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On the Detection of Imprinted Quantitative Trait Loci in Experimental Crosses of Outbred Species

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ABSTRACT

In this article, the quantitative genetic aspects of imprinted genes and statistical properties of methods to detect imprinted QTL are studied. Different models to detect imprinted QTL and to distinguish between imprinted and Mendelian QTL were compared in a simulation study. Mendelian and imprinted QTL were simulated in an F2 design and analyzed under Mendelian and imprinting models. Mode of expression was evaluated against the H₀ of a Mendelian QTL as well as the H₀ of an imprinted QTL. It was shown that imprinted QTL might remain undetected when analyzing the genome with Mendelian models only. Compared to testing against a Mendelian QTL, using the H₀ of an imprinted QTL gave a higher proportion of correctly identified imprinted QTL, but also gave a higher proportion of false inference of imprinting for Mendelian QTL. When QTL were segregating in the founder lines, spurious detection of imprinting became more prominent under both tests, especially for designs with a small number of F₁ sires.

Parental genomes undergo modifications during gametogenesis. The result is that some genes inherited from one parent are not completely expressed, if at all. This phenomenon of genomic imprinting has been shown to influence several genes and traits in animals (including humans, Morison et al. 2001) as well as plants (Alleman and Doctor 2000) and insects (Lloyd et al. 1999).

Genome scans have revealed a number of genes or quantitative trait loci (QTL) contributing to genetic variation in many species. Genome scans can also be used to search for imprinted QTL provided that the parental origin of alleles can be traced back from the F₂ to the F₁ parents (Knott et al. 1998). This prerequisite excludes F₂ crosses between inbred lines because the F₁ parents are all heterozygous for the same marker alleles. Methods to detect imprinted QTL have been described for outbred crosses by Knott et al. (1998) and successfully applied to genome scans by Jeon et al. (1999) and in a modified form by de Koning et al. (2000). Nezer et al. (1999) used a maximum-likelihood algorithm to detect QTL with specific LOD scores for imprinted QTL against Mendelian QTL. The quantitative genetics of imprinted QTL and the statistical properties of tests to detect imprinted QTL and distinguish between Mendelian and imprinted QTL have not been studied in great detail. In this study, we first outline some of the quantitative genetic aspects of a (partially) imprinted QTL and subsequently we describe the results of a simulation study. The objective of the simulation study was twofold: (1) determine empirically the power for detection of imprinted QTL in outbred F₂ designs under Mendelian or imprinting models and (2) quantify the risk of spurious detection of imprinted QTL under different tests.

THEORY

Quantitative genetics of an imprinted gene: For a Mendelian gene with additive effect a and dominance effect d and with frequency p for the positive allele A and q for the negative allele B, the population mean under random mating is

\[ M = a(p - q) + 2pqd \] (1)

(Falconer and Mackay 1996). The average allele substitution α is

\[ \alpha = a + d(q - p). \] (2)

The single gene variance is

\[ V_c = 2pq[a + d(q - p)]^2 + (2pqd)^2 \] (3)

(Falconer and Mackay 1996).

Now consider a biallelic gene with partial maternal imprinting (preferential expression of paternally inherited allele). This imprinting effect (i) will be apparent in the two groups of heterozygous individuals (AB and BA, first allele coming from sire). The genetic value for AB individuals can be denoted d + i and for BA individuals as d − i. The population mean is identical to (1) but the average allele substitution effect has to

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be specified for the sex through which the allele will be transmitted:
\[
\alpha_z = a + i + d(q - p) \\
\alpha_z' = a - i + d(q - p) = \alpha_z - 2i.
\] (4)

The single gene variance becomes
\[
V_{gi} = [p^2a^2 + pq(d + \delta)^2 + pq(d - \delta)^2 + q^2a^2] \\
- [a(p - q) + 2dpq]^2 \\
= 2pq[a + d(q - p)]^2 + 2pqd^2 + (2pqd)^2.
\] (5)

When there is complete imprinting, \( i \) will be equal to \( a \) and \( d \) will be zero. For a gene with exclusive paternal expression \( \alpha_z' \) becomes zero and the paternal allele substitution effect \( (\alpha_z) \) becomes 2a. For complete imprinting, the single gene variance \( V_{gi} \) (5) reduces to
\[
V_{gi} = 4pqd^2. \quad (6)
\]

