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MAPPING QTL WITH COVARIATES

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Abstract

Quantitative trait loci (QTL) analysis is an effective tool for locating regions of the genome associated with a trait. Quantitative trait data are complex, and when statistically testing for the location of a QTL, the distribution of the test statistic is typically unknown. Historically, asymptotic thresholds have been difficult to derive for QTL analysis. Permutation testing has successfully provided significance thresholds for QTL analysis, but the need for exchangeability among the observations limits these empirically derived thresholds to simple linear models and does not permit the inclusion of important covariates in the model. We address the limitation of permutation theory for supplying empirically derived QTL significance threshold using a novel bootstrap threshold that is appropriate for multiple regression based interval mapping models. Simulation studies demonstrate that the proposed bootstrap thresholds improve detection and estimation of additive effects in QTL studies.

Keywords: Quantitative trait loci analysis, mixture models, permutation testing, bootstrap, multiple regression

1. Introduction

A primary goal of genetic research is locating genes and understanding their function. Quantitative trait locus (QTL) mapping relies on statistical models to identify significant associations between phenotypes or quantitative traits and genetic markers. One popular statistical model for QTL analysis is interval mapping (IM), which utilizes a mixture model based on normal distributions while incrementally searching the genome for QTL (Lander and Botstein 1989; 1994). However, it is known that mixture distributions do not follow the regularity conditions required for the likelihood ratio test statistic to achieve an asymptotic χ^2 null distribution (McLachlan and Peel 2001). Furthermore, there is a multiple testing issue in QTL mapping, and when the Bonferroni multiple testing correction is applied it can be too conservative when there is a large number of tests. Alternative multiple testing corrections often require independence among tests, which is not the case for QTL mapping.

Theoretical approximations for the significance threshold are technically appropriate for QTL studies; however, formulations for theoretical thresholds are complex, specific to the experimental design, and difficult to derive. For example, Lander and Botstein (1989; 1994) supplied an approximate theoretical threshold for quantitative trait data specifically from a backcross (BC) experimental design by assuming an infinite number of markers and a large number of offspring. Rabai, Goffinet et al. (1994) established a more general formula to calculate thresholds for interval mapping models using Davies approximations, but the derived formula is mathematically challenging and dependent on the parameters of the experiment.

Empirical significance thresholds based on permutation theory (Fisher 1935), as proposed by Churchill and Doerge (1994), were simpler to implement than the theoretical thresholds for a

variety of experimental designs. Permutation thresholds are established using randomized phenotypes, based on sampling without replacement, to represent the experimental data as having no phenotypic association with the genotypes. As such, an analysis of the randomized data provides test statistics from the null distribution. Repeated randomization and analysis of the data provide the distribution of the test statistics under the null hypothesis. The largest test statistic from each of the many randomizations provides an extreme value distribution from which an empirical 100(1-α)% level significance level can be determined. Experimentwise thresholds provide detection of the presence of a QTL while controlling the overall type I error rate to be α or less.

Because interval mapping as presented by Lander and Botstein (1989; 1994) is based on a single QTL model, mapping polygenetic quantitative traits presents a challenge using this method. To address this issue, Doerge and Churchill (1996) proposed two modifications to their permutation threshold approach that control for known QTL in the genome. The conditional empirical threshold (CET) permutes the trait values within the genotypes of known QTL. The residual empirical threshold (RET) uses linear regression to remove the effects of known QTL and then analyzes the residuals to identify new QTL using empirical thresholds (Doerge and Churchill 1996). Alternatively, the interval mapping model was extended (composite interval mapping; CIM) independently by (Jansen 1993; Zeng 1993; 1994) through the use of covariates to control for additional QTL. The composite interval mapping model, like the interval mapping model, is based on a mixture of normal distributions and is probably the most commonly used method for QTL analysis; however, this model fails to include fixed factors beyond marker cofactors that are believed to affect the phenotype, e.g. a covariate for gender to control for QTL located on the sex chromosomes. An extended composite interval mapping (ECIM) model mimics composite interval mapping by expanding the mixture distribution model to include additional covariates, along with markers covariates. The ECIM model is available in "R\qtl" (Broman, Wu et al. 2003), a package in the "R" statistical analysis software (R Development Core Team 2009). As is the case with interval mapping, CIM and its extensions provide test statistics that fail to follow a predictable theoretical distribution. Unlike interval mapping, and because of the covariates, the phenotypes in any CIM/ECIM procedure are not exchangeable. Thus, the use of Churchill and Doerge (1994) empirical thresholds is not a theoretically valid option for providing significance thresholds (Churchill and Doerge 2008). As a solution, we present a novel bootstrap threshold algorithm that provides accurate resampling based QTL significance thresholds for CIM and ECIM analysis methods that include fixed covariates, and in turn fail to satisfy the necessary exchangeability requirement of permutation thresholds.

