A Rank-Based Nonparametric Method for Mapping Quantitative Trait Loci in Outbred Half-Sib Pedigrees: Application to Milk Production in a Granddaughter Design

Wouter Coppieters,* Alexandre Kvasz,* Frédéric Farnir,* Juan-Jose Arranz,* Bernard Grisart,* Margaret Mackinnon and Michel Georges*

*Department of Genetics, Faculty of Veterinary Medicine, University of Liège, 4000 Liège, Belgium and 1Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh, EH9 3J T, United Kingdom

Manuscript received November 6, 1997
Accepted for publication March 30, 1998

ABSTRACT

We describe the development of a multipoint nonparametric quantitative trait loci mapping method based on the Wilcoxon rank-sum test applicable to outbred half-sib pedigrees. The method has been evaluated on a simulated dataset and its efficiency compared with interval mapping by using regression. It was shown that the rank-based approach is slightly inferior to regression when the residual variance is homoscedastic normal; however, in three out of four other scenarios envisaged, i.e., residual variance heteroscedastic normal, homoscedastic skewed, and homoscedastic positively kurtosed, the latter outperforms the former one. Both methods were applied to a real data set analyzing the effect of bovine chromosome 6 on milk yield and composition by using a 125-cM map comprising 15 microsatellites and a granddaughter design counting 1158 Holstein-Friesian sires.

RECENT developments in DNA marker technology, such as the discovery of microsatellites (Weber and May 1989), random amplified polymorphic DNA (RAPDs; Williams et al. 1990), and amplified fragment length polymorphism (AFLPs; Vos et al. 1995) as abundant sources of well-dispersed genetic markers, have boosted the construction of marker maps across a broad taxonomic range. Not only are such maps now available for human and model organisms such as mouse and rat but for a number of agriculturally important animal and plant species as well.

These maps increasingly are applied to locate genes underlying inheritable phenotypes of interest. Several of the most relevant phenotypes are continuously distributed quantitative traits involving multiple polygenes or quantitative trait loci (QTL), as well as nongenetic effects. Experimental back- and intercrosses are often the preferred design to map QTL. However, in a number of agriculturally important species (notably cattle and pine trees), reproductive cycles and breeding designs have led to the generation of extensive half-sib pedigrees that are readily available for QTL mapping. A well-documented example of this is the so-called granddaughter design to map genes underlying milk production in commercial cattle populations (Weller et al. 1990). This design takes advantage of the numerous paternal half-brother pedigrees that exist in dairy cattle populations, generated as part of the applied progeny-test breeding design.

A number of mapping methods have been applied to such half-sib designs, including single-marker regression (e.g., Cowan et al. 1990), interval mapping using regression (e.g., Knott et al. 1996), and maximum likelihood methods (e.g., Georges et al. 1995). All these methods share a common assumption, namely the residual normal distribution and homoscedasticity of the analyzed phenotypes or transformations thereof. These approaches therefore are not suitable for phenotypes that are known not to satisfy this normality assumption. Moreover, deviations from normality for traits that generally are assumed to be quasi-normally distributed are likely to affect the power and robustness of these conventional approaches.

Recently, Kruglyak and Lander (1995a) described a nonparametric QTL interval mapping approach based on the Wilcoxon rank-sum test applicable in experimental crosses. This method provided a robust alternative to conventional approaches, applicable to normally distributed traits with minimal loss of power and extending the scope of QTL mapping to a variety of traits not normally distributed, such as counts generated by a Poisson process, truncated data, probabilities, and qualitative data.