**Detection of imprinted QTL in outbred \( F_1 \) designs:**

The analyses of crosses between outbred species are based mainly on the line-cross methodology proposed by Haley et al. (1994), assuming that the founder lines may segregate at the marker loci, but are fixed for alternative alleles at the QTL. Assuming Mendelian expression, an additive effect \( (a) \) and a dominance effect \( (d) \) are estimated using least squares as
\[
y_j = m + ap_j + dp_j + e_j, \quad (7)
\]
where \( y_j \) is the trait score of individual \( j \), \( m \) is the population mean, \( a \) and \( d \) are the estimated additive and dominant effects of a putative QTL at the given location, \( p_j \) is the conditional probability of animal \( j \) to carry two alleles of line 1, \( p_j \) is the conditional probability of animal \( j \) to be heterozygous, and \( e_j \) is the residual error. The calculations of these probabilities and QTL effects are described in detail by Haley et al. (1994).

To test for imprinting, Knott et al. (1998) added the contrast between the two types of heterozygous individuals as an additional component to model (7):
\[
y_j = m + ap_j + dp_j + ip_j + e_j. \quad (8)
\]

Variables are as in (7), with the extension that \( i \) is the estimated imprinting effect and \( p_i \) is the conditional probability that individual \( j \) is heterozygous and inherited the line 1 allele from its sire. de Koning et al. (2000) proposed a reparameterization of (8) by introducing the conditional probabilities that an individual inherited a line 1 allele through its sire (\( p_{pat} \)) or through its dam (\( p_{mat} \)):
\[
p_{pat} = p_i + p \\
p_{mat} = p - p_i.
\] (9)

Model (8) can be rewritten with a specific maternal and paternal QTL component as
\[
y_j = m + ap_{pat} + dp_{pat} + dp_{mat} + e_j, \quad (10)
\]
where \( a_{pat} \) is the paternally inherited QTL effect and \( a_{mat} \) is the maternally inherited QTL effect. Models (8) and (10) are identical in terms of total variance explained by the model. de Koning et al. (2000) proposed to scan the genome with reduced imprinting models with exclusive paternal or maternal expression:
\[
y_j = m + ap_{pat} + e \quad \text{or} \quad y_j = m + a_{mat}p_{mat} + e. \quad (11)
\]

**Simulation study**

**Simulation details:** The outline of the simulation study is comparable to that of Alfonso and Haley (1998), who investigated the effect of mating design and segregation of QTL alleles in the founder lines on the power of detecting Mendelian QTL. \( F_1 \) individuals were generated by random mating of 20 sires from line 1 to 80 different dams (4 dams per sire) from line 2, each having five offspring. For most of the simulations 20 \( F_1 \) sires and 80 \( F_1 \) dams (4 dams per sire) were randomly mated to produce 400 \( F_2 \) offspring (5 offspring per dam). We also simulated an extreme design, where only 2 \( F_1 \) sires were mated to 80 \( F_1 \) dams (40 dams per sire). Marker data were simulated for all animals for a 100-cM chromosome with 11 evenly spaced markers. To have fully informative markers with regard to line of origin as well as optimal distinction of parental origin for the marker alleles in the \( F_2 \), eight alleles were simulated for every marker, with four line-specific alleles segregating at equal frequencies in the two founder lines. An additive, a dominant (\( a = d \)), a paternally expressed, or a maternally expressed biallelic QTL was simulated at 46 cM. Founder lines were either fixed for alternative QTL alleles or segregating at frequencies of 0.80 and 0.20 for the positive allele in lines 1 and 2, respectively. Imprinted QTL were simulated with exclusive uniparental expression and no dominance (i.e., complete imprinting). The phenotype of an individual was further determined by 10 unlinked biallelic QTL, each with an effect of 0.25 and segregating at a frequency of 0.5 in both founder lines, giving an expected additive genetic variance of 0.31 (Alfonso and Haley 1998). An additional environmental component was sampled from a normal distribution with a variance of 0.47 and added to the genetic (QTL) value of an individual to obtain the phenotype (Alfonso and Haley 1998). QTL effects were varied between 0.25 and 1.0. The simulated QTL effects and their expected genetic variances following Equation 3 or 5 for the different genetic models are summarized in Table 1. One thousand replicates were simulated and analyzed for every alternative. For every mating design, an alternative with-
out a QTL was simulated to validate the use of chromosome-wide 5% significance thresholds.