2. Bootstrap Significance Threshold Algorithm

A sufficient condition for an exact and unbiased permutation test is the exchangeability of the observations that exists when the probability of a joint result is the same, regardless of the ordering of the observations (Good 2000). When a multiple regression-based interval mapping model is considered, the assumption of exchangeability is no longer met because each phenotype is associated with a unique set of covariates (Good 2000). Efron (1979) introduced a parallel nonparametric resampling method known as "the bootstrap." Bootstrap samples are drawn from the data in a manner that generates an empirical null distribution of a test statistic. Because the

bootstrap procedure models the null distribution, it is flexible and can be used to establish critical values, or significance thresholds, for complicated data structures (Davison and Hinkley 1997).

The bootstrap significance threshold algorithm that we propose mirrors the empirical threshold algorithm of Churchill and Doerge (1994) by selecting and saving the maximum LOD score from a genome scan of the resampled data. The bootstrap significance threshold is determined from the 100(1-α) percentile of the distribution of the maximum LOD scores as generated from a number of resampled datasets. The bootstrap threshold algorithm differs from the empirical permutation threshold algorithm in that it attempts to model the null distribution of the maximum LOD score by removing the estimated additive effect of the QTL at the current map location and then generating a new data set based on a bootstrap resampling of the centered residual effects. For each test location, the residuals are generated by subtracting the all of the parameter estimates from the values of the phenotype. In interval mapping models, the additive effect is modeled as a mixture distribution; therefore, the estimates of the QTL genotype component classification variable are used to weight the additive effects when generating the residuals. The residuals are centered to mimic the model's assumption that the error terms have a mean of zero and the centered residuals are bootstrapped (Davison and Hinkley 1997). Because this is a computationally expensive procedure, one can improve the speed and consistency of the bootstrap threshold algorithm by relying on the indices of the individuals to perform a single bootstrap of the residuals at each test location in a single genome scan. The bootstrap residuals and the estimates of the fixed covariate effects, except for the additive and dominance effects, along with the estimated mean, are combined to generate a bootstrap data set under the null hypothesis of no QTL effects at the current test location. A genome scan is conducted by generating a bootstrap data set at each test location along the genome and the maximum LOD score is selected. When the data are exchangeable, results obtained using the bootstrap procedure are asymptotically equivalent to the results obtained using permutation procedure. Therefore, the bootstrap threshold algorithm gives rise to similar significance thresholds as those determined by using the permutation-based empirical threshold algorithm when the data meet the exchangeability requirement.

3. Simulations

Three simulation studies were conducted based on a differing number of simulated QTL. Five hundred backcross data sets of 300 individuals were simulated. In these simulations, 300 individuals were selected to generate adequate data to obtain estimates for all the model parameters. A consideration when utilizing a multiple regression based model, as the number of individuals' decreases, the estimates obtained by the model and the bootstrap threshold procedure will have larger variances. The bootstrap threshold procedure is not limited by the number of individuals in the experiment in terms of obtaining a threshold; however, since the bootstrap threshold is based on the model estimates, it is sensitive to the ability to estimates these values. Therefore, the bootstrap threshold requires a sample size large enough to obtain reasonable estimates for all the terms in the model. Each data set consisted of a single chromosome comprised of thirty markers at 10cM intervals, and every backcross individual was randomly classified into one of five covariate classes. Phenotypes were simulated based on the additive effect of the QTL alleles, the classification of the fixed covariate with an additive effect of 0.5, and additive random error terms sampled from a log normal distribution with a mean of

zero and a variance of one. The log normal error was selected for the distribution of the error terms because it is a strongly skewed distribution, which will challenge the proposed bootstrap threshold's ability to establish appropriate thresholds beyond a "best case" normal error distribution.