In this article, we describe the adaptation of this method to half-sib pedigrees in outbred populations and apply it to milk production in a granddaughter design. A computer program to implement this approach has been developed and is available from the authors upon request.
MATERIALS AND METHODS

A QTL interval mapping procedure based on the Wilcoxon rank-sum test—general principles: To measure the evidence in favor of a QTL at a given map position, Kruglyak and Lander (1995a) define the following statistic (illustrated for an \((A \times B) \times A\) backcross),

\[
Z_n(s) = Y_n(s) / \sqrt{\text{Var}(Y_n(s))},
\]

where

\[
Y_n(s) = \sum_{i=1}^{n} [n + 1 - 2 \cdot \text{rank}(i)] - [P(g_i(s)|g_l,g_r) - P(g_i(s)|g_l,g_s)].
\]

In which \(n\) is the number of progeny; \(\text{rank}(i)\) is the rank by phenotype of progeny \(i\); \(P(g_i(s)|g_l,g_r)\) is the probability that progeny \(i\) has genotype AA at map position \(s\) given its genotype at the left \(g_l\) and right \(g_r\) flanking markers; \(P(g_i(s)|g_l,g_s)\) is the probability that progeny \(i\) has genotype AB at map position \(s\) given its genotype at the left \(g_l\) and right \(g_s\) flanking markers; and

\[\text{Var}(Y_n(s)) = \left( \frac{n^3 - n}{12} \right) \left( 1 - 2P(g_i(s)|g_l,g_s) \right)^2.\]

This occurs in the case of missing genotypes or when the offspring has the same marker genotype as the sire, and the QTL at chromosome position \((L,g_l,g_r)\) is the likelihood of the pedigree data for linkage phase. The ratio of all information needed to compute \(P(g_i(s)|g_l,g_s)\) in a given interval when dealing with experimental crosses, information from more distant markers is considered in the outbred half-sib situation, when closer markers are not fully informative. This occurs in the case of missing genotypes or when the offspring has the same marker genotype as the sire, and the dam is either not genotyped or has the same heterozygous genotype as well. In the former case, part of the information can be recovered by considering marker allele frequencies in the population.

Calculation of \(P(g_i(s)|g_l,g_s)\) requires knowledge of the sire's marker linkage phase. In the absence of grandparental marker information, the most likely linkage phase is first estimated from the marker genotypes of the offspring. This is accomplished by calculating the likelihood of the pedigree data under the 2\(^2\) possible phases (assuming \(x\) informative markers) as follows (Georges et al. 1995):

\[
L_i = \prod_{j=1}^{x} \left[ \sum_{k=1}^{m} P(k|j) \sum_{n=1}^{N} \text{AFM}_n \right],
\]

where \(L_i\) is the likelihood of the pedigree data for linkage phase \(i\); \(\prod_{j=1}^{x}\) is the product over all \(x\) informative markers; \(\sum_{k=1}^{m}\) is the sum over all possible sire's genotypes \(k\); \(P(k|j)\) is the probability of gamete \(k\) given Mendelian laws, phase \(i\), and recombination rates between adjacent markers; \(\theta_i\) to \(\theta_k\); \(\prod_{j=1}^{x}\) is the product over all \(m\) markers within the synteny group; \(\text{AFM}_n\) is the population frequency of the obligate maternal marker allele of marker \(m\), given the paternal gamete \(k\).

All marker phases are a priori considered to be equally likely; i.e., linkage equilibrium is assumed to be reached between all markers. The marker phase maximizing the likelihood of the pedigree data is considered the true one and is selected for further analysis.

As pointed out by Kruglyak and Lander (1995a),

\[
Y_n(s) = \left( \frac{n^3 - n}{12} \right) \left( 1 - 2P(g_i(s)|g_l,g_s) \right)^2.
\]

While \(1 - 2P(g_i(s)|g_l,g_s)\) or the expected value of \(1 - 2P(g_i(s)|g_l,g_s)^2\) over all possible genotypes is computed easily for experimental crosses, its calculation is more cumbersome in outbred designs as it will depend on marker allele frequencies and genotype of the founder sire. The value of \(1 - 2P(g_i(s)|g_l,g_s)^2\) is therefore calculated for each half-sib pedigree by simulating all possible offspring and calculating a frequency weighted mean of \(1 - 2P(g_i(s)|g_l,g_s)^2\).

