ON THE POWER OF QTL DETECTION IN OUTBRED POPULATIONS

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SUMMARY
Simulation was used to study the power of detection of a fairly young (mutation occurred 50 generations prior to pedigree recording) medium-sized (substitution effect of ≈ 0.5 phenotypic STD) bi-allelic (favourable allele frequency of 0.2) QTL, in a small (10 cM) or very small (1 cM) chromosomal region using haplotypes of increasing size from 10 evenly spaced multi-allelic markers (5 alleles of approximately equal frequencies for each marker) in a general pedigree. The power of detection was low with the chosen simulation parameters, but the shape of the power curves indicated that fine mapping was feasible. For both chromosome lengths, the statistical power was found to decrease with increasing haplotype sizes.

Keywords: quantitative trait loci, haplotypes

INTRODUCTION
Literature reports that haplotype-based tests are usually less powerful than marker-based tests (Long and Langley 1999; Bader 2001), especially when many infrequent haplotypes are present, although Akey et al. (2001) conclude that the use of haplotypes can significantly improve the power and robustness of mapping. This paper studies the power of the QTL detection using single markers and haplotypes of increasing size, when trying to detect a QTL of moderate size in a typical outbred population, on a small or very small region on a chromosome.

MATERIALS AND METHODS
Simulation. Simulation was used to generate data which matched closely an experimental population, composed of 3973 Coopworth sheep born between 1978 and 2002, 412 of which are founder animals. No selection pressure was applied to the QTL simulated. The methodology was as follows: 10 founder animals, 5 males and 5 females, were generated. The 20 founder haplotypes created comprised 10 markers evenly spanning an interval of either 10 cM or 1 cM, plus one in the middle (5 cM or 0.5 cM). For 50 generations, the animals of the previous generation were mated randomly to create 500 new animals. Afterwards, the number of alleles of the middle marker was reduced to 2 (hence simulating our bi-allelic QTL) with approximate allelic frequencies of 0.2 and 0.8, while the number of alleles for the other 10 markers was reduced to 5, with approximate equal allelic frequencies. In the process, care was taken to insure that the collapsing wouldn’t break marker associations. Next, 412 animals of the last generation simulated were used as the founders of our experimental population, and the gene flow was simulated through the real pedigree. A QTL was then simulated by adding to the measure of a trait recorded in the real population a value equal to 0.5 x the raw standard deviation of this trait in the population (0.853) per QTL allele simulated for each animal with a non-missing trait value (2400 animals). This entire process was repeated 200 times for both chromosome lengths (10 cM or 1 cM). An extra 100 times per chromosome length, with no QTL effect, was generated to create the null distributions.
Model. Model fitting was divided into 2 steps. Firstly, the following mixed model was applied to each of the simulated populations: \( y = Xb + Zu + e \), where \( y \) is a vector of trait measurements, \( b \) is a vector of fixed effects (including contemporary group, birth date and sex), \( u \) is a vector of random genetic additive effect, \( e \) is a vector of random residuals, \( X \) and \( Z \) are incidence matrices. Secondly, the haplotype probabilities of 300 of the youngest animals of the experimental pedigree with non-missing trait values were estimated using the EM-based HAPLO.EM S-PLUS/R routine (http://mayoresearch.mayo.edu/mayo/research/staff/schaid_dj.cfm). Each 10-marker haplotype was then broken up successively into smaller haplotypes consisting of blocks of adjacent marker haplotypes of increasing size, i.e., 10 single markers, 9 haplotypes of 2 markers, 8 haplotypes of 3 markers, ..., up to the full haplotype of 10 markers (for instance, the haplotype [ABCDEFGHJ] would be broken into [A] [B] [C] [D] [E] [F] [G] [H] [I] [J], [AB] [BC] [CD] [DE] [EF] [FG] [GH] [HI] [IJ], ..., up to [ABCDEFGHIJ]). The residuals obtained from the first step for these 300 animals were modelled for each size of haplotype \( \{10,3,1\} \) and rank of the haplotype as: \( r = H_{p,s}\beta + e \), where \( r \) is a vector of residuals from the mixed model, \( H_{p,s} \) is a \((300 \times n)\) matrix whose columns represent each of the \( n \) different haplotypes of length \( s \) and of rank \( p \) observed in the population of 300 animals, and whose element \( \{i,j\} \) is the probability that a haplotype chosen at random on animal \( i \) is equal to the haplotype \( j \), \( \beta \) is a vector of fixed regression coefficients, \( e \) is a vector of residuals. To investigate the influence of our haplotype estimation method, the haplotypes used to compute \( H_{p,s} \) were either the estimated haplotypes, or directly the simulated haplotypes. Single markers do not need to be estimated and so \( H_{p,1} \) is identical in both cases. The test statistic for the overall marker effect was taken to be \(-\log_e (P-value)\) of the F-statistic of this multivariate regression.