The three models utilized in these studies are interval mapping (Lander and Botstein 1989; 1994), composite interval mapping (Jansen 1993; Zeng 1993; 1994), and extended composite interval mapping. Interval mapping (Lander and Botstein 1989; 1994) models the QTL effect using a mixture model where the component distributions are Gaussian with a common error variance having a mean phenotype based on the linear model

$$y_i = \mu + Q_q\alpha + \varepsilon_i, \tag{3.1}$$

where y_i is the mean phenotypic value term for individual $i = \{1, 2, ..., n\}$, μ represents the mean effect, Q_q is a coded value for the QTL genotype $q = \{1, 2, ..., n_Q\}$, α represents the additive effect of an allelic substitution, and ε_i is the random error. An extension to interval mapping is composite interval mapping (Jansen 1993; Zeng 1993; 1994), where the mean phenotype is modeled using a multiple regression containing terms for marker covariates that control for additional QTL in the genome. The mean phenotype for this model can be written as

$$y_i = \mu + Q_q\alpha + \sum_{k=1}^{K} M_{ik}\beta_k + \varepsilon_i, \tag{3.2}$$

where M_{ik} is a coded value for the covariate markers $k = \{1, 2, ..., K\}$, β_k is a vector of parameters for the marker covariates, and all other parameters are as defined for the interval mapping model (Zeng 1994). Extended composite interval mapping expands the mean phenotype to include additional fixed covariates beyond markers. The model is

$$y_i = \mu + Q_q\alpha + X\beta + \varepsilon_i, \tag{3.3}$$

where X is the design matrix for the fixed effects (including marker covariates), β is a vector of fixed effects, and all other parameters are as defined for the interval mapping model. In each simulation, the simulated fixed covariate effect was included in the extended composite interval mapping model for each simulation study.

The first simulation contained no QTL so that an experimental Type I error rate, the proportion of simulations that contain at least one false positive result anywhere on the chromosome, could be determined. The permutation-based empirical threshold (Churchill and Doerge 1994) is used to determine significant effects for an interval mapping model (3.1) and the proposed bootstrap threshold is established for an extended composite interval mapping model (3.3). Genomewide significance thresholds were determined by selecting the ninety-fifth percentile of the empirical maximum LOD score distribution generated using 500 resampled data sets. The experimentwise Type I errors obtained in this simulation are provided in Table 1. The extended composite interval mapping model (3.3) using the proposed bootstrap threshold produced estimated Type I error rates generally close to the theoretical experimentwise Type I error rate of 5%. The estimated Type I error rates obtained for the interval mapping model (3.1) using the permutation-based empirical threshold (Churchill and Doerge 1994) are slightly more conservative compared to the simulated Type I error rate for the extended composite interval mapping model utilizing the bootstrap threshold.

The second simulation was conducted under the same simulation parameters as the first study; only a single QTL with an additive effect of 0.5 was placed 145cM (between Markers 15 and 16) from the left end of the chromosome. The simulated data sets were evaluated using the interval mapping model (3.1) with the permutation-based empirical threshold (Churchill and Doerge 1994), and the extended composite interval mapping model (3.3) with the bootstrap threshold. The percentage of significant results, based on a threshold set at the 95th percentile, at each testing location is shown in Figure 1. The mean and standard deviations for the estimated parameter values at the test location closest to the known QTL are given in Table 2. The difference in detection of the QTL between the extended composite interval mapping model (3.3) using the proposed bootstrap threshold and the interval mapping model (3.1) using the empirical threshold (Churchill and Doerge 1994) was 13.6%. This simulation demonstrates that if a fixed covariate is influencing the phenotype, a model that includes this covariate is more effective at locating a QTL. An additional benefit of using an extended composite interval mapping model (3.3) is that estimates for the effect of the covariate can be obtained, see Table 2.

The final simulation incorporated two QTL at opposite ends of the chromosome. This study differed from the previous two backcross simulations in that the fixed covariate for this model included two levels with an effect of 1.5, along with a log normal error with a mean of zero and a variance of two. The first QTL was located 95cM from the left end of the chromosome with an additive effect of 0.85 and the second QTL was placed 185cM from the left end of the chromosome with an additive effect of 0.55. This study involved an interval mapping model (3.1) with CET and RET thresholds (Doerge and Churchill 1996), as well as a composite interval mapping model (3.2) and the extended composite interval mapping model (3.3) using the bootstrap threshold. Marker 10 was used as the conditioning marker for the CET and RET thresholds and as a marker cofactor in the composite interval mapping (3.2) and extended composite interval mapping (3.3) models. The results for this study are shown in Figure 2. The mean and the standard deviation for the parameter estimates are provided in Table 3 for the test location nearest the known QTL location. The extended composite interval mapping model (3.3) with the bootstrap threshold outperformed the other interval mapping methods by at least 13% when locating the second QTL. The RET and CET thresholds condition on the first marker, removing the ability to determine accurate parameter estimates for this QTL, whereas for the composite interval mapping (3.2) and the extended composite interval mapping (3.3) models parameter estimates are available for the first QTL as well.

