QTL Analysis using Bayesian Interval Mapping

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Abstract

Bayesian interval mapping of QTL library R/qtlbim provides Bayesian analysis of multiple quantitative trait loci (QTL) models. This includes posterior estimates of the number and location of QTL, and of their main and epistatic effects. This document assumes some familiarity with QTL and with Bayesian methods. In addition it provides graphical diagnostics that can help investigate several "better" models. It also provides a 1-D and 2-D genome scan. The R/qtlbim package provides plotting facilities for results generated by the analytical tools in the R/qtlbim package. These plotting facilities include time series plots of QTL model characteristics as basic MCMC diagnostic plots, visual tools for comparison of putative QTL models and exploratory plots whose purpose is the aid in the identification of likely QTL. This library R/qtlbim requires R/qtl 1.03.

1 Overview

> library(qtlbim)
> data(qbHyper)
> hyper <- qb.cross(qbHyper)

This vignette describes the MCMC sampling routines and some of the plotting facilities available through the R/qtlbim package. The purpose of these plots is to provide graphical tools for

1. exploring putative single and multiple QTL,
2. producing interpretable graphics of the relative evidence in favor of a set of putative QTL,
3. visual diagnostics of the MCMC model selection algorithm.

This package is now quite stable. Over the past year, since our "beta" release, there been numerous incremental improvements, yielding faster computation and smaller R objects. Most notably, the external directory and files created by qb.mcmc are now removed immediately (planned later upgrades will eliminate their need). Users with "old" style MCMC samples will be warned to upgrade using qb.legacy. [The old qb.remove is retained for compatibility, but is not needed for new qb objects.] Another important improvement is that values (results) from all routines are now self contained. The qb object contains the pertinent aspects of the cross object used to create it, and routines such as qb.scanone produce self-contained objects. This makes intermediate results more transportable. In addition, some objects have been made more compact, and R code efficiency has improved. Those interested in specific improvements can examine ChangeLog.txt in the R library area.

This document walks through the R/qtlbim package by demonstrating the following major functions:
creation of Bayesian samples from the posterior using MCMC sampling; use of plot and summary tools to examine genetic architecture; data management in R/qtlbim.

2 Hyper Data Demo

This document focuses on the hyper dataset from R/qtl. One way to produce a qb object is to run the hyper demo by typing
demo(qb.hyper.tour)
at the R prompt. Alternatively a qb object can be created by the following sequence of commands.
# First load the library
library(qtlbim)

# Now load the hyper data set.
data(hyper)

# Select just the columns in the hyper data set corresponding to
# chromosomes one through 19. This means that we have dropped
# the X-chromosome from our analysis, since R/qtlbim does not
# properly handle X yet.
hyper = subset(hyper, chr=1:19)

# Calculate genotype probabilities.
hyper = qb.genoprob(hyper, step=2)

# Now run the MCMC model selection algorithm. Running this command
# may take as much as 15 minutes on slower computers.
qbHyper = qb.mcmc(hyper, pheno.col = 1, seed = 1616)

The comments in the code above describe the meaning of each step in the sequence of commands used to produce qbHyper. The option seed sets the random number seed so that this run can be repeated exactly. The demo(qb.hyper.tour) or the qb.mcmc command above creates a qb object called qbHyper that is used throughout this vignette.

3 Bayesian QTL Mapping

This section describes in more detail how to create Markov chain Monte Carlo (MCMC) samples from the Bayesian posterior to be used for QTL mapping.

The function qb.genoprob creates a grid of putative QTL positions throughout the entire genome and compute the genotypic probabilities of the same. It would also compute genotypic probabilities for missing markers. In general, the genotypes at different loci are unobserved except at completely informative markers but the probability distribution can be inferred from the observed marker data using the multipoint method (Jiang and Zeng 1997).

In the simplest case, where defaults in the next subsections are used, MCMC samples are created with the following call:

qbHyper <- qb.mcmc(hyper, pheno.col = 1)

Named arguments to qb.data and qb.model, described in the next two subsections, can be passed through qb.mcmc. Otherwise, default values are used. The next subsections detail the qb.data, qb.model and qb.mcmc routines.