**Analyses:** For every replicate, the coefficients of line origin were estimated following Haley et al. (1994). Subsequently, the best Mendelian QTL was estimated using (7) and the best imprinted QTL were estimated using both the paternal and maternal models of (11), respectively. For each of these three models, a chromosome-wide 5% threshold against the $H_0$ of no QTL was imposed to claim a significant QTL. Thresholds were obtained by permutation tests (Churchill and Doerge 1994) with 10,000 permutations for every 20th replicate and subsequent averaging over the 50 thresholds. For the significant replicates of the reduced imprinting models (11), imprinting was tested in the following manner:

**Alternative a:** $H_0$, Mendelian QTL ($i = 0$ or $a_{pat} = a_{mat}$); $H_a$, imprinted QTL. It was tested whether a full model (Equations 8 and 10) explained significantly more variance than a Mendelian model (7). This test, which is referred to as $F_{Mend}$, was performed at the best QTL position from the reduced model. $F_{Mend}$ is an $F$-test with 1 d.f. in the numerator and $n - 4$ (n is the number of $F_2$ individuals) d.f. in the denominator. This test was first described by Knott et al. (1998), with the exception that in this study $F_{Mend}$ is carried out against a Mendelian QTL at the position of the best imprinted QTL, which is not necessarily the best position of the Mendelian QTL.

**Alternative b:** $H_0$, imprinted QTL (e.g., $H_a$: $a_{mat} = d = 0$ when evaluating a model with exclusive paternal expression); $H_1$, Mendelian QTL. It was tested, at the position of the best imprinted QTL, whether the specific reduced model (11) explained the same amount of variance as the full model (8 and 10) at that position. This test, which is referred to as $F_{red}$, is an $F$-test with 2 d.f. in the numerator and $(n - 4)$ d.f. in the denominator. For both alternatives a and b, a tabulated $F$ value corresponding to $P = 0.05$ was imposed to respectively infer (a) or reject (b) imprinting.

**Alternative c:** Imprinting was inferred when both a and b pointed toward imprinting ($H_a$ was rejected under alternative a but under alternative b $H_a$ was not rejected).

### RESULTS

**Detection of imprinted QTL:** The results of the simulations with imprinted QTL are summarized in Table 2. All replicates showed significant QTL under both the Mendelian and the correct imprinting model for QTL effects of 0.50 or larger (Table 2). However, for a QTL effect of 0.25, only 83% of the replicates showed significant QTL under a Mendelian model while under the imprinting model all replicates showed significant QTL. When founder lines were segregating for the positive QTL allele with frequencies of 0.80 and 0.20, respectively, the Mendelian model had 40% lower power compared to the correct imprinting model to detect imprinted QTL with an effect of 0.25 (Table 2).

Under the extreme design with two $F_1$ sires, there was consistently more power to detect maternally expressed QTL compared to paternally expressed QTL (Table 2). Across all simulations, $F_{Mend}$ had better power to correctly identify imprinted QTL for larger QTL effects, while $F_{red}$ had higher power to distinguish imprinted QTL for smaller QTL effects.

The estimates of QTL effects and position were comparable for the Mendelian and imprinting analyses for all simulated imprinted QTL, although the estimates from the Mendelian analyses had higher standard deviations (Table 2).

**Detection of Mendelian QTL:** The results for simulations without a QTL confirmed that using the 5% chromosome-wide thresholds for the $H_0$ of no QTL was sufficient to keep the type I error <5% (Table 3). When founder lines were fixed for different QTL alleles, all replicates showed significant QTL for effects >0.50 under both the Mendelian and imprinting models. Under the $F_{Mend}$ imprinting is inferred if $H_0$ is rejected; i.e., the column in Table 3 represents the type I error for that specific test. However, under $F_{red}$ imprinting is inferred if $H_0$ is accepted and $H_1$ is rejected, i.e., the type II error. Both $F_{Mend}$ and $F_{red}$ performed generally well in identifying the simulated QTL as being Mendelian for QTL effects of 0.50 and 0.75. The proportion of spuriously identified imprinted QTL was higher for purely additive QTL compared to dominant QTL (Table 3). Applying both thresholds restricted the spurious detection of imprinting to 5% of the replicates or less.

