Exploring Alternative Models for Sex-Linked Quantitative Trait Loci in Outbred Populations: Application to an Iberian × Landrace Pig Intercross

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ABSTRACT

We present a very flexible method that allows us to analyze X-linked quantitative trait loci (QTL) in crosses between outbred lines. The dosage compensation phenomenon is modeled explicitly in an identity-by-descent approach. A variety of models can be fitted, ranging from considering alternative fixed alleles within the founder breeds to a model where the only genetic variation is within breeds, as well as mixed models. Different genetic variances within each founder breed can be estimated. We illustrate the method with data from an F2 cross between Iberian × Landrace pigs for intramuscular fat content and meat color component *a*. The Iberian allele exhibited a strong overdominant effect for intramuscular fat in females. There was also limited evidence of one or more regions affecting color component *a*. The analysis suggested that the QTL alleles were fixed in the Iberian founders, whereas there was some evidence of segregation in Landrace for the QTL affecting *a* color component.

The mammalian sex chromosomes, X and Y, are relatively poor in gene content. About 690 genes are known in the human X chromosome and only 77 in the Y chromosome as of April 2002 (HUBBARD et al. 2002). Nevertheless, >200 diseases are currently listed in the OMIM database as being caused by deficiencies in genes located in the human X chromosome (HAMOSH et al. 2002), and ~40 diseases in domestic animals (NICHOLAS et al. 2000) are listed. There also exists increasing evidence of quantitative trait loci (QTL) linked to chromosome X in mice as well as in livestock species. RANCE et al. (1997a,b) reported a QTL affecting growth in the mouse X chromosome. In pigs, a sex-linked QTL affecting backfat has been reported in at least five independent experiments (KNOTT et al. 1998; HARLIZIUS et al. 2000; ROHRER 2000; BIDANDEL et al. 2001a; MALEK et al. 2001b). Further, the porcine X chromosome seems also to be involved in meat quality-related traits (MALEK et al. 2001a) as well as in follicle-stimulating hormone levels and testes size (BIDANDEL et al. 2001b; ROHRER et al. 2001).

The aforementioned porcine QTL results were obtained in crosses between divergent breeds, and the methodology employed was a regression-based approach (KNOTT et al. 1998). This analysis is done within sex and alternative fixed alleles within each founder breed are assumed. Nevertheless, there exists genetic variation within as well as between lines in crosses between divergent outbred populations. Furthermore, the QTL analysis of sex chromosomes poses special statistical challenges because of the dosage compensation phenomenon and because of the limited homology between X and Y chromosomes. The dosage compensation phenomenon consists of the random inactivation of one of the two female X chromosomes in different cell lineages. This results in female mosaicism for loci located in the differential chromosome region. A review of the molecular mechanisms involved in the X inactivation process is in AVNER and HEARD (2001). From a QTL mapping point of view, a consequence is that the female genotype can be modeled as the sum of one-half its individual allelic effects (LYNCH and WALSH 1998, p. 716). This model results in a female genetic variance that is one-half that of males although no differences in mean between sexes are predicted. This model also implicitly assumes that the allelic effect in females is strictly proportional to the percentage of cells on which that allele is active. Departures from this simple model can be allowed for by adding a term for dominance interaction in females.

All in all, it is important to allow for genetic variation within breeds in analyzing outbred lines, but it is also desirable to model dosage compensation explicitly. The objectives of this work are (1) to present a coherent and general theory that allows us to analyze X-linked QTL in general pedigrees, including crosses between outbred lines and (2) to report the QTL analysis of sex chromosomes in an F2 cross between the Iberian × Land-
between divergent outbred breeds. First we recall the main phenotype is always of the dam’s origin (dosage compensation and in computing the problem. Suppose now for a gene located in the differential sex-linked gene in crosses; interaction. Note that the allele contributing to a male’s phenotype is always of the dam’s origin \( g^2 \). The parameters \( \psi^i \) and \( \psi^2 \) should add up to 1; it is biologically possible to think of under- or overexpression of female alleles with respect to male alleles but this effect would be captured by the overall sex effect \( \mu_s \) and \( \mu_e \). The usual model to account for mammalian dosage compensation as explained before (Lynch and Walsh 1998) is retrieved setting \( \psi^i = \psi^2 = \frac{1}{2} \). Nevertheless, (5) is general and allows for values other than \( \psi = \frac{1}{2} \).