Across family analysis: In practice, the available pedigree material is composed most often not of one half-sib pedigree but of a series of such half-sibs, such as in the grand-daughter design (Weller et al. 1995). In outbred populations, however, the different sibships cannot be assumed to segregate for the same QTL or even QTL alleles; i.e., one cannot assume locus and allelic homogeneity across families.

Rather than analyze the pedigrees separately, however, and reduce power by multiple testing, the individual \(Z_n(s)\) scores were squared and summed over all \(k\) families yielding a chi-squared statistic with \(k\) degrees of freedom:

\[
\sum_{i=1}^{k} [Z_n(s)]^2 = \chi^2(k).
\]

Interval mapping by regression: The rank-sum-based approach (hereafter referred to as method RS) was compared with interval mapping by using regression (hereafter referred to as method MR for multipoint regression; Knott et al. 1996). For each half-sib family, \(j\), phenotypes were regressed on \(P(g_i(s)|g_l,g_s)\), calculated as described above, yielding least-squares estimators of the \(y\) intercept, \(\beta_0\), and the slope, \(\beta_1\), the latter being an estimator of the QTL allele substitution effect in the corresponding family, \(j\). The ratio

\[
\Sigma_{i=1}^{k} \text{SSR}/k \left( \Sigma_{i=1}^{k} \text{SSE}/(n - 2k) \right)
\]

was used to measure the evidence in favor of a segregating QTL at chromosome position \(s\). \(n\) is the total number of observations, \(k\) is the number of half-sib families, and \(\text{SSR}\) (sum of squares regression) measures the variability in the phenotype attributed to the segregation of a hypothetical QTL at position \(s\) in family \(j\), and \(\text{SSE}\) (sum of squares error) measures the residual or unexplained phenotypic variability in family \(j\). This ratio can be shown to be distributed as an \(F\) statistic under the null hypothesis of no QTL at the corresponding chromosome position.

Significance thresholds: For both the RS and MR methods, chromosome-wise significance thresholds were determined from the distribution of the test statistic over 10,000 permutations (simulated data set) or 100,000 permutations (real data set) of the phenotypes (or ranks) as suggested by Churchill and Doerge (1995). Phenotypes were permuted within family. For each permutation, the highest value of the test statistic over the entire chromosome was retained to yield “chromosome-wise” distributions of the test statistic under the null hypothesis. For the real data set, a Bonferroni correction was applied to the chromosome-wise significance level, considering that chromosome 6 represents 1/29 of the bovine au-
and variance 1; i.e., plus a value drawn from a normal distribution with mean 0 model 1 as reference.

**QQ** which the individual belongs (\(\text{mean} = 0\)) and (5) homoscedastic, (Table 1). Within each model, we compared the relative merits of skewness, positive kurtosis or more peaked around the center the corresponding power (\(1^\text{st}\) was determined such that

\[
\sigma_i^2 = \frac{\text{variance}^2}{\text{degrees of freedom}}, \text{ with variance } \sigma_i^2 = 2n \text{ and mean } n. \text{ Individual phenotypic values were generated as the mean of the genotypic class to which the individual belongs (\(Q\text{Q} = a - n\), \(Qq = 0 - n\), or \(qq = -a - n\)) plus a value drawn from a chi-squared distribution with \(n\) degrees of freedom, obtained by summing \(n\) squared values drawn from a standard normal.

**Homoscedastic, positive kurtosis:** Excess of kurtosis was simulated by assuming that the residual effect was distributed as a Student’s \(t\)-distribution with \(n\) degrees of freedom, with variance

\[
\sigma_i^2 = \frac{n}{(n-2)} \text{ and } \text{mean } 0. \text{ Individual phenotypic values were generated as the mean of the genotypic class to which the individual belongs (\(Q\text{Q} = a, Qq = 0, \text{or } qq = -a\)) plus a value drawn from a \(t\)-distribution with \(n\) degrees of freedom, i.e.,

\[
Z_i = \left(\frac{Y_i - \mu_i}{\sigma_i}\right) \frac{\sqrt{n}}{\sqrt{n-2}}.
\]

