SIGNIFICANCE THRESHOLDS IN GENOME SCANS – WHICH THRESHOLD TO USE TO MAXIMISE THE ACCURACY OF SUBSEQUENT MARKER ASSISTED SELECTION

B. Hayes¹, P.J. Bowman² and M.E. Goddard¹²

¹Institute of Land and Food Resources, University of Melbourne, Parkville, VIC 3052
²Victorian Institute of Animal Science, Department Natural Resources and Environment, Attwood, VIC 3049

SUMMARY
Our aim was to investigate the effect of the level of stringency of quantitative trait loci (QTL) detection in a genome scan on the accuracy of subsequent marker assisted selection (MAS). A genome scan and subsequent MAS were performed in a population of simulated pigs. The accuracy of MAS was greatest when QTL with \( P < 0.10 \) (on an individual gene basis) in the genome scan were used in MAS. More stringent thresholds reduced the proportion of genetic variance accounted for by detected QTL. Lowering the significance threshold from \( P < 0.10 \) to \( P < 0.25 \) resulted in a large number of false positive results, with the consequence that the variance accounted for by marked QTL was overestimated, and accuracy of MAS reduced. While criteria \( P < 0.10 \) maximised the accuracy of MAS, it may not be the most profitable threshold. Using more stringent thresholds greatly reduced the number of markers to be typed without greatly reducing accuracy of MAS.

Keywords: QTL, MAS, significance level.

INTRODUCTION
Many economically important traits in livestock are quantitative in nature and controlled by QTL and environmental factors. If genetic markers could be found which were linked to these QTL, this information could be used for MAS. One method of finding genetic markers linked to QTL is a genome scan, where markers are placed at intervals along the entire genome. An important question is how many QTL to take from the genome scan and use in the MAS program. The number of QTL detected in a genome scan is controlled by the number of segregating genes which affect the trait, the power of the experiment, and the level of stringency of the statistical test used to set the size of the significance threshold, above which a QTL is ‘detected’. If the number of segregating genes affecting a quantitative trait is relatively large, the stringency of the significance threshold is likely to be the most important factor controlling the number of QTL detected. The less stringent the threshold, the greater the number of QTL detected, and the higher the proportion of genetic variance exploited by MAS using these detected QTL. The ‘cost’ of using less stringent thresholds is the higher number of false positives detected. False positives reduce the accuracy of MAS, as the variance explained by marked QTL is overestimated. Our aim is to investigate the effect of the level of significance of QTL detection in a genome scan on the accuracy of subsequent MAS. Genome scans and MAS were carried out in a simulated population of pigs.

MATERIALS AND METHODS
Simulation of the evolution of a quantitative trait. The genetic variance of a population of pigs is increased by mutations which have additive effects. Each pig has a genome of 18 chromosomes. On
each chromosome there were 9 loci, 4 of which were QTL and 5 of which were markers. Every second locus was a QTL. Initially, all QTL and marker loci had a 0 allele. The effect of the 0 allele at a QTL on the quantitative trait was sampled from a gamma distribution. The effect of the 0 allele at a QTL on fitness was 0. To create an offspring, for each parent in a mating pair, a gamete was formed from its chromosome pairs by sampling the number of crossovers for each chromosome pair from a Poisson distribution, with mean of 1 (so distance between adjacent markers was 25cM). Crossover points were randomly positioned along chromosome pairs. The haploid gametes were mutated, with the mutation rate for markers of $1.4 \times 10^{-3}$/ locus–gamete–generation and for QTL of $2.5 \times 10^{-6}$/ locus–gamete–generation. If a locus was mutated, a new allele was added. If the locus was a QTL, the effect of the QTL on fitness and the quantitative trait was sampled from the bivariate gamma distribution, scale parameter 0.23 and shape parameter 1, with the correlation between the absolute values of fitness and quantitative effects of 0.4. Fitness effects were always negative, while the effect on the quantitative trait had an equal chance of being negative or positive. The true breeding value of an offspring was the sum over the 72 QTL loci of $\frac{1}{2}$ the value of the allele inherited from the sire + $\frac{1}{2}$ the value of the allele inherited from the dam. Fitness was calculated the same way. Offspring survived to breed the next generation if $\exp(-\text{fitness})/\max\text{fitness} > \text{dev}$, where max fitness is the fitness of the offspring with the highest fitness in the population and dev is uniform random deviate between 0 and 1. One thousand generations of natural selection on fitness were followed by ten generations of artificial selection on the quantitative trait. During natural selection, the effective population size was 1000, during artificial selection this was reduced to 100. Artificial selection was on phenotype. The phenotype of an animal was its true breeding value plus a random deviate $\sim N(0, \sigma^2)$. The value of $\sigma^2$ was either 2 or 6. Heritability of the trait was 0.25 or 0.1.

QTL mapping. In generation 1010, the top sire was selected and 200 progeny were bred from this sire for a genome scan. For each of the four marker brackets on the 18 chromosomes, the sire’s progeny were separated into those that inherited the sire’s paternal bracket and those that inherited the sire’s maternal bracket. Recombinants were ignored. If the difference between the average of the phenotypes of the two groups exceeded the significance threshold, a QTL was detected. The location of a detected QTL was considered to be at the centre of the bracket with the largest estimated effect on the quantitative trait. Five significance thresholds of decreasing stringency were set by permutation testing (Churchill and Doerge 1994). The probabilities of a false positive for the five thresholds when testing an individual marker bracket were 0.0008 (corresponding to less than 5% false positives for the whole experiment), 0.014 (less than 5% false positives for each chromosome tested), 0.05, 0.10 and 0.25. The false discovery rate (FDR) is the expected proportion of detected QTL that are in fact false positives (Weller 1998). FDR was calculated for each significance level as $m P_{\max}/n$, where $P_{\max}$ is the largest $P$ value of QTL which exceed the significance threshold, $n$ is the number of QTL which exceed the significance threshold and $m$ is the number of chromosomes (18).