3.1 Number of bootstrap samples

One consideration for any resampling algorithm is the number of resampled data sets that should be generated. In a QTL study, this question is especially important due to the large number of tests that are conducted for each resampled data set in a QTL analysis study. To investigate the effect of the number of bootstrapped date sets on the proposed bootstrap threshold algorithm, a 95% bootstrap significance threshold was established for a differing number of bootstrapped data sets in two of the backcross simulations described in Section 3. For the backcross simulation that included no QTL, a single covariate with an effect of 0.5, and a log normal error distribution with a variance of one, as few as 250 bootstrap samples could be collected for the extended composite interval mapping model (3.3) with no significant increase in the estimated Type I error rate, see Table 4. When two QTL with effect sizes of 0.85 and 0.55

were introduced into the backcross simulation, there was also no loss in the ability to locate the QTL using as few as 250 bootstrap samples, see Figure 3. However, based on lack of variability in simulated data sets, it is recommended that at least 500 bootstrap samples be utilized in practice, and, if possible, 1000 bootstrap samples as is recommended for the permutation-based empirical threshold (Churchill and Doerge 1994; Doerge and Churchill 1996) to ensure the Type I error rate.

4. Hereditary Spherocytosis

Hereditary spherocytosis is an inherited skeletal membrane deformity of a red blood cell and is the most common hemolytic anemia in people from Northern Europe (Peters, Swearingen et al. 2004). Peters, Swearingen et al. (2004) crossed individuals from the $C3H^{wan/+}$, a line with a new hereditary spherocytosis mutation, and *Mus musculus castaneus* (CAXT/Ei) inbred line of mouse to produced an F_2 intercross population to establish the location of QTL involved in a hereditary spherocytosis mutation. Five phenotypic measurements related to red blood cells were collected: red blood cell count (RBC), mean corpuscular hemoglobin content (MCHC), percent hematocrit (HCT), hemoglobin level (HGB), and mean corpuscular volume (MCV). A genomewise QTL study was conducted that included gender and body weight as additive covariates for all five traits using the Pseudomarker software (Sen and Churchill 2001; Wu, Sen et al. 2009). An initial genomewide scan by Peters, Swearingen et al. (2004), utilizing 93 individuals, suggested the existence of a QTL on chromosome 12 using the MCV phenotype were performed. Based on the results of their initial scan, additional markers were included on chromosome 12, thus providing a total of 96 markers and an additional 90 animals were genotyped for makers on chromosome 12 only. A secondary analysis was conducted using the full set of 183 individuals with gender and weight once again included as covariates in the analysis model. Peters, Swearingen et al. (2004) located significant QTL (a LOD score greater than 2.2) on chromosome 6 and chromosome 12 for MCV, and two suggestive QTL (a LOD score between 2 and 2.2) on chromosomes 7 and 19 for MCHC. No other significant QTL were located for the remaining phenotypes.

These data were reanalyzed in this study using an interval mapping model (3.1) (Lander and Botstein 1989; 1994) with an empirical threshold (Churchill and Doerge 1994) and an extended composite interval mapping analysis (3.3), including covariates for gender and weight, using the proposed bootstrap threshold. The bootstrap and empirical thresholds (Churchill and Doerge 1994) are determined based on 1000 resampled data sets and established at the 95^{th} and 90^{th} percentiles of the resulting maximum LOD score distribution. A fine mapping analysis utilizing the 183 animals typed for 8 markers on chromosome 12 was conducted for each of the five phenotypes. Significant results were obtained for the mean corpuscular volume (MCV) and percent hematocrit (HCT) (Figures 4 and 5). Significant QTL for MCV were identified with both analysis methods; however, a significant QTL was identified for HCT at the 90^{th} percentile using the extended composite interval mapping model (3.3) with the bootstrap threshold that was not detected using the interval mapping model (3.1) with the empirical threshold (Churchill and Doerge 1994). The parameter estimates obtained for the largest LOD scores for the interval mapping model (3.1) and the extended composite interval mapping model (3.3) are shown in Table 5.

The estimated locations of the putative QTL for MCV and HCT are identical for each analysis method, but the estimated LOD scores for MCV and HCT are larger for the extended composite interval mapping model (3.3), indicating that this model is a better fit. From the parameter estimates obtained from the extended composite interval mapping model (3.3), it appears that weight has a relatively small effect on both MCV and HCT; however, gender was determined to have a relatively large effect on both traits (see Table 5). For MCV and HCT, the parameter estimate for the additive effect of the QTL was estimated to be slightly smaller in magnitude by the interval mapping model (3.1) as compared to the extended composite interval mapping model (3.3), while the dominance effect was estimated to be larger in magnitude. The difference in estimates of the genetic effects is evidence of how covariates that alter the expression of the phenotype can bias the genetic estimates if the covariates are not included in the analysis model. If covariates that do not significantly affect the expression of the phenotype are included in the model, the variance estimates for the genetic effects will increase however since these effects are also modeled in the bootstrap threshold they should not alter the ability to detect significant QTL; however, biologically unimportant covariate effects were not investigated in this study. This analysis demonstrates the importance of including influential biological cofactors into an analysis because their presence in the model can improve the model fit, thus increasing the value of LOD scores and, in unison with the proposed bootstrap threshold, resulting in a better ability to identify significant QTL.