**Homoscedastic, negative kurtosis:** Negative kurtosis was simulated by assuming that the residual effect was distributed as a hemispherical distribution with \(a\) and variance

\[
\sigma_i^2 = 2pq^2, \text{ where } p \text{ and } q : 0.25 \text{ (Q) and } q = 0.75 \text{ (q), respectively. Founder-sire therefore had an a priori probability } 2pq = 0.375 \text{ to be heterozygous Qq for the QTL. Following Falconer's notation (Fal coner and Mackay 1996) and assuming additively acting alleles, the average phenotype values of the QQ, Qq, and qq genotypic classes were set at \(a\), \(d = 0\), and \(-a\), respectively. Assuming Hardy-Weinberg equilibrium, this yields an average effect of an allele substitution, \(a\) = \(a\), and a variance attributable to the segregation of the QTL:

\[
\sigma_i^2 = 2pq^2.
\]

The value of \(a\) was determined such that

\[
h^2 = \frac{\sigma_i^2}{\sigma^2} = \frac{\sigma_i^2}{\sigma_i^2 + \alpha_k^2} = \frac{2pq^2}{2pq^2 + \alpha_k^2}
\]

reached a constant percentage, or

\[
a = \sqrt{\frac{h^2}{\alpha_k^2} \frac{\sigma^2}{2pq(1-h^2)}}.
\]

\(h^2\) was set at 9.4% for all simulations, corresponding to an \(a\) value of 0.55. Five scenarios were considered to model the residual variance, \(\sigma_i^2:\) (1) homoscedastic, normal residual variance, (2) heteroscedastic, normal residual variance, (3) homoscedastic, skewed, or asymmetric residual variance, (4) heteroscedastic, positive kurtosis or more peaked around the center than the density of the normal curve, and (5) homoscedastic, negative kurtosis or flatter around the center than the density of the normal curve.

**Homoscedastic normal residual variance** Individual phenotypic values were generated as the mean of the genotypic class to which the individual belongs (\(Q\text{Q} = a, Qq = 0, \text{or } qq = -a\)) plus a value drawn from a normal distribution with mean \(0\) and variance 1; i.e., \(\sigma_i^2\) was set at one.

**Heteroscedastic normal residual variance** Individual phenotypic values were generated as the mean of the genotypic class to which the individual belongs (\(Q\text{Q} = a, Qq = 0, \text{or } qq = -a\)) plus a value drawn from normal distributions with mean 0 and variances of \(\sigma_{\text{QQ}}^2 = 1, \sigma_{\text{Qq}}^2 = \tau, \text{ and } \sigma_{\text{QQ}}^2 = s\), such that

\[
\sigma_i^2 = pq^2 + 2\tau q + q^2.
\]

Homoscedastic, skewed residual variance: Skewness was simulated by assuming a residual effect distributed as a chi-squared distribution with \(n\) degrees of freedom, with variance

\[
\sigma_i^2 = 2n \text{ and mean } n. \text{ Individual phenotypic values were generated as the mean of the genotypic class to which the individual belongs (\(Q\text{Q} = a - n, \text{or } Qq = 0 - n\), or \(qq = -a - n\)) plus a value drawn from a chi-squared distribution with \(n\) degrees of freedom, obtained by summing \(n\) squared values drawn from a standard normal.

Homoscedastic, negative kurtosis: Negative kurtosis was simulated by assuming that the residual effect was distributed as a Student’s \(t\)-distribution with \(n\) degrees of freedom, with variance

\[
\sigma_i^2 = \frac{n}{(n-2)} \text{ and } \text{mean } 0. \text{ Individual phenotypic values were generated as the mean of the genotypic class to which the individual belongs (\(Q\text{Q} = a, Qq = 0, \text{or } qq = -a\)) plus a value drawn from a \(t\)-distribution with \(n\) degrees of freedom, i.e.,

\[
Z_i = \left(\frac{Y_i - \mu_i}{\sigma_i}\right) \frac{\sqrt{n}}{\sqrt{n-2}}.
\]

where \(s\) is a random number between 0 and 1.