Accuracy of MAS. For each marker bracket with a significant effect, the effect of the four possible sire haplotypes (paternal, maternal, paternal-maternal recombinant and maternal-paternal recombinant) were estimated from the 200 progeny by solving the equation, $[Z'Z + \mathbf{I}] \mathbf{u} = [Z'\mathbf{y}]$, where $Z$ is a design matrix allocating records to haplotypes, $\mathbf{I}$ is used to approximate $\mathbf{G}$, the matrix of
haplotype co(variances), \( \lambda = \frac{\sigma^2_e}{\sigma^2_g} \) where \( \sigma^2_e \) is the error variance and \( \sigma^2_g \) is the within sire variance for the QTL, \( \mathbf{u} \) is a vector of the estimates of the haplotype effects, and \( \mathbf{y} \) is a vector of phenotypic records. A further 500 progeny were bred from the sire used in the genome scan. These progeny were genotyped for the marker haplotypes surrounding the detected QTL. The breeding values of a progeny were estimated as the sum over the marked QTL of the estimates for the marker haplotypes which the progeny carried. The correlation of estimated breeding values with true breeding values for these 500 progeny was the accuracy of MAS. Three approaches were used to estimate \( \sigma^2_g \) for each QTL. DIRECT estimated \( \sigma^2_g \) as the square of the QTL effect estimated from the genome scan. BAYES estimated \( \sigma^2_g \) as an average of the estimate from DIRECT and the mean of a prior distribution of QTL effects. The prior distribution was the distribution of QTL effects estimated from meta-analysis of the results of QTL mapping experiments (Hayes and Goddard 2001). TRUE was the true value of \( \sigma^2_g \). Results are the average of fifty replicates.

RESULTS AND DISCUSSION
The distribution of QTL effects in the simulated population in generation 1010 were very similar to the distribution of QTL effects estimated from the results of mapping experiments (Hayes and Goddard 2001), eg. the largest 5% of QTL explained 55% of genetic variance in the population.

![Figure 1. Number of QTL detected in the genome scan and accuracy of MAS.](image)

The accuracy of estimated breeding values in 500 progeny against the number of genes detected in the genome scan, for the different methods of estimating the variance, is shown in Figure 1. The
The accuracy of MAS rises rapidly as the significance threshold for QTL detection is lowered from $P<0.0008$ to $P<0.014$. This is a result of the greater number of QTL detected explaining a greater proportion of the within sire variance. When DIRECT was used to estimate the variance from each QTL, the greatest accuracy of MAS was obtained when $P<0.10$. Lowering the stringency from $P<0.10$ to $P<0.25$ greatly increased the FDR, Table 1. This indicates that the majority of additional QTL detected by lowering the significance from $P<0.10$ to $P<0.25$ are false positives. These additional QTL therefore explain very little of the additive variance (also indicated by the plateau of accuracy using TRUE to estimate the variance), and in fact reduce the accuracy of MAS as the proportion of variance accounted for by detected QTL is overestimated.

**Table 1. False discovery rates for different significance thresholds ($h^2=0.25$)**

<table>
<thead>
<tr>
<th>$P$ value</th>
<th>QTL detected</th>
<th>False discovery rate (FDR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0008</td>
<td>0.35</td>
<td>0.04</td>
</tr>
<tr>
<td>0.014</td>
<td>1.3</td>
<td>0.20</td>
</tr>
<tr>
<td>0.05</td>
<td>3.2</td>
<td>0.24</td>
</tr>
<tr>
<td>0.1</td>
<td>4.9</td>
<td>0.34</td>
</tr>
<tr>
<td>0.25</td>
<td>9</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Using the BAYES estimate of the variance when estimating haplotype effects increased the accuracy of MAS slightly at all significance levels. One effect of using BAYES to estimate the variance was that the accuracy of MAS did not decline with $P<0.25$. The Bayesian prior allows for the possibility of detected QTL with a small variance actually being false positives (Hayes and Goddard 2001).

When the heritability was lowered from 0.25 to 0.1, fewer QTL were detected in the genome scan for the same significance thresholds. The accuracy of MAS was greatly reduced. However, the pattern of change in accuracy with significance threshold was the same as when $h^2=0.25$.

**IMPLICATIONS**

The accuracy of MAS was greatest when $P<0.10$ was the criteria for taking QTL from a genome scan, if the QTL variance was estimated by DIRECT. While the $P<0.10$ threshold gave the greatest accuracy, it may not be the most profitable criteria for taking QTL from a genome scan to MAS. For example, using criteria $P<0.05$ would reduce the number of markers to be typed from 10 to 6 while only reducing accuracy by 8%.

**ACKNOWLEDGEMENTS**

The author’s are grateful for funding from a grant from the Pig Research and Development Corporation, grant number US43, “Marker assisted selection for profitable pigs”.

**REFERENCES**