From (4) and (5), together with a generalization of (3), it can be seen that the genetic covariances between any two crossed individuals are

\[
\text{Cov}(g_i, g_j) = \Pr(g_i^2 = g^2_i \in A)\sigma_{gA}^2 + \Pr(g_i^2 = g^2_j \in B)\sigma_{gB}^2
\]

(6a)

if \( i \) and \( j \) are males,

\[
\text{Cov}(g_i, g_j) = \sum_{k=1}^{2}\psi^i[k] \Pr(g_i^2 = g^2_i \in A)\sigma_{gA}^2\]

\[\quad + \Pr(g_j^2 = g^2_j \in B)\sigma_{gB}^2\]

(6b)

when \( i \) is a male and \( j \) is a female, and

\[
\text{Cov}(g_i, g_j) = \sum_{k=1}^{2}\psi^i[k] \Pr(g_i^2 = g^2_i \in A)\sigma_{gA}^2
\]

\[\quad + \Pr(g_j^2 = g^2_j \in B)\sigma_{gB}^2\]

(6c)

for both \( i \) and \( j \) being females, where \( \Pr(g_i^2 = g^2_i \in A) \) is the probability of alleles \( g_i^2 \) and \( g^2_i \) being IBD and being of breed origin \( A \), similarly for \( \Pr(g_j^2 = g^2_j \in B) \), and \( \sigma_{gA}^2 \) (\( \sigma_{gB}^2 \)) is the variance of the gene effects in breed \( A \) (\( B \)). We define, similarly, \( p_{ij} = \Pr(g_i^2 \in A) \) when \( i \) is a male and \( p_{ij} = \sum_{k=1}^{2}\psi^k \Pr(g_i^2 \in A) \) otherwise. Equations 6a–6c can then be included into (3) to obtain the genetic covariance matrix \( \mathbf{G} \) of a sex-linked gene in crosses; likewise, we compute \( \mathbf{c} \), the means’ vector. As stated above, some interesting consequences follow from (6). Provided that \( \psi = \frac{1}{2} \), female genetic variance is halved with respect to that in males (Lynch and Walsh 1998), except if the female is completely inbred. Similarly, the expected genetic covariance between female full-sibs is \( 3\sigma_{g}^2/4 \) with limits \( [\sigma_{g}^2/2, \sigma_{g}^2] \), because they always share the male allele, or \( \sigma_{g}^2/4 \) between female and male full-sibs, with limits \([0, \sigma_{g}^2/2]\).

The coefficients \( \Pr(g_j^2 = g^2_j \in A) \), \( \Pr(g_j^2 = g^2_j \in B) \), \( \Pr(g_j^2 \in A) \), and \( \Pr(g_j^2 \in B) \) were obtained via a modification of the algorithm fully described previously (Pérez-
APPLICATION TO AN F$_2$ PIG CROSS

Experimental design and genotyping: Full details of the experiment are given elsewhere (Ovilo et al. 2000b; Pérez-Enciso et al. 2000a). Briefly, the pedigree consisted of three pure Iberian boars, 31 pure Landrace sows, 79 F$_1$ individuals (6 sires and 73 sows), and 577 F$_2$ animals, the so-called IBMAP pedigree. A number of meat-quality-related traits were measured. Here we illustrate the proposed method with the analyses corresponding to two traits highly relevant to meat quality: intramuscular fat percentage in longissimus muscle (IMF) and Minolta meat color component, $a^*$. The two founder breeds were highly divergent for these traits (Serra et al. 1998). Founder, F$_0$, and 338 F$_2$ individuals were typed for five microsatellites. The female map was SW949–50–SW2126–27–SW2456–7–SW2476–25–SW1608 (distances in cM). These distances were obtained with the CRIMAP software (Green et al. 1990). Only the first marker (SW949) was in the pseudoautosomal region.