Figure 1 illustrates the expected phenotypic distributions of offspring from heterozygous founder-sires, Qq, for the five examined models. Offspring are sorted in two genotypic classes depending on the QTL allele transmitted by the sire (Q or q). Each class therefore comprises two subpopulations QQ (25%) and Qq (75%) for the Q class and Qq (25%) and qq (75%) for the q class.

At least 200 datasets (ranging from 200 to 866) were simulated under each of the five models of residual variation and analyzed with the RS and MR methods. Permutations were used to estimate the significance levels reached for each of these analyses (Churchill and Doerge 1995). For each replicate, 10,000 permutations were performed and analyzed with the RS and MR methods to yield a dataset-specific, chromosome-wise distribution of the RS and MR statistics under the null-hypothesis, allowing us to measure the P value of the unpermuted data under the null hypothesis of no QTL. Average P values over replicates were calculated for each of the five models. For each model, the proportion of datasets yielding a P value less than 0.05 (\(= a\)) was used to measure the corresponding power (1 - \(= a\)) of the RS and MR methods (Table 1). Within each model, we compared the relative merits of the RS vs. MR methods by applying the Wilcoxon matched pairs test on all resulting pairs of P values (Hollander and Wolfe 1973). Within each method, the effect of the model on the power to detect the QTL was evaluated by using the Mann-Whitney U test (Hollander and Wolfe 1973), using model 1 as reference.

**Real dataset:** The real data set was a Holstein-Friesian granddaughter design comprising 1158 sons distributed over 29 paternal half-sib families, partially described in Spelman et al. (1996). The number of sons per family ranged from 11 to 153.

All animals were genotyped for a battery of 15 previously
Figure 1.—Phenotypic distributions of offspring from heterozygous Qq sires, sorted according to the QTL allele inherited from the sire (Q or q), assuming (a) a heteroscedastic normal residual variance ($r^2_s$); (b) a homoscedastic, skewed residual variance ($x^2_s$); (c) a homoscedastic, positively kurtosed residual variance ($t_s$); and (d) a homoscedastic, negatively kurtosed residual variance (hemicircular residual variance). The phenotypic distributions of the $q^?$ offspring are shown (thick black lines) and compared with the corresponding distribution assuming a homoscedastic normal residual variance (thick gray lines). The corresponding distributions of the $Q^?$ offspring are shown as thin lines. Each class therefore comprises two subpopulations: $QQ$ (25%) and $Qq$ (75%) for the $Q^?$ class and $Qq$ (25%) and $qq$ (75%) for the $q^?$ class. The differences between the means of the $Q^?$ and $q^?$ populations, corresponding to the effect of the Q to q allele substitution, equal 0.5 $\sigma_P$.

Simulated data: Using the approach described in materials and methods, we simulated GDDs segregating for a QTL explaining a fixed 9.4% of the phenotypic variance (corresponding to $a = 0.5\sigma_P$) but with five alternative residual components: homoscedastic normal, heteroscedastic normal, homoscedastic skewed, homoscedastic positive kurtosis, and homoscedastic nega-

\[
\begin{align*}
\text{RESULTS}
\end{align*}
\]
TABLE 1
Comparison of the power and precision of the RS and MR QTL mapping methods under five models of residual variance

<table>
<thead>
<tr>
<th>Model</th>
<th>Model 1 (r = 2, s = 4)</th>
<th>Model 3 (χ²)</th>
<th>Model 4 (t₂)</th>
<th>Model 5 (0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS</td>
<td>MR</td>
<td>RS</td>
<td>MR</td>
<td>RS</td>
</tr>
<tr>
<td>Replicates</td>
<td>866</td>
<td>200</td>
<td>500</td>
<td>200</td>
</tr>
<tr>
<td>Average P value</td>
<td>0.24</td>
<td>0.23</td>
<td>0.20</td>
<td>0.23</td>
</tr>
<tr>
<td>1 − β (α = 0.05)</td>
<td>0.34</td>
<td>0.37</td>
<td>0.37</td>
<td>0.34</td>
</tr>
<tr>
<td>SD position (cM)</td>
<td>24.1</td>
<td>22.6</td>
<td>21.7</td>
<td>22.8</td>
</tr>
<tr>
<td>RS vs. MR</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Model 1 vs. Model 3 (RS)</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Model 1 vs. Model 3 (MR)</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
</tbody>
</table>