When founder lines were segregating at 0.80 and 0.20, respectively, the power to detect QTL was reduced (Table 3). There was little difference in power between the paternal and maternal imprinting models. The proportion of replicates with spurious imprinting was up to 11% for $F_{Mend}$ and 22% for $F_{red}$ (Table 3). Imposing both tests to infer imprinting kept the proportion of spurious imprinting <6%. Analyses with QTL effects between

### Table 1

**Variance ($\sigma^2_{qtl}$) and proportion of total variance ($h^2_{qtl}$) explained by the simulated QTL in the $F_2$, under different genetic models**

<table>
<thead>
<tr>
<th>QTL effect</th>
<th>Additive QTL</th>
<th>Dominant QTL</th>
<th>Imprinted QTL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\sigma^2_{qtl}$ ($h^2_{qtl}$)</td>
<td>$\sigma^2_{qtl}$ ($h^2_{qtl}$)</td>
<td>$\sigma^2_{qtl}$ ($h^2_{qtl}$)</td>
</tr>
<tr>
<td>1</td>
<td>0.50 0.39</td>
<td>0.75 0.49</td>
<td>1.00 0.56</td>
</tr>
<tr>
<td>0.75</td>
<td>0.28 0.27</td>
<td>0.42 0.35</td>
<td>0.56 0.42</td>
</tr>
<tr>
<td>0.50</td>
<td>0.13 0.14</td>
<td>0.19 0.19</td>
<td>0.25 0.24</td>
</tr>
<tr>
<td>0.25</td>
<td>0.03 0.04</td>
<td>0.05 0.06</td>
<td>0.06 0.07</td>
</tr>
</tbody>
</table>
Detection and characterization of imprinted QTL

<table>
<thead>
<tr>
<th>Simulation details</th>
<th>Power&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Estimated effects&lt;sup&gt;c&lt;/sup&gt;</th>
<th>QTL position&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Imprinting inferred</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mend. ± SD</td>
<td>Imp. ± SD</td>
<td>Mend. ± SD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20/80</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2/80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

QTL were simulated under an imprinting model and analyzed under Mendelian (Mend.) and imprinting (Imp.) models for 400 F<sub>2</sub> individuals with different designs, QTL effects, and allele frequencies.

<sup>a</sup> Proportion of replicates significant at the 5% chromosome-wide level against the H<sub>0</sub> of no QTL.

<sup>b</sup> Estimates and empirical standard deviations, calculated with the replicates that exceed the 5% chromosome-wide significance level.

<sup>c</sup> Proportion of replicates where tests of both full vs. Mendelian (P<sub>Mend</sub>) and reduced vs. full (P<sub>red</sub>) indicate imprinting.

0.50 and 0.25 revealed that detection of spurious imprinting, when applying only P<sub>red</sub>, was as high as 29% of the replicates for a QTL effect of 0.35 (data not shown). For smaller QTL effects, the proportion of spurious imprinting decreased as a result of lower power to detect any QTL effect.

For the extreme design, with only two F<sub>1</sub> sires and segregating founder lines, the power to detect QTL under the Mendelian model was lower than that for the design with 20 F<sub>1</sub> sires, for effects of 0.50 and 0.75 (Table 3). P<sub>Mend</sub> gave levels of spurious imprinting up to 35%, whereas P<sub>red</sub> indicated imprinting for 24% of the replicates (Table 3). Even when both tests were imposed, spurious imprinting was detected for up to 13% of the replicates under the model with maternal expression (Table 3).

**Estimated QTL effects:** When founder lines were segregating at 0.80 and 0.20, respectively, the estimated dominance effects were much smaller than the estimated additive effects (data not shown). The estimates of the additive effect were empirically shown to follow

\[ \hat{a} = \Delta f \times a, \]  

where \( \hat{a} \) is the estimated QTL effect, \( \Delta f \) is the difference in allele frequency between the founder lines, and \( a \) is the simulated QTL effect. The estimated dominance effects were empirically shown to be proportional to the squared difference in allele frequency between the founder lines,

\[ \hat{d} = \Delta f^2 \times d, \]  

where \( \hat{d} \) is the estimated QTL effect and \( d \) is the simulated dominance effect. This shows clearly that the power to detect dominance effects is compromised when founder lines are segregating.