3.1 Specifying data

The function qb.data specifies the traits to be analyzed, their underlying distribution, the random and/or fixed covariates and whether to standardize or to use a boxcox transformation. Note that, the cross object can have several phenotypes and some of which could be used as covariates.

qbData <- qb.data(hyper, pheno.col = 1, trait = "normal",
boxcox = F, standardize = F)

3.2 Defining the model

The function qb.model defines the model which is a Cockerham epistatic model. For mapping a $K+1$ genotypes per loci, there are $K$ main effects and $K^2$ epistatic effects. For a backcross population with two segregating genotypes $bqbq$ and $Bqbq$ at locus $q$ the coefficients are

$$x_{iq1} = z_{iq} - 0.5$$
$$x_{iqq1} = x_{iq1}x_{iq1}$$

where $z_{iq}$ denotes the number of $bq$ alleles. For an intercross derived from two inbred lines, there are three segregating genotypes $bqbq$, $bqBq$ and $BqBq$. The coefficients of the Cockerham model are as follows:
\[ x_{iq1} = z_iq + 1, \quad x_{iq2} = (1 - x_{iq1})(1 + x_{iq1}) - 0.5 \quad \text{and} \quad x_{iqq'} = \begin{cases} x_{iq1}x_{iq'} & k = 1 \\ x_{iq1}x_{iq'1} & k = 2 \\ x_{iq2}x_{iq'} & k = 3 \\ x_{iq2}x_{iq'2} & k = 4 \end{cases} \]

It also specifies if epistasis is considered, the expected number of main effect QTLs (main.qtl), the prior number of total QTLs on all chromosome which includes QTLs with only epistatic effect, the maximum number of QTLs allowed per chromosome. The maximum number of QTLs allowed per chromosome has a default of \( l_0 + 3\sqrt{l_0} \) where \( l_0 \) is main.qtl in a non-epistatic and mean.qtl in an epistatic model. The interval between two flanking QTLs for each chromosome and the fixed covariate(s) interacting with QTLs are also specified. Typically a real data set has several traits which can be considered as covariates. By setting the max.qtl=0 (the maximum number of QTLs allowed) a traditional Bayesian model selection can be performed. This provides a more relevant and shorter set of covariates. This set of covariates can then be used to for QTL mapping. For our case, as we have only a couple of covariates, we include both in the model. The main.qtl argument of the qb.model function refers to the prior number of main effect QTL. This is obtained generally from a previous rudimentary non-Bayesian analysis. For example, R/qtl can be used to analyze this data without epistasis and the number of QTLs detected could be used as the prior number of QTLs.

\[
\text{qbModel} \leftarrow \text{qb.model}(\text{hyper}, \text{epistasis} = \text{TRUE}, \text{main.nqtl} = 3, \\
\quad \text{interval} = \text{rep}(5, \text{nchr(cross)}), \text{chr.nqtl} = \text{rep}(2, \text{nchr(cross)}), \\
\quad \text{depen} = \text{FALSE}, \text{prop} = c(0.5, 0.1, 0.05))
\]

### 3.3 Running MCMC

The function qb.mcmc creates MCMC samples on the data and model specified. The results are saved in a specified directory, default being the current directory ("."). The genoupdate parameter of the qb.mcmc function should be switched on when there are many missing marker genotypes, as for the hyper dataset. For our case, we have only 7% of the marker genotypes missing so we are not updating the genotypes. There are two choices of the MCMC algorithm namely, Metropolis-Hastings algorithm and the Gibbs sampler. The M-H algorithm samples from a proposal density approximating the conditional posterior distributions whereas Gibbs sampler samples directly from the conditional posterior distribution. The M-H algorithm is a lot faster compared to the Gibbs sampler. We would saving 3000 samples which is the default n.iter. It is worth mentioning that n.iter does not specify the total number samples drawn from the posterior distribution but merely indicates the number of samples that would be saved and used for posterior analysis. The first n.burnin samples are discarded to allow the chain to converge to the posterior distribution and after that every n.thin sample is considered to reduce serial correlation resulting in a total of n.burnin + n.iter*n.thin samples drawn.

### 3.4 Examining a qb Object

Please note that the plot and summary routines in this package all use the S3 generic method. That is, we create an object with a call to qb.xxx and then plot it using the generic plot command. Manual pages show the complete set of command and plot options.