*Model 1 is homoscedastic normal; 2, heteroscedastic normal; 3, homoscedastic skewed; 4, homoscedastic positively kurtosed; and 5, homoscedastic negatively kurtosed.

† Standard deviation of the most likely QTL position for all simulations with chromosome-wise P values less than 0.05.

‡ Comparison of P value distribution between methods, within models (Wilcoxon Matched Pairs Test).

§ Comparison of P value distribution between models, within methods (RS or MR, Mann-Whitney U test).

RS, rank-sum-based approach; MR, multipoint regression.

The relative merit of the RS and MR methods was evaluated by using the Wilcoxon matched pairs test as described in materials and methods. As expected, multiple regression is superior to the rank-sum approach under the basic model of homoscedastic normal residual variance (P = 0.000014). The loss of power when using the rank-based method is estimated at 8% at α-value of 0.05. The MR method proved also significantly superior to the RS method in the negative kurtosis model (model 5; P = 0.000001); the loss of power with the RS method was estimated at 14% at α-value of 0.05. For the three remaining scenarios, however, the RS approach outperformed MR, the gain in power ranging from 8 to 20% at α-value of 0.05 (Table 1).

The effect of the model on the power to detect the QTL was evaluated by using the Mann-Whitney U test (see materials and methods), by using model 1 as reference. Comparisons were performed separately for the RS and MR approach. Interestingly, MR appears to be quite insensitive to the nonnormality of the residual variation, as the distribution of P values under the alternative models is never significantly different from those obtained under the basic model. This is likely due to the

TABLE 2
Primer pairs used for amplification of BTA6 microsatellite markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>UP-Primer (5′-3′)</th>
<th>DN-Primer (5′-3′)</th>
<th>θ from previous marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>ILSTS090</td>
<td>TAGTACCATACCCAGGTAGG</td>
<td>GCCAAACACACAAGTGTCG</td>
<td>0</td>
</tr>
<tr>
<td>URB16</td>
<td>AGCTTTCTCTACGGGTTCG</td>
<td>CCGACAGGACTGAGCTACGA</td>
<td>0.219</td>
</tr>
<tr>
<td>BM1329</td>
<td>TGTCTTCAAGTCCAACAGT</td>
<td>AACACCCGAGGCTCATCC</td>
<td>0.018</td>
</tr>
<tr>
<td>BM143</td>
<td>ACCTGGGAGGCCTCATATC</td>
<td>CTGCGGGACAGTTCTCTTATG</td>
<td>0.142</td>
</tr>
<tr>
<td>TGLA37</td>
<td>CATTCCAATCCCCATCTCTGAG</td>
<td>TTTAATTTCTATGAGACCTGTA</td>
<td>0.061</td>
</tr>
<tr>
<td>ILSTS097</td>
<td>AGAATTCCCGCTCAAGGC</td>
<td>GTCTTTTTGATCTTTAGGT</td>
<td>0.105</td>
</tr>
<tr>
<td>BM4528</td>
<td>AGAAATCCATACGATCATGACA</td>
<td>AGGAACAGGTATGAGATTTTGA</td>
<td>0.011</td>
</tr>
<tr>
<td>BM4621</td>
<td>CAAAATTCATCTTCTCTGGCT</td>
<td>TTTGATACGAGGGGCTCATC</td>
<td>0.033</td>
</tr>
<tr>
<td>RM028</td>
<td>CTAGCTGTAGCGGTCTGAGAAG</td>
<td>ATCTGTCAGCCTGGCTGAGAG</td>
<td>0.023</td>
</tr>
<tr>
<td>BM415</td>
<td>GTCTTCCCTTCTGCTTGGT</td>
<td>GAGCTACTACACACAGGAG</td>
<td>0.022</td>
</tr>
<tr>
<td>KCA5</td>
<td>CAGTTTAAACATCTGTGTAAGAATA</td>
<td>AGAGCTTGGACATCAATGACAA</td>
<td>0.079</td>
</tr>
<tr>
<td>ILSTS087</td>
<td>AGAGAGCATGAGACTGACGG</td>
<td>CTGGCTTTGCTTGGAGACG</td>
<td>0.030</td>
</tr>
<tr>
<td>BM4311</td>
<td>TCCACTTCTCCCTCTCTCCTC</td>
<td>GAAATATAGTGTGCTGAGG</td>
<td>0.011</td>
</tr>
<tr>
<td>BP7</td>
<td>GACCTTTCTCCTGCCCTCTCTCT</td>
<td>TTTTATCGCTGCTTGGGC</td>
<td>0.018</td>
</tr>
<tr>
<td>BM2320</td>
<td>GTTCCAGACAGACAGTAGAG</td>
<td>CCCATGCTCTCCGTACTTC</td>
<td>0.250</td>
</tr>
</tbody>
</table>
fact that significance levels are deduced from phenotype permutations rather than from the theoretical distribution of the test statistic. Using RS, on the contrary, significant increases in detection power are observed for models 2, 3, and 4 (respectively 9, 12, and 23% at $\alpha$-value of 0.05; Table 1), while the distribution of $P$ values does not differ significantly between models 1 and 5.