**Further analyses:** Results of additional simulations of additive QTL for a population of 800 F<sub>2</sub> individuals as well as for a mating design with five F<sub>1</sub> sires and 16 F<sub>1</sub> dams are summarized in Table 4.

For the design with 800 F<sub>2</sub> individuals, there was better power to detect smaller QTL effects individually, both under fixation and segregation of founder lines, compared to a design with 400 F<sub>2</sub> individuals. For QTL effects between 0.25 and 0.75 and fixation of founder lines, there was considerably less spurious imprinting compared to the design with 400 F<sub>2</sub> individuals (Tables 3 and 4). However, for a QTL effect of 0.15, up to 32% of the replicates showed spurious imprinting following
TABLE 3
Detection and characterization of Mendelian QTL

<table>
<thead>
<tr>
<th>Simulation details</th>
<th>Power&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Imprinting inferred</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mend. Mat. Pat.</td>
<td>Mat. Pat.</td>
</tr>
<tr>
<td>No. males/ females F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>F&lt;sub&gt;Mend&lt;/sub&gt;</td>
<td>F&lt;sub&gt;red&lt;/sub&gt;</td>
</tr>
<tr>
<td>20/80</td>
<td>A, additive QTL; D, dominant QTL with a = d (frequency of positive QTL allele in F&lt;sub&gt;0&lt;/sub&gt;).</td>
<td></td>
</tr>
<tr>
<td>No QTL</td>
<td>0.05 0.05 0.05</td>
<td>0.03 0.05 0.03</td>
</tr>
<tr>
<td>A 0.75 (1.0/0.0)</td>
<td>1.0 1.0 1.0</td>
<td>0.05 0.00 0.00</td>
</tr>
<tr>
<td>A 0.50 (1.0/0.0)</td>
<td>1.0 1.0 1.0</td>
<td>0.06 0.01 0.00</td>
</tr>
<tr>
<td>A 0.25 (1.0/0.0)</td>
<td>0.85 0.64 0.62</td>
<td>0.07 0.28 0.06</td>
</tr>
<tr>
<td>D 0.75 (1.0/0.0)</td>
<td>1.0 1.0 1.0</td>
<td>0.05 0.00 0.00</td>
</tr>
<tr>
<td>D 0.50 (1.0/0.0)</td>
<td>1.0 0.99 1.0</td>
<td>0.05 0.01 0.00</td>
</tr>
<tr>
<td>D 0.25 (1.0/0.0)</td>
<td>0.96 0.61 0.60</td>
<td>0.06 0.15 0.04</td>
</tr>
<tr>
<td>A 0.75 (0.8/0.2)</td>
<td>0.99 0.94 0.91</td>
<td>0.11 0.11 0.06</td>
</tr>
<tr>
<td>A 0.50 (0.8/0.2)</td>
<td>0.91 0.72 0.71</td>
<td>0.07 0.24 0.06</td>
</tr>
<tr>
<td>A 0.25 (0.8/0.2)</td>
<td>0.37 0.25 0.38</td>
<td>0.05 0.19 0.05</td>
</tr>
<tr>
<td>D 0.75 (0.8/0.2)</td>
<td>0.98 0.88 0.87</td>
<td>0.09 0.09 0.03</td>
</tr>
<tr>
<td>D 0.50 (0.8/0.2)</td>
<td>0.9 0.66 0.67</td>
<td>0.08 0.19 0.06</td>
</tr>
<tr>
<td>D 0.25 (0.8/0.2)</td>
<td>0.39 0.24 0.25</td>
<td>0.07 0.17 0.06</td>
</tr>
<tr>
<td>2/80</td>
<td>A 0.50 (1.0/0.0)</td>
<td>1.0 0.99 0.99</td>
</tr>
<tr>
<td>No QTL</td>
<td>0.05 0.05 0.04</td>
<td>0.03 0.04 0.03</td>
</tr>
<tr>
<td>A 0.75 (0.8/0.2)</td>
<td>0.92 0.94 0.82</td>
<td>0.33 0.17 0.13</td>
</tr>
<tr>
<td>A 0.50 (0.8/0.2)</td>
<td>0.83 0.77 0.71</td>
<td>0.18 0.24 0.13</td>
</tr>
<tr>
<td>A 0.25 (0.8/0.2)</td>
<td>0.40 0.28 0.32</td>
<td>0.07 0.21 0.07</td>
</tr>
<tr>
<td>D 0.75 (0.8/0.2)</td>
<td>0.89 0.82 0.77</td>
<td>0.35 0.15 0.12</td>
</tr>
<tr>
<td>D 0.50 (0.8/0.2)</td>
<td>0.83 0.65 0.67</td>
<td>0.19 0.19 0.12</td>
</tr>
<tr>
<td>D 0.25 (0.8/0.2)</td>
<td>0.47 0.30 0.32</td>
<td>0.09 0.20 0.08</td>
</tr>
</tbody>
</table>