The qbHyper is an object of class qb to which we can apply the generic summary or plot routines. We defer plots to later sections. Here we show only the summary:

```r
> summary(qbHyper)

Bayesian model selection QTL mapping object qbHyper on cross object hyper
had 3000 iterations recorded at each 40 steps
with 1200 burn-in steps.
MCMC runs saved in qb object.
Trait bp ( 1 ) treated as normal .
Trait was not standardized.
Epistasis was allowed.
Prior number of QTL: 3 main, 6 total, with 13 maximum.
Minimum distance between QTL:

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<td>3.91</td>
<td>6.31</td>
<td>6.67</td>
</tr>
<tr>
<td>9.08</td>
<td>13.80</td>
<td>14.20</td>
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</tbody>
</table>
```

3
4 Plotting MCMC History

The final command above produced the \texttt{qbHyper} object by running the MCMC model selection algorithm: 
\begin{verbatim}
qbHyper = qb.mcmc(hyper, pheno.col = 1, genoupdate=TRUE)
\end{verbatim}

Consequently a logical first step in exploring the results of the analysis is to examine this MCMC chain. The plotting tools in \texttt{R/qtlbim} provide a method for visually inspecting the history of the MCMC run. The command
\begin{verbatim}
plot(qb.coda(qbHyper))
\end{verbatim}
shows a default view of the MCMC chain as a time series. Each iteration of the MCMC chain represents a single model; therefore, we can explore the history of the MCMC chain by plotting time series for relevant model features. The time series plotted by \texttt{qb.coda} show the sampling histories for

1. number of QTL in each model model (\texttt{nqtl}),
2. mean phenotype according to each model (\texttt{mean}),
3. environmental variability under each model (\texttt{envvar}),
4. variance explained under each model (\texttt{var}) and

It is possible to plot a different subset of the model characteristics above, by using the optional argument \texttt{variables} in the \texttt{qb.coda} function. For example, in order to view just the number of QTL (\texttt{nqtl}) and the model means, use the following command. The results of the following command are shown in Figure ??.
\begin{verbatim}
plot(qb.coda(qbHyper, variables = c("nqtl","envvar")))
\end{verbatim}
Figure 1: Diagnostic Plot for a MCMC run.
MCMC sampled loci

plot(qb.loci(subset(qbHyper, chr=c(3,4))), labels=TRUE)

QTL are from single QTL models appearing as samples in the MCMC chain. In the plot, the actual markers are shown with the actual markers appearing as samples in the MCMC chain. In the plot, the actual locations of possible QTL are jittered slightly in order to give a sense of the density of putative QTL in the vicinity of each marker. The code

plot(qb.loci(qbHyper))

will produce a plot with all chromosomes. In order to view a subset of the chromosomes, the parameter chr to the generic subset routine can be used to limit the plot to a selected set of chromosomes. The horizontal (blue) lines in the plot show the locations of markers. The markers themselves can be labelled by using the parameters markers in the function.

plot(qb.loci(subset(qbHyper, chr=c(3,4))), labels=TRUE)

Figure ?? shows the result of this command.

Figure 2: A jittered plot of quantitative trait loci, showing only chromosomes 3 and 4, with locations and marker labels.

5 A Plot of QTL by chromosome

From a biological perspective it may be interesting to view the location of possible QTL along the chromosome. The function qb.loci shows a plot of quantitative trait loci for each chromosome. The QTL are from single QTL models appearing as samples in the MCMC chain. In the plot, the actual locations of possible QTL are jittered slightly in order to give a sense of the density of putative QTL in the vicinity of each marker. The code

plot(qb.loci(qbHyper))

will produce a plot with all chromosomes. In order to view a subset of the chromosomes, the parameter chr to the generic subset routine can be used to limit the plot to a selected set of chromosomes. The horizontal (blue) lines in the plot show the locations of markers. The markers themselves can be labelled by using the parameters markers in the function.

plot(qb.loci(subset(qbHyper, chr=c(3,4))), labels=TRUE)

Figure ?? shows the result of this command.
6 Summary Plots for Sampled Models

The function \texttt{qb.BayesFactor} produces a composite (4-by-2) summary plot of the models sampled by the MCMC chain. These plots are useful as an initial tool for examining the evidence in favor of multiple QTL models and in determining the locations of QTL. Figure ?? shows the plot produced by the command \texttt{qb.BayesFactor(qbHyper)}. The function of each of these plots is described below.

1. The plot appearing in the upper-left of the figure represents a plot of the prior distribution for the number of QTL involved in models (shown as a broken blue line) against the corresponding posterior probabilities (shown as a histogram).