Estimates of the precision in the estimation of QTL positions were also compared. Table 1 shows the standard deviation of the most likely QTL position for all simulations yielding a signal exceeding the 5% chromosome-wise significant threshold. Comparing the difference between real and estimated position by using the Mann-Whitney U test, we found no evidence for a significant effect either of the statistical method or of the model for the underlying residual variance. In essence, precision was as poor in all circumstances, standard deviations of the estimated position being 20 to 25 cM. While the actual position of the QTL was at 62 cM counting from the first marker, the estimates ranged from 0 to 118 cM, i.e., the entire chromosome length. A total of 95% of the estimates were within 43 cM (=1.9 $\sigma$) from the actual position.

**Real data:** Table 2 and Figure 2 show the most likely marker map as obtained from our genotypes. The map covers 125 cM (Kosambi) with average interval of 9 cM. The most likely order was in agreement with Kappes et al. (1996). The same figure also compares information content when (1) exploiting marker allele frequency estimates to extract information from noninformative marker genotypes, and (2) when ignoring this information, i.e., when considering all microsatellite alleles to be equally frequent in the population. It can be seen that more than 80% of the maximal information is extracted for the central part of the chromosome; however, the information content drops at both extremities of the chromosome. Moreover, the figure shows that information content is improved only marginally by considering marker allele frequencies. This is especially true in the central, denser part of the marker map.

Figures 3a and 3b summarize the location score profiles obtained for the five different milk production traits by using both RS and MR approaches. Generally speaking, both methods clearly yield very similar curves for all traits along the entire chromosome length. For protein percentage, the location scores maximize at the same chromosome position (48 cM) using both approaches. The associated experiment-wise significance levels are $P = 0.03$ for RS and $P = 0.01$ for MR, therefore slightly superior for the latter.

**Figure 2:** Information content (in percentage of the theoretical maximum) map along the length of bovine chromosome 6 when using (+ + +) or ignoring (− − −) marker allele frequencies. Marker names and corresponding map position in centimorgans are shown along the x-axis.
These results are in agreement with the report of a QTL affecting milk production on the centromeric half of chromosome 6, first identified by Georges et al. (1995) and later confirmed in independent studies in Holstein-Friesian by Spelman et al. (1996) and Kühn et al. (1996), in Finnish Ayrshire by Vilkki et al. (1997), and in Norwegian Red by Gomez-Raya et al. (1996). A detailed analysis of this chromosome region in the corresponding pedigree material is given in Spelman et al. (1996).