QTL were simulated under a Mendelian model and analyzed under Mendelian (Mend.) and imprinting [maternal/paternal (Mat./Pat.)] models for 400 F<sub>2</sub> animals with different designs, QTL effects, and allele frequencies.

<sup>a</sup>A, additive QTL; D, dominant QTL with a = d (frequency of positive QTL allele in F<sub>0</sub>).

<sup>b</sup>Proportion of replicates significant at the 5% chromosome-wide level against the H<sub>0</sub> of no QTL.

<sup>c</sup>Proportion of replicates significant at the 5% chromosome-wide level and for which a full model explains significantly more variance (P < 0.05) than a Mendelian QTL at the position of the best QTL under the respective model.

<sup>d</sup>Proportion of replicates significant at the 5% chromosome-wide level and for which a full model does not explain significantly more variance (P < 0.05) than a QTL with a single parental effect at the position of the best imprinted QTL.

<sup>e</sup>Proportion of replicates where both tests of full vs. Mendelian (F<sub>Mend</sub>) and reduced vs. full (F<sub>red</sub>) indicate imprinting.

F<sub>red</sub> under the model with maternal expression (Table 4). Under segregation of founder lines, there was considerable spurious imprinting for a QTL effect of 0.25, indicating that also for larger F<sub>2</sub> populations spurious detection of imprinting can be a problem. For the design with five F<sub>1</sub> sires, the proportion of spuriously detected imprinted QTL was lower compared to the design with two F<sub>1</sub> sires, but still considerably higher compared to the design with 20 F<sub>1</sub> sires (Table 4).

**DISCUSSION**

**Detection of imprinted QTL:** For imprinted and Mendelian QTL with the same QTL effect there was a higher power to detect imprinted QTL as compared to Mendelian QTL (Tables 2 and 3). This is not surprising given that the variance explained by an imprinted QTL is larger than that of a Mendelian QTL (Table 1). It could, however, be argued that, on average, the effects of imprinted genes are expected to be smaller than those for Mendelian genes, because for an imprinted gene, only one allele is expressed.

For smaller QTL effects and when founder lines are segregating for the same QTL alleles, it was demonstrated that the reduced imprinting models had higher power to detect imprinted QTL than standard Mendelian models (Table 2). Consequently, it is not surprising that performing QTL analyses with reduced imprinting models reveals imprinted QTL that remained undetected under a Mendelian model as found by de Koning et al. (2001). However, for practical situations this would imply testing three different models. We did not impose an additional Bonferroni correction for these tests. Since the three models are correlated, it would be over-
Additional analyses for additive Mendelian QTL

TABLE 4

<table>
<thead>
<tr>
<th>Simulation details</th>
<th>Power$^a$</th>
<th>Imprinting inferred</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. males/ females F1</td>
<td>(frequency)</td>
<td></td>
</tr>
<tr>
<td>20/160</td>
<td>0.50 (1.0/0.0)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>0.25 (1.0/0.0)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>0.15 (1.0/0.0)</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>0.50 (0.8/0.2)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>0.25 (0.8/0.2)</td>
<td>0.66</td>
</tr>
<tr>
<td>5/80</td>
<td>0.75 (0.8/0.2)</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>0.50 (0.8/0.2)</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>0.25 (0.8/0.2)</td>
<td>0.41</td>
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QTL were simulated under a Mendelian model and analyzed under Mendelian (Mend.) and imprinting [maternal/paternal (Mat./Pat.)] models for 800 (20/160) and 400 (5/80) F1 animals with different QTL effects and allele frequencies.