5. Summary

A novel bootstrap threshold procedure was developed for multiple regression based interval mapping models. In simulation studies, the bootstrap threshold was shown to maintain an appropriate Type I error rate and successfully identified QTL when they were present. The Type I error rate was maintained even when the error distribution was skewed, which demonstrates the flexibility of the bootstrap to adapt to a nonsymmetric error distribution. Since the distribution of the error terms in a quantitative trait analysis are usually unknown, the ability of the bootstrap threshold to adjust to a variety of error distributions is a favorable property. The bootstrap threshold procedure is easy to implement and is asymptotically equivalent to the empirical threshold when the data are exchangeable. It is recommended that at least 500 bootstrap samples should be used to establish a threshold with the appropriate Type I error rate, although in simulation studies as few as 250 samples could be utilized with little effect on the Type I error rate.

Incorporating biologically important covariates into the analysis by utilizing additional fixed covariates can help improve the detection of QTL. As shown in the simulation studies and the QTL analysis on traits involved in hereditary spherocytosis, including terms for fixed covariates increased the LOD scores at the QTL location and in conjunction with the proposed bootstrap threshold resulted in an improved ability to identify significant QTL. Utilizing more advanced models that include biological cofactors in this way can better partition the variance and thus make QTL effects easier to detect. The proposed bootstrap threshold can be easily adapted to many model structures, which would allow for more detailed analysis of data from QTL experiments.

6. Acknowledgements

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Table 1. Estimated Type I error rates obtained for the 95% experimentwise permutation-based empirical threshold were slightly more conservative compared to the 95% experimentwise bootstrap threshold. The estimate of the experimentwise Type I error rate based on 500 simulated data sets, Pr(number of false positives > 0), for a backcross simulation study that included no QTL, a single covariate with an effect of 0.5, and lognormal random error with a mean of zero and a variance of one. Each simulated data set consisted of 300 individuals and a single chromosome with 30 markers spaced 10cM apart. The models and thresholds used in this simulation are the extended composite interval mapping model (3.3) with the bootstrap threshold (ECIM BT) and the interval mapping model (3.1) with the empirical (permutation) threshold (IM ET).

Model	ECIM BT	IM ET
Type I error	4.2%	3.8%

Table 2. The average parameter estimates and estimated power based on 95% experimentwise threshold for the marker or test location nearest to the known QTL location, 145cM from the left end of the chromosome, in a simulated backcross design of a single chromosome with 30 markers spaced 10cM apart. The QTL had an additive effect of 0.5, the covariate was simulated with an effect of 0.5 and the random error was drawn from a log normal distribution with a variance of one. Estimates are based on 500 simulated data sets of 300 individuals. The models and thresholds used are the extended composite interval mapping model (3.3) with the bootstrap threshold (ECIM BT) and the interval mapping model (3.1) with the permutation-based empirical threshold (IM ET). The values in the parenthesis are the empirical standard deviation of the estimates.

	Additive Effect	Covariate	Error	Empirical Power
Simulated Values	0.50	0.50	1.00	
ECIM BT	.50 (0.12)	0.50 (0.05)	0.97 (0.19)	0.874
IM ET	0.49 (0.15)	NA	1.14 (0.17)	0.738

Table 3. The average parameter estimates and estimated power based on 95% experimentwise threshold, for the marker or test location nearest to the two known QTL locations, 95cM and 185 cM from the left end of the chromosome, in a simulated backcross design of a single chromosome with 30 markers spaced 10cM apart. The QTL were simulated with additive effects of 0.85 and 0.55, along with a single covariate with an effect of 1.5. The random error was drawn from a log normal distribution with a variance of 1. Estimates are based on 500 simulated data sets of a single chromosome with 30 markers spaced 10cM apart for 300 individuals. The models and thresholds used are: an extended composite interval mapping model (3.3) with the bootstrap threshold and a marker covariate at marker 10 (ECIM BT), a composite interval mapping model (3.2) with the bootstrap threshold and a marker covariate at marker 10 (CIM BT), an interval mapping model (3.1) with a residual empirical threshold conditioned on marker 10 (IM RET), and an interval mapping model (3.1) using the conditional empirical threshold conditioned on marker 10 (IM CET). The values in parenthesis are the empirical standard deviation of the estimates.