Statistical analysis strategy: A variety of analysis strategies can be envisaged using the mixed-model methodology described here and in previous works, depending on the aim of the study (Pérez-Enciso and Varona 2000; Ponz et al. 2001). Here we used a two-step analysis, the purpose being to illustrate a possible way to spare computing cost but minimizing the risk of discarding genome regions containing significant QTL. Thus, first we fitted the two most extreme models that can be envisaged in (1), a model where alternative alleles are assumed to be fixed in each founder breed ($\sigma_A^2 = \sigma_B^2 = 0$),

Model c1:  \[ y = XB + ca + u + \epsilon, \]

and a model that presupposes no genetic differences between breeds ($a = 0$ and $\sigma_A^2 = \sigma_B^2$),

Model v1:  \[ y = XB + u + \epsilon. \]

Above, $u$ is the polygenic genetic value, distributed as $N(0, \text{Ge}, \sigma^2_s)$, where $\text{Ge}$ is the usual numerator relationship matrix, $\sigma^2_s$ is the infinitesimal genetic variance, and $u$ is a random genetic effect for segment $s$. Model c1 did not allow for dominance in the female genotype; i.e., model (6) was simplified as $y_i = \mu + 0.5g_A^i + 0.5g_B^i + \epsilon$. In Model v1, it is assumed that $g_A$ and $g_B$ are distributed identically $g_A = g_B = u \sim N(0, \text{Ge}, \sigma^2_s)$, with $\text{Ge}$ computed as shown in (6) setting $\sigma_A^2 = \sigma_B^2 = \sigma^2_s$. The effects included in $\beta$ were sex, batch (five levels), and carcass weight as covariate. These two models were fitted at successive segments of 5 cM, i.e., IBD probabilities were obtained for segments 0–5, 5–10, . . . , 105–110 cM. This preliminary analysis can be carried out to preselect traits showing promissory QTL.

In a second step, a more thorough analysis was done. Model c1 as above plus three additional models were fitted in successive 2-cM segments in the chromosome region where the previous analysis was suggested as more likely to contain the QTL. The remaining models fitted were

Model c2:  \[ y = XB + ca + c'd + u + \epsilon; \]

Model m1:  \[ y = XB + ca + u + u + \epsilon; \]

and

Model m2:  \[ y = XB + ca + u + u + u + \epsilon. \]

Model c2 is a model where dominance in female genotypes is allowed for as shown in (5), $c'$ is a vector containing the probabilities of having received an allele from each of the two founder breeds, and it contains a zero for all male coefficients. Model c2 assumes fixed alleles within breed. The latter two models, m1 and m2, are mixed models where additive genetic differences between and within breeds are allowed for. Model m1 still presupposes that additive variances are equal in both founder breeds ($\sigma_A^2 = \sigma_B^2$), although in this case it is assumed that mean allelic effects between breeds can be different. Finally, Model m2 also allows for the possibility that $\sigma_A^2$ and $\sigma_B^2$ are different and they are estimated separately. In this latter case $\operatorname{Var}(g) = \operatorname{Var}(u_A) + \operatorname{Var}(u_B) = \text{Ge} \sigma_A^2 + \text{Ge} \sigma_B^2$, where $\text{Ge}$ is a matrix consisting of elements $\Pr(g^A_i = g^B_i \in A)$ obtained from (6), and similarly for $\text{Ge}$. A brief description of the models is outlined in Table 1.