- **Additive QTL (frequency of positive QTL allele in F0).**
- **Proportion of replicates significant at the 5% chromosome-wide level against the H0 of no QTL.**
- **Proportion of replicates significant at the 5% chromosome-wide level, for which a full model explains significantly more variance ($P<0.05$) than a Mendelian QTL at the position of the best QTL under the respective model.**
- **Proportion of replicates significant at the 5% chromosome-wide level, for which a full model does not explain significantly more variance ($P<0.05$) than a QTL with a single parental effect at the position of the best imprinted QTL.**
- **Proportion of replicates where both tests of full vs. Mendelian ($F_{Mend}$) and reduced vs. full ($F_{red}$) indicate imprinting.**

For the design with an extremely low number of F1 sires and the QTL allele segregating in the founder lines, there is considerably less power to detect paternally expressed QTL compared to maternally expressed QTL. This is because with only two F1 sires, there is an increased risk that one or both F1 sires are homozygous for their QTL alleles or have a different phase between line origin and QTL effect. The number of F1 parents is an important factor to take into account when founder lines are not fixed for their QTL.

**Detection of Mendelian QTL:** Alfonso and Haley (1998) performed an extensive simulation study on the detection of Mendelian QTL in F2 designs. The estimated powers in Table 3 correspond generally well with those reported by Alfonso and Haley (1998). The estimated QTL effects reported by Alfonso and Haley (1998) follow approximately the expectations denoted in (11) and (12).

**Detection of spurious imprinted QTL:** The simulations of the Mendelian QTL showed that spurious detection of imprinting is a serious problem for smaller QTL effects, when founder lines are segregating, and for mating designs with a low number of F1 sires (Table 3). Obviously, design is not an issue when founder lines are completely fixed for their QTL alleles, but for experimental crosses in livestock this is not a very likely scenario. For most scenarios, the test of Knott et al. (1998) (alternative a) is more conservative, while $F_{red}$ similar to de Koning et al. (2000), is more liberal and can give higher rates of spurious imprinting. However, $F_{Mend}$ gave the highest rates of spurious imprinting for larger QTL effects in designs with two F1 sires. This shows clearly that both tests have their flaws, although $F_{Mend}$ performs better on average for the scenarios considered in this study. For smaller QTL effects, the $H_0$ of $F_{red}$ appears to be too robust against a purely additive Mendelian QTL. Imposing both tests to infer imprinting kept the level of spurious imprinting <6% for the design with 20 F1 sires. This could be an ad hoc solution to control the spurious detection of imprinting, but better alternatives should be investigated (e.g., Lee et al. 2001). Imposing both tests to the simulations with imprinted QTL resulted in a proportion of correctly identified imprinted QTL that was close or equal to the smaller of the two proportions identified by the individual tests (Table 2). This indicates that the power to detect imprinted QTL would not be greatly affected by imposing both tests.

The designs with only two or five F1 sires resulted in high proportions of spuriously imprinted QTL, even when both tests (alternative c) were imposed (Tables 3 and 4). Although the detection of imprinted QTL was reasonable compared to the design with 20 F1 sires, the results for the Mendelian QTL clearly indicate that
these designs are unsuitable for the detection of imprinted QTL when founder lines are segregating. It is not straightforward to provide a yardstick for the minimum number of F₁ parents of each sex that should be used to circumvent the risks of detection of spurious imprinting. However, the results here indicate that with only two or five F₁ sires, not only the power to detect QTL is affected, but also the risk of detection of spurious imprinting is increased. Although this study focused on the effect of mating design in the F₁, the results are also applicable for the mating design of the F₀. In practice, it might seem cost effective to restrict the number of F₀ and F₁ parents to the minimum number that is necessary to obtain the desired number of F₂ individuals. Our study, however, indicates that this is not the best strategy when one of the objectives of a study is to test for imprinting effects.