**DISCUSSION**

In this article, we have adapted a nonparametric QTL mapping method based on sum of ranks that was described previously for experimental crosses (Kruglyak et al. 1995).
and Lander 1995a) to outbred half-sib pedigrees. This is particularly relevant for mapping QTL in specific livestock and plant species where such pedigrees routinely are generated within the context of specific breeding designs. It extends the scope of QTL mapping in these pedigrees to a variety of not normally distributed traits, including counts generated by a Poisson process, truncated data, and probabilities and qualitative data (Kruglyak and Lander 1995a).

We confirm that this approach (the RS method) can be applied conveniently to normally distributed traits with minimal loss of power when compared to parametric methods. In the simulated example, we noticed a loss of power of 8% at α-value of 5% when compared to the MR method. When simulating nonnormal or heteroscedastic residuals, however, the RS method outperformed the MR method in three out of four scenarios (models 2–4: heteroscedastic normal, homoscedastic skewed, and homoscedastic positively kurtosed). Interestingly, this was shown not to be due to a loss of power of the MR approach, which proved to be relatively robust in the scenarios that we simulated, but rather to a gain of power when applying the RS method. Our interpretation of this finding is that in the three scenarios where RS proved superior to MS, the phenotypic distribution is characterized by “outliers” when compared to the normal distribution (see Figure 1). These outliers contribute excessively to the residual variation, while the bulk of the observations actually are more centered around the mean (and therefore less variable) when compared to the normal distribution. When using ranks rather than the actual phenotypes, the contribution of the outliers to the residual variation is tempered, therefore increasing the ratio QTL variance/residual variance and concomittantly increasing the power to detect the QTL.

A disadvantage of the rank-based methods is the fact that these do not provide convenient estimates of QTL effects. These methods therefore are suitable for the detection of QTLs but have to be complemented with alternative methods, such as least-squares or maximum likelihood techniques when quantifying the QTL effects.

Recently, a number of QTL mapping methods that account for multiple linked or unlinked QTL have been proposed. These include two QTL models (e.g., Haley and Knott 1992), composite interval mapping (Zeng 1993), and multiple QTL mapping (Jansen 1993). Rank-based approaches have been described to test three or more classes, including the Kruskal-Wallis test and the Jonckheere-Terpstra test, which would allow a two-QTL model to fit. Alternatively, it might be interesting to explore the possibility to use regression techniques directly on ranks, which, if applicable, would allow inclusion of additional markers as cofactors in the model.

Assuming paternal half-sib pedigrees, the proposed method allows for missing genotypes in the “dams.” In such cases, estimates of marker allele frequencies can be used to improve inference about the identity of the transmitted paternal chromosome. However, it is shown that when performing multipoint analyses with dense marker maps, this contributes only a marginal improvement of the information content. The benefit of including marker allele frequency is therefore doubtful. Indeed, errors in the estimation of the marker allele frequencies may even cause an increase in type I errors or a loss of power if accounting for inaccuracies in the frequency estimates (Charlier et al. 1996).

As expected, the precision in the estimation of the QTL position using both proposed parametric and nonparametric approaches is mediocre. This illustrates the need to develop alternative strategies for fine-mapping QTL in outbred populations.

We acknowledge the financial support of Holland Genetics, Livestock Improvement Corporation, the Vlaamse Rundvee Vereniging, and the Ministère des Classes Moyennes et de l’Agriculture, Belgium. Continuous support from Nanke den Daas, Brian Wickham, Denis Volckaert, and Pascal Leroy is greatly appreciated. We thank Johan van Arendonk, Richard Spelman, Henk Bovenhuis, Marco Bink, Dave Johnson, and Dorian Garrick for fruitful discussions.

LITERATURE CITED


Kühn, C., R. Weikard, T. Goldammer, S. Grupe, I. Olsaker et al.
Half-Sib Nonparametric QTL Mapping


Communicating editor: Z-B. Zeng