The likelihood ratio (LR) between hierarchical models provides insight about the genetic nature of the QTL. Model c2 compared to c1 permits us to evaluate whether dominance occurs. It should be noted, nonetheless, that only two genotypes are possible in F$_2$ females in the particular design of our experiment, “AB” and “BB,” where $B$ stands for the Landrace allele, and thus the dominance parameter should be interpreted broadly to include sex × allele interaction. Similarly, the LR of model m1 over c1, or m2 over c1, is aimed at assessing whether there is evidence of genetic segregation within breeds. The comparison of m1 with v1 can be used to identify differences between mean allelic effects for each breed. Finally, unequal genetic variances within breed are tested comparing m2 with m1. Note that these two latter models are hierarchized, given that $\sigma_A^2$ and $\sigma_B^2$ can be reparameterized as $\sigma^2_A + \sigma^2_B$ and $\sigma^2_A - \sigma^2_B$, respectively. Finally, we found evidence that there might be two chromosome regions affecting color component $a^*$.
TABLE 1
Models used in the QTL analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>Infinitesimal genetic effect</th>
<th>Allelic action between breeds</th>
<th>Variance within breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>c1</td>
<td>Yes</td>
<td>Additive</td>
<td>No: alleles fixed</td>
</tr>
<tr>
<td>c2</td>
<td>Yes</td>
<td>Dominant</td>
<td>No: alleles fixed</td>
</tr>
<tr>
<td>v1</td>
<td>Yes</td>
<td>None</td>
<td>Yes: equal variance in both breeds</td>
</tr>
<tr>
<td>m1</td>
<td>Yes</td>
<td>Additive</td>
<td>Yes: equal variance in both breeds</td>
</tr>
<tr>
<td>m2</td>
<td>Yes</td>
<td>Additive</td>
<td>Yes: different variance in each breed</td>
</tr>
</tbody>
</table>

(see below) and we thus fitted a two-segment model to refine the statistical evidence. Details are presented as necessary in the next section.

It should be stressed that the method uses all available marker and pedigree information, which is particularly relevant for estimating the genetic variances . All individuals in our IBMAP pedigree were directly or indirectly related and the exact relationship coefficients were computed, except that founders were taken as unrelated. The parameters of interest, and the residual variance were obtained by maximizing the log-likelihood

\[
L = -\frac{1}{2} \left[ \text{Constant} + \log |V| + (y - X\beta)' V^{-1}(y - X\beta) \right],
\]

using a simplex algorithm.

RESULTS AND DISCUSSION

Application to pig data: The plots of the likelihood ratios for every chromosome position are shown in Figure 1, using the two extreme models, a completely fixed model (c1) and a random model (v1). It should be recalled that Models c1 and v1 are not hierarchized and thus the LR values for each model are not comparable.

![Figure 1](image-url)  
Figure 1.—Preliminary 5-cM segment scans for the traits analyzed. Results with a fixed model (c1) are represented by a solid line and random model results (v1) by a dashed line. Twice the likelihood ratio of the corresponding model over a model that contained only the fixed effects plus the infinitesimal genetic value is plotted. (a) Intramuscular fat percentage (IMF); (b) meat color components. The markers were located in positions 0, 51, 78, 85, and 110; the differential chromosome region begins in position 51. See Table 1 for model description.
The trend was similar with either v1 or c1 models for IMF but there was also a striking difference in the maxima positions identified for each model in color component $a^b$. The results of the detailed analysis are in Figure 2 and in Table 2. Neither Model m1 nor m2 improved upon Model c1 for IMF, thus strongly suggesting that alternative alleles are fixed in each founder breed. The most relevant result concerning this trait is that the model including a dominance effect in (5), Model c2, was far more likely than the simpler strictly additive Model c1. The value 12.7 corresponds, approximately, to a critical value of 0.1% for a chi square with 2 d.f., respectively. Interestingly, the Iberian allele was distinctly overdominant: the females, being heterozygous for Iberian/Landrace alleles, had a significantly larger amount of IMF than the average males and females homozygous for IMF.