In our simulation study we considered a relatively simple pedigree structure, which facilitated the use of regression methods and enabled a large-scale simulation study. Due to the approximations involved in regressions methods, one may want to explore data from real QTL experiments in more detail with more advanced methods that can handle complex pedigree structures (e.g., HOESCHELE *et al.* 1997; SILLANPÄÄ and ARJAS 1999). The results obtained in our study will also apply to the more advanced methods of analysis.

In the simulation study, we used fully informative markers and complete imprinting to prevent effects other than those under evaluation from causing any differences in results between models. Both assumptions are unlikely to be met in the analysis of experimental crosses between outbred lines. Uninformative markers lead to an increase in the effective average marker spacing, resulting in a generally lower power. In cases where line origin can be derived, but parental origin cannot, this might compromise correct characterization of the QTL. When a QTL displays partial imprinting, the power to distinguish between imprinted and Mendelian QTL will be a function of the difference between the paternally and maternally inherited alleles. Furthermore, \( F_{Mend} \) and \( F_{red} \) are expected to give conflicting answers, because \( F_{red} \) assumes complete imprinting while \( F_{Mend} \) does not.

**The effect of the null hypothesis:** The \( H₀ \) of \( F_{Mend} \) is that of a Mendelian QTL whereas the \( H₀ \) of \( F_{red} \) is that of an imprinted QTL. The results of the simulation study indicate a relationship between the power of the design to detect QTL and the power to discriminate between Mendelian and imprinted QTL. When the power to detect QTL reduces, both \( F_{Mend} \) and \( F_{red} \) favor the acceptance of their respective \( H₀ \), leading to different conclusions, depending on the \( H₀ \) of the test. MALECOT (1999) demonstrated that the choice of the null hypothesis is never objective, but a result of experiences and ideas of a researcher or a group of researchers. When testing for imprinting, the \( H₀ \) of the test clearly affects the conclusion. The null hypothesis that genes, and hence QTL, show Mendelian expression may be the most reasonable \( H₀ \) when one is the first researcher to study a new genetic phenomenon. It could, however, be argued that this is partly because, if not all, genetical research of the 20th century was based on Mendelian principles. The Mendelian principles provide no explanation for reciprocal differences that are observed in crossbreeding and that may be attributable to genomic imprinting. Genomic imprinting has been studied only during the last decade and on the basis of recent findings (e.g., DE KONING *et al.* 2000) it should no longer be considered a rare phenomenon.

Furthermore, it could be argued whether the inference of the mode of expression of a QTL should be tested with the same stringent criteria as the existence of that QTL. In other words, is spurious inference of imprinting for a Mendelian QTL (or vice versa) just as serious as spurious detection of a QTL? The discrepancies between the tests as a result of different \( H₀ \)'s make it unlikely that the issue of testing the mode of expression of a QTL can be solved in a classical testing framework. An appealing alternative is to adopt a Bayesian approach (MALECOT 1999), where QTL are assigned prior probabilities to show Mendelian or uniparental expression on the basis of knowledge about the proportion of imprinted genes among identified genes.

As science progresses and new observations accumulate, the effect of the subjective parts (i.e., the assumptions and \( H₀ \)) is expected to diminish (MALECOT 1999). With regard to the detection of imprinted QTL, the new information should come not only from independent replicates of QTL studies, but especially from expression studies that can provide proof for imprinting at the molecular level.

**Implications:** The simulation study showed that, compared to detecting Mendelian QTL, the successful detection and inference on mode of inheritance of a QTL put more demands on the design of the experiment as well as the interpretation of the results. Because the possibility to test for imprinting effects in QTL experiments was only recently described by KNOTT *et al.* (1998), most QTL mapping experiments to date are not optimized to detect imprinted QTL. It is recommended that researchers include tests for imprinting whenever possible, but critically reflect upon their results with regard to the design of the experiment and the probability of segregation of QTL alleles within founder lines. This holds not only for F₂ crosses between outbred species but also for making strategic backcrosses to test for imprinting effects following CLAPCOTT *et al.* (2000). This strategy relies on finding a QTL in a certain backcross and not in the reciprocal backcross. This is no problem when using completely inbred mice strains, but when it is not completely sure that all F₁ individuals will be heterozygous for the QTL, the design must be
optimized to minimize the spurious detection of imprinted QTL.

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