Power analysis. The \( \alpha = 0.05 \) thresholds were computed using the 95-percentile of the P-values obtained on 100 repetitions of the simulations without a QTL effect simulated, using all results available. As observations were not independent (each simulated population provided from 1 to 10 values, depending on the size of the haplotypes considered), 2000 bootstrap samples of the 100 populations were taken, and the thresholds were adjusted for possible bias. The power for each haplotype group (size and rank of the haplotype), and the 2 chromosome lengths was then calculated.

RESULTS AND DISCUSSION
Significance thresholds. For the null distributions, no significant difference was observed between the log P-values obtained when the haplotypes were estimated, and when the simulated haplotypes were used (\( P=0.17 \) and 0.49 for the chromosomes of 10 cM and 1 cM respectively). As a consequence of the reasonably high P-values and to increase the number of observations, results from both analyses (between 200 and 1800 values depending on haplotype size) were used to compute the thresholds. Thresholds were found statistically different from each other according to haplotype size and chromosome length, and were then computed separately for each category. The threshold values ranged from 1.197 (for a chromosome length of 10cM and a single marker) to 1.612 (for a chromosome length of 10 cM and a haplotype of 9 markers).
QTL. Advanced Statistical Approaches

Power analysis. No significant differences were found between the power values computed using the simulated haplotypes or the estimated ones as the former were generally equal or sometimes only slightly higher than the later. As a result, only the power values obtained with the estimated haplotypes were reported.

Table 1. Power results for each different haplotype ranks (R) for each haplotype size (S), using the estimated haplotypes for the two genome lengths (G, cM)

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Table 1 shows the power obtained for each rank and haplotype size for both chromosome lengths. The power levels are low with the simulation parameters chosen, but the curving shape of the plots (Figure 1) indicates that fine mapping is possible. A general descending trend of power with increasing haplotype size can clearly be identified, and is consistent with previous findings in literature. Differences can be tested using binomial distributions with 200 observations.

There are at least two possible causes of a reduction of the power occurring when haplotypes increase in size. With the 10 cM chromosome, the probability of recombination between two adjacent markers after 50 generations is \( \approx 0.42 \). A higher average number of haplotypes of each size are associated with the QTL, and haplotypes of smaller size closer to the QTL explain the phenotype better. This behaviour is observed in the results, where the power of detection of larger haplotypes is better than the power of detection of smaller ones far away from the QTL, but worse than those close to the QTL. With the 1cM chromosome, the probability of recombination between two adjacent markers is
much lower (≈ 0.05), meaning a smaller average number of haplotypes of any size, but with an amount of linkage disequilibrium high enough so that larger haplotypes do not explain much more than smaller ones at least in this case where 5 alleles per marker are present (in the case of bi-allelic markers, this conclusion could be erroneous). Here, the loss of power can be explained by a higher number of degrees of freedom for the regression. This hypothesis is reinforced by the fact that, in this case, the power is always lower for larger haplotypes, no matter the distance from the QTL. To illustrate this point, Figure 1 shows the power results for the 3 first haplotype sizes, respectively for a chromosome length of 10 cM and 1cM. In any case, more simulations (with varying number of generations from the mutation, varying number of alleles per marker, and varying distance between markers) are needed to test this hypothesis.

Figure 1. Power of detection of the QTL for chromosome lengths of 10 cM and 1cM with single markers and haplotypes of size 2 and 3 markers.

Conclusion. This small simulation shows that the method employed in this study can be used for the study of fine mapping approaches in livestock. In accordance with previous studies in literature, the power of detection of the QTL decreased when larger haplotypes were used. A hypothesis has been advanced to explain this behaviour, but more simulations are needed to confirm it. Also, other methods of analysis preventing an increase of degrees of freedom when larger haplotypes are used or methods of haplotype grouping (Tzeng 2004) should be investigated.

REFERENCES