	Additive Effect 1	Additive Effect 2	Covariate	Error	Empirical Power 1	Empirical Power 2
Simulated Values	0.85	0.55	1.50	1.00		
ECIM BT	1.21 (0.20)	0.64 (0.25)	1.48 (0.23)	1.27 (1.41)	0.952	0.738
CIM BT	1.18 (0.22)	0.59 (0.28)	NA	1.55 (1.33)	0.948	0.600
IM RET	NA	0.42 (0.19)	NA	1.56 (1.33)	NA	0.526
IM CET	NA	1.02 (0.24)	NA	1.58 (1.33)	NA	0.122

Table 4. The empirical experimentwise Type I error rate, Pr(number of false positives > 0), for a backcross (BC) simulation study with no QTL effects. The extended composite interval mapping model (3.3) for the backcross is used with a varying number of bootstrap samples to set the 95% experimentwise significance threshold. The backcross simulation included a single covariate with an effect of 0.5 and a lognormal error distribution with a variance of one. Estimates were obtained using 500 simulated data sets for a single chromosome with 30 markers spaced 10cM apart for 300 individuals.

No. of Samples	1000	500	250
Type I error	4.2%	4.4%	4.4%

Table 5. Table of parameter estimates for the maximum LOD score on chromosome 12 for the mean corpuscular volume (MCV) and percent hematocrit (HCT). The models used for this analysis are an interval mapping (IM) model (3.1) utilizing an empirical threshold and an extended composite interval mapping (ECIM) model (3.3) employing the bootstrap threshold. The estimates were selected for the location with the largest LOD score, and the range is established as the first and last location with LOD scores larger than the 95% significance threshold. The range is not provided for the IM model for HCT because the LOD score for this location was not above the 95% empirical threshold value

	Location	Range	Add. Effect	Dom. Effect	Gender	Weight	Error SD	LOD score
MCV								
ECIM	32	0-50	2.60	-1.83	-1.78	0.04	6.54	3.52
IM	32	0-50	2.43	-2.01	NA	NA	6.60	3.31
HCT								
ECIM	29	29-30	1.58	-1.03	-2.73	0.08	5.51	1.99
IM	29		1.37	-1.32	NA	NA	5.69	1.29

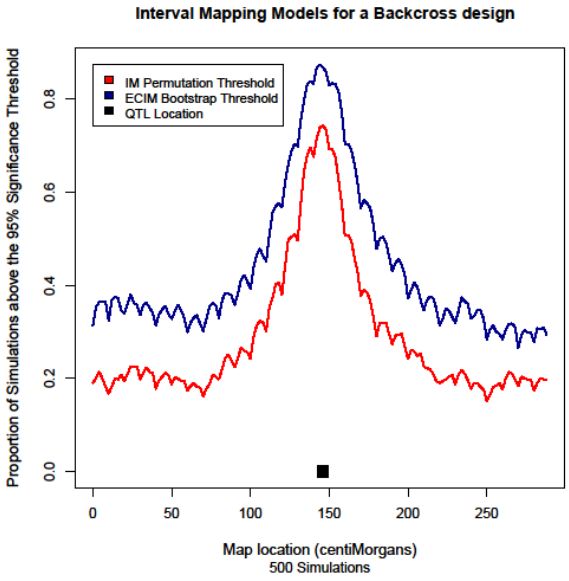


Figure 1. The proportion of simulated data sets with significant test statistics using interval mapping (IM) (3.1) and permutation thresholds, and extended composite interval mapping (ECIM) (3.3) with bootstrap thresholds based on a 95% experimentwise threshold for 500 simulated backcross data sets that contained one QTL located 145cM from the left end of the chromosome with an additive effect of 0.5 and a single covariate with an effect of 0.5. The random error was drawn from a log normal distribution with a variance of 1. Each simulated data set consisted of a single chromosome with 30 markers spaced 10cM apart for 300 individuals.