### Table 2

<table>
<thead>
<tr>
<th>Trait</th>
<th>Model</th>
<th>2 LRT (Sex)</th>
<th>Position</th>
<th>$a$</th>
<th>$d$</th>
<th>$\sigma_a^2$</th>
<th>$\sigma_b^2$</th>
<th>$\sigma_{a^b}^2$</th>
<th>$\sigma_e^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMF</td>
<td>c1</td>
<td>$-0.08 \pm 0.03$</td>
<td>110</td>
<td>0.097</td>
<td>0.002</td>
<td>-</td>
<td>-</td>
<td>0.06</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>c2</td>
<td>$0.09 \pm 0.03$</td>
<td>110</td>
<td>0.057</td>
<td>0.002</td>
<td>$0.26 \pm 0.01$</td>
<td>-</td>
<td>0.08</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>m1</td>
<td>$-0.08 \pm 0.03$</td>
<td>110</td>
<td>0.097</td>
<td>0.002</td>
<td>-</td>
<td>-</td>
<td>0.06</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>m2</td>
<td>$-0.07 \pm 0.02$</td>
<td>110</td>
<td>0.101</td>
<td>0.002</td>
<td>-</td>
<td>-</td>
<td>0.06</td>
<td>0.21</td>
</tr>
<tr>
<td>$a^b$</td>
<td>c1</td>
<td>$-0.52 \pm 0.20$</td>
<td>50</td>
<td>0.58</td>
<td>0.06</td>
<td>-</td>
<td>-</td>
<td>0.28</td>
<td>2.39</td>
</tr>
<tr>
<td></td>
<td>c2</td>
<td>$-0.36 \pm 0.21$</td>
<td>52</td>
<td>0.16</td>
<td>0.02</td>
<td>$0.77 \pm 0.15$</td>
<td>-</td>
<td>0.24</td>
<td>2.40</td>
</tr>
<tr>
<td></td>
<td>m1</td>
<td>$-0.52 \pm 0.20$</td>
<td>50</td>
<td>0.58</td>
<td>0.06</td>
<td>-</td>
<td>-</td>
<td>0.27</td>
<td>2.39</td>
</tr>
<tr>
<td></td>
<td>m2</td>
<td>$-0.18 \pm 0.20$</td>
<td>100</td>
<td>0.30</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
<td>0.45</td>
<td>2.44</td>
</tr>
</tbody>
</table>
As far as the color component $a^c$ is concerned, the likelihood maxima are quite distinct depending on the model of choice. The bordering areas between the pseudoautosomal and the differential regions are more significant when the data are analyzed via the fixed Model $m_2$ whereas the mixed Model $m_2$ suggests instead the significant result on backfat in this data set (results not presented).

As far as the color component $a^c$ is concerned, the likelihood maxima are quite distinct depending on the model of choice. The bordering areas between the pseudoautosomal and the differential regions are more significant when the data are analyzed via the fixed Model $m_2$ whereas the mixed Model $m_2$ suggests instead the significant result on backfat in this data set (results not presented).

Application to general pedigrees: This two-step method (a step to compute relationship coefficients and a step to obtain the maximum-likelihood estimates) can be applied to pedigrees of any complexity. We and others (George et al. 2000; Pérez-Enciso and Varona 2000; Pratt et al. 2000) have shown it to be quite a robust and powerful approach in a variety of situations. The only limitation, either for autosomal or sex-linked QTL, lies in computing the IBD coefficients in (6). The Markov chain Monte Carlo (MCMC) method that we have applied is valid for any pedigree complexity as long as markers are available for all individuals. Nevertheless, the method is able to deal with a limited number of missing markers if there are at least parents or offspring genotyped (i.e., the method may get stuck if several generations of individuals have not been typed). We are currently developing a more general MCMC algorithm that allows for a far larger flexibility in terms of missing information and that also block updates a series of marker phases jointly, which is important if markers are tightly linked. We plan also to generalize a Bayesian fine-mapping strategy (M. Pérez-Enciso and D. Milan, unpublished data) to sex-linked QTL.

