chromosome is not expected to improve the precision of the estimate and may make it worse.

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