2. The plot in the upper-right shows Bayes factor ratios. These are the ratios of posterior probabilities to prior probabilities. For pairs of values along the horizontal axis of this plot, the member of the pair with a larger Bayes factor ratio should be interpreted as more likely. The vertical arrows give an indication of the strength of evidence: weak (BF = 3), moderate (BF = 10) or strong (BF = 30).

3. The second row conveys information in terms of the pattern of chromosomes involved in the models.

4. The third row addresses the frequency of sampling each chromosome.

5. The fourth row show relative importance of epistatic pairs. Here the "6.15", or chr 6 by chr 15, epistatic pair is by far the strongest.

As with other plot functions in the \texttt{R/qtlbim} package, it is possible to limit attention of a subset of chromosomes using the generic \texttt{subset} routine. The \texttt{subset} argument \texttt{pattern} can be used to limit the models plotted to those involving a specified list of chromosomes. For example the command \texttt{qb.BayesFactor(subset(qbHyper,\texttt{pattern=c(2,3,17)}) considers only those models involving chromosomes 2, 3 and 17. Repeats in the pattern sequence indicate multiple QTL on the same chromosome.

7 The Plot Demo

The plot demo (\texttt{demo(qb.plot.tour}) gives a sample of the plots available in the \texttt{R/qtlbim} package. Running the plot demo requires, the \texttt{lattice} package, which is loaded when you attach the \texttt{R/qtlbim} package. To start the plot demo, use the command
\texttt{demo(qb.plot.tour)}

The plot demo begins by giving a generic plot for the \texttt{qb object} \texttt{qbHyper}. The \texttt{R/qtlbim} generic \texttt{qb} plot is analogous to the generic \texttt{R} plot for linear model objects. Where the generic plot for a linear model object shows a sequence of graphics whose purpose is to aid in the initial results of model fitting, the generic plot function for \texttt{qb} objects shows a sequence of graphics whose purpose is to give an initial assessment of the results produced by the MCMC algorithm. The generic plot for the \texttt{qb object} \texttt{qbHyper} created above is shown with the command
\texttt{plot(qbHyper)}

The generic plot function shows a sequence of plots that include time series plots of the mccm chain, jittered plots of QTL by chromosome and others. The sequence of plots appearing in the plot demo is listed below.

The list of plots shown by the generic plot function.

1. A time series plot of the mcmm chain runs. This is shown in Figure ??, where it was created by the command \texttt{plot(qb.coda(qbHyper))}.

2. A jittered plot of QTL by chromosome. This plot is identical to the plot in Figure ?? produced by the command \texttt{plot(qb.loci(qbHyper))}.

3. A model selection plot by chromosome. This plot is identical to \texttt{plot(qb.BayesFactor(qbHyper))} shown in Figure ??.

4. Plot of QTL posterior for loci plus smooth estimates of QTL effects. This plot is the same as the plot generated by \texttt{plot(qb.hpdone(qbHyper))}. Figure ?? shows the result of this command.

5. A plot of epistatic effects if such effects are allowed. Figure ?? shows the result of the command \texttt{plot(qb.epistasis(qbHyper))}.

6. Summary diagnostics as histograms and boxplots by number of QTL. This final diagnostic plot can be generated separately by the command \texttt{plot(qb.diag(qbHyper))}. Figure ?? shows the result of this command.
Figure 3: Paired plots of posteriors as bars overlaid by priors as blue lines (left panels) with Bayes factor ratios to the least likely model (right panels). Models in right panel can be compared by vertical separation as scale is geometric. Blue arrows on right panels indicate weak, moderate or strong Bayes factors for ratios of 3, 10 or 30, respectively. Rows convey information about (1) number of QTL, (2) chromosome pattern of QTL, (3) chromosomes, (4) epistatic pairs.

strong
Figure 4: A paired plot of posterior scan for loci above a scan of marginal genotypic means by locus. In upper panel, black is overall posterior, blue is for main effects and purple is for epistasis. In lower panel, blue is for AA, purple for AB, and red for BB genotype at scanned locus.
Figure 5: A plot of epistatic effect by pair using Cockerham effects. Only stronger epistatic pairs are shown. Blue line at median; box contains 50% of samples for epistatic pair. Percent below pair indicates percent of MCMC samples with this epistatic pair.
Figure 6: A set of diagnostic plots. Default has mean, unexplained variance ("envvar"), explained variance ("var"), and heritability ("herit"). Left panels show density plot and horizontal box plot for all samples. Right panels show box plots by number of QTL.
8 Data Management