Estimating dosage compensation parameter ($\psi$): The most straightforward assumption in mammals is to set $\psi^f = \psi^m = \frac{1}{2}$ (Lynch and Walsh 1998). This was the value that we used here but the reader should be cautious in interpreting the results. This model implies that the fact that one allele is inactivated in 50% of the cells is equivalent to halving its effect on the phenotypic scale, which is by no means evident, even allowing for dominance. Another consequence of this model is that the additive genetic variance in the females is one-half that of the males, as discussed above. In principle, one could estimate the dosage compensation parameter $\psi$ from (6) at different values of $\psi$ and maximizing the likelihood. The justification for not taking $\psi$ for granted is that there are some genes, at least a dozen in humans, that are known to escape X-inactivation (Heard et al.)
QTL Analysis of Sex Chromosomes

1997), but also allow a more flexible relationship between the phenotypic scale and the degree of inactivation. Unfortunately, our experimental design prevents us from estimating \( \psi \) when treating the QTL effect as fixed because no reciprocal crosses of Iberian females by Landrace males were carried out and thus the dominance effect and \( \psi \) are confounded. This is not the case in the random IBD approach (e.g., Model v1) because then matrix \( V \) depends nonlinearly on \( \psi \) but is independent of \( d \). We computed \( G \) for two extreme values, \( \psi = 1 \) and \( \psi = 0.5 \), and the trait IMF in a limited number of mixed models and genome positions but we observed that the likelihood was barely affected. Probably as expected, \( \psi \) cannot be estimated accurately from these types of data, but a positive reading shows that the method is robust to departures from the true \( \psi \). However, it will be exciting to ascertain whether \( \psi \) can be estimated in carefully designed experiments using the approach developed here. It is also clear that the theory presented here can be applied to model imprinting with only a few changes, e.g., setting \( \psi^1 = 1 \) and \( \psi^2 = 0 \) for male imprinting.

In conclusion, our work adds to previous methods of QTL analysis for sex chromosomes in three main aspects: first, a model for dosage compensation is used such that both the pseudoautosomal and the nonhomologous region are studied using a coherent statistical modeling; second, a series of increasingly sophisticated genetic models have been applied, showing that model choice is a critical aspect of QTL detection and is specially relevant for sex chromosome analysis; and third, it uses all available pedigree information to compute the IBD probabilities conditional on marker information. We have illustrated the theoretical approach with an analysis of original pig real data, and we have explored a variety of models. From this analysis, we can conclude that the distal part of the differential region contains one (or more) QTL affecting IMF and, perhaps, the \( a^b \) color component. As far as genetic action is concerned, the analyses with these traits, as well as other traits analyzed (our unpublished results), all lead to the conclusion that the genetic variance within the Iberian line used was zero. This agrees with expectations due to the small number of Iberian founders, together with the high relationship coefficient between the Guardyerbas boars, 0.75 on average. It should be recalled that all Guardyerbas individuals are highly related; their inbreeding coefficient is >0.3 on average (Rodriguez et al. 1997). In addition, there is evidence for overdominance (IMF) and that genetic segregation might exist within Landrace founders (\( a^b \) color component).

Finally, and given that the power to detect sex-linked QTL is lower than for autosomes, it would be most interesting to carry out a joint analysis like that of porcine SSC4 (Wallig et al. 2000) using our approach. If more than two founder breeds are involved, the theory can be extended; e.g., (6a) can be replaced by

\[
\text{Cov}(g, g') = \sum_{w=1}^{w_{\text{threshold}}} \Pr(g_s = g'; g_i \in W)\sigma_{W}^2
\]

for the remaining equations.

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