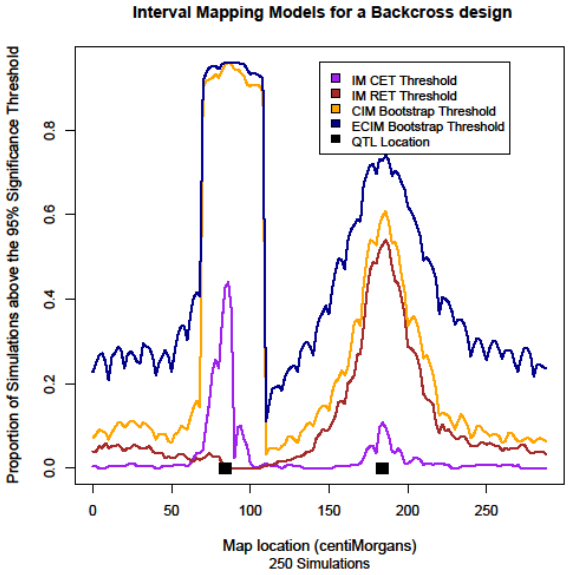


Figure 2. The proportion of simulated data sets for which a test point or marker was determined to be significant based on a 95% experimentwise threshold for 500 simulated backcross data sets that contain two QTL with additive effects of 0.85 and 0.55 and located 95cM and 185cM from the left end of the chromosome. A single covariate was simulated with an effect of 1.5 and the random error was drawn from a lognormal distribution with a variance of 1. Each simulated data set consisted of a single chromosome with 30 markers spaced 10cM apart for 300 individuals. The models and thresholds used are: the interval mapping model (IM) (3.1) with the CET and RET thresholds, the composite interval mapping model (CIM) (3.2) with the bootstrap threshold, and the extended composite interval mapping model (ECIM) (3.3) with the bootstrap threshold. For CIM and ECIM models, a marker covariate was established at marker 10 with a window of 40cM. RET is the residual empirical threshold and CET is the conditional empirical threshold.

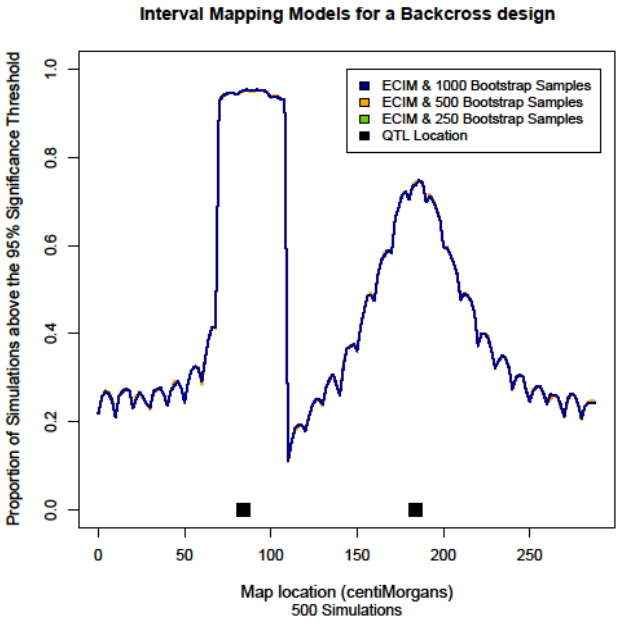
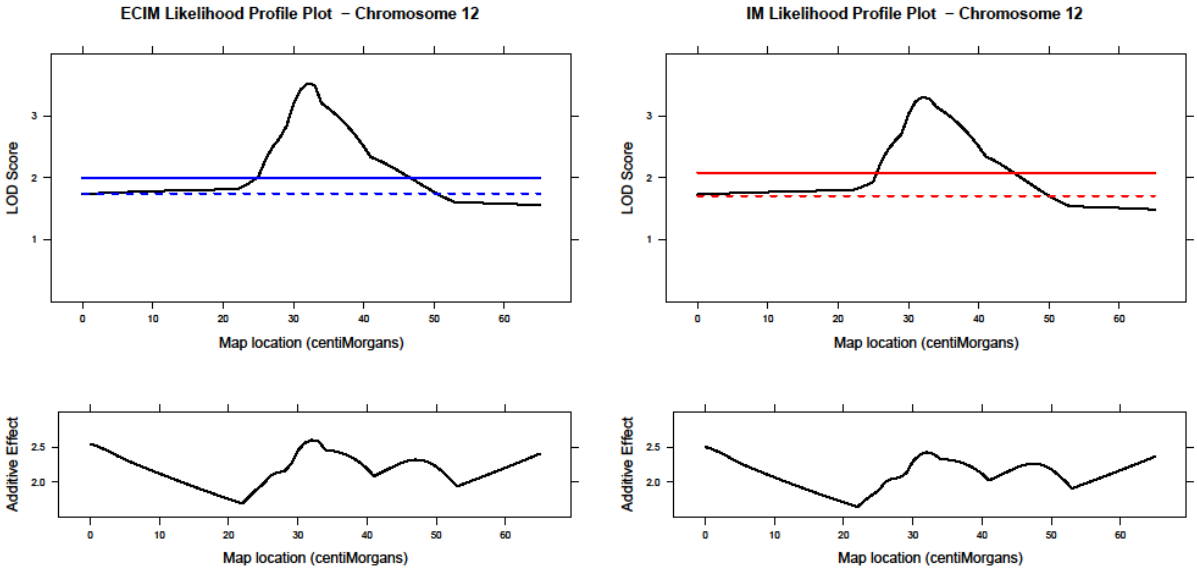
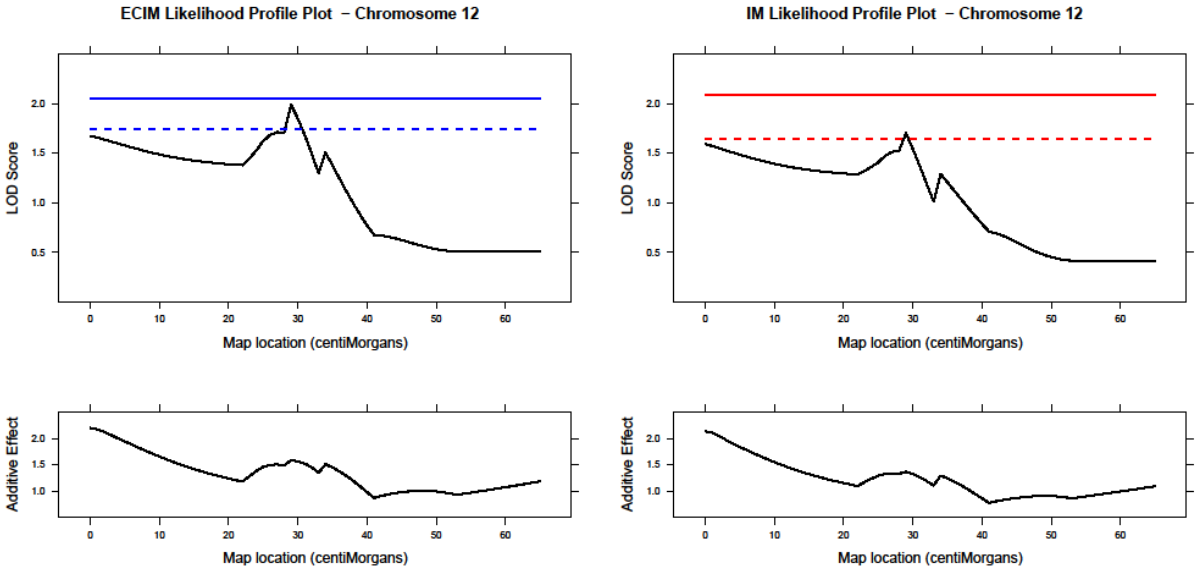


Figure 3. Investigation of the effect of the number of bootstrap samples in a simulated backcross experiment with QTL effect sizes of 0.85 and 0.55. The extended composite interval mapping model (3.3) for the backcross is used with a varying number of bootstrap samples to set the 95% experimentwise significance threshold. The backcross simulation included a single covariate with an effect of 0.5 and a lognormal error distribution with a variance of one. Estimates were obtained using 500 simulated data sets for a single chromosome with 30 markers spaced 10cM apart for 300 individuals.



a) ECIM with Bootstrap Threshold **b) IM with Empirical Threshold**

Figure 4. A likelihood profile plot of chromosome 12 for mean corpuscular volume (MCV) for an F_2 intercross in mouse. An interval mapping (IM) model (3.1) utilizing an empirical threshold and an extended composite interval mapping (ECIM) model (3.3) employing the bootstrap threshold were utilized to search for significant associations between MCV and a putative QTL genotype. Each upper graph consists of a plot of the LOD scores for each model. The two horizontal lines represent the 95% (the solid horizontal line) and the 90% (the dashed horizontal line) significance thresholds based on 1000 samples from the null distribution derived using the listed resampling procedure. The lower graphic is a plot of the estimated additive effect for each location along the chromosome.



a) ECIM with Bootstrap Threshold **b) IM with Empirical Threshold**

Figure 5. A likelihood profile plot of chromosome 12 for percent hematocrit (HCT) for an F_2 intercross in mouse. The models used for this analysis are an interval mapping (IM) model (3.1) utilizing an empirical threshold and an extended composite interval mapping (ECIM) model (3.3) employing the bootstrap threshold. Each upper graphic consists of a plot of the LOD score for each test location along chromosome 12 for each model. The two horizontal lines represents the 95% (the solid horizontal line) and the 90% (the dashed horizontal line) significance thresholds based on 1000 resampled data sets. The lower graphic is a plot of the estimated additive effect for each location along the chromosome.