8.1 Data Simulation

R/qtlbim has an inbuilt function \texttt{qb.sim.cross} to simulate a backcross or F2 data set of class \texttt{cross} (see R/qtl help pages for details). The following chunk of code generates a data set of 100 individuals of F2 mating design. These individuals are genotyped for 11 not equally spaced markers on 20 chromosomes. There are 7 QTLs, two on chromosome 1 and one each on chromosomes 3,5,7,10 and 19. QTL numbers 1.3 and 4 have additive main effects of 0.5, -0.5 and 0.5 and numbers 2 and 4 have dominant main effects of 0.5 and -0.5. QTL numbers 4 and 5 have an additive-additive interaction of -0.7 and numbers 6 and 7 have an additive-dominant interaction of 1.2. Two covariates, a binary fixed covariate and an ordinal random are generated with their corresponding coefficients as 0.5 and 0.07. G x E (gene x environment) interaction is also considered with the fixed covariate. A normal phenotype and an ordinal phenotype with 3 categories are measured. 7% of the genotypes are randomly missing.

```r
> cross <- qb.sim.cross(len = rep(100, 20), n.mar = 11,
+ eq.spacing = F, n.ind = 100, type = "f2",
+ ordinal = c(0.3, 0.3, 0.2, 0.2), missing.geno = 0.03,
+ missing.pheno = 0.07, qtl.pos = rbind(c(1,
+ 15), c(1, 45), c(3, 12), c(5, 15), c(7,
+ 15), c(10, 15), c(12, 35), c(19, 15)),
+ qtl.main = rbind(c(1, 0.5, 0), c(2, 0, 0.5),
+ c(3, -0.5, 0), c(4, 0.5, -0.5)), qtl.epis = rbind(c(4,
+ 5, -0.5, 0, 0, 0), c(6, 8, 0, 1.2, 0,
+ 0)), covariate = c(0.5, 0.07), gbye = rbind(c(7,
+ 0.8, 0)))
```

By using the function \texttt{qb.sim.cross} a list is attached to cross object named "qtl". This list is typically not a part of the \texttt{cross} object as described in \texttt{read.cross} of the R/qtl library and is generated only with the \texttt{qb.sim.cross()} function.

```r
> names(cross)
[1] "geno"  "pheno"  "qtl"
```

The \texttt{cross$qtl} contains information about the true values which can be compared to after the analysis.

```r
> summary(cross$qtl)
```

\$pos

<table>
<thead>
<tr>
<th>chr</th>
<th>pos</th>
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</thead>
<tbody>
<tr>
<td>qtl.1</td>
<td>1 15</td>
</tr>
<tr>
<td>qtl.2</td>
<td>2 45</td>
</tr>
<tr>
<td>qtl.3</td>
<td>3 12</td>
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<td>5 15</td>
</tr>
<tr>
<td>qtl.5</td>
<td>7 15</td>
</tr>
<tr>
<td>qtl.6</td>
<td>10 15</td>
</tr>
<tr>
<td>qtl.7</td>
<td>12 35</td>
</tr>
<tr>
<td>qtl.8</td>
<td>19 15</td>
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</table>

\$herit.main

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</thead>
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<td></td>
</tr>
<tr>
<td>main.2</td>
<td>0.06077263 0.00000000</td>
<td></td>
</tr>
<tr>
<td>main.3</td>
<td>0.06201289 0.00000000</td>
<td></td>
</tr>
<tr>
<td>main.4</td>
<td>0.06201289 0.0310644</td>
<td></td>
</tr>
</tbody>
</table>

\$herit.epis

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<th>qtl.b</th>
<th>aa</th>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>epis.2</td>
<td>6 8 0.00000000 0.08929856 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
$herit.cov
  fix.cov  ran.cov
0.03100644 0.03472722

$herit.gbye
  qtl  add dom
GxE.1 7 0.03968825 0

The summary of the cross object summary is shown below.

> summary(cross)

  F2 intercross

  No. individuals: 100
  No. phenotypes: 4
  Percent phenotyped: 91 97 91 94

  No. chromosomes: 20
  Autosomes: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

  Total markers: 220
  Percent genotyped: 97
  Genotypes (%): AA:26.1 AB:49.3 BB:24.6 not BB:0 not AA:0

### 8.2 Cleaning Up

Often we want to keep a qb object around for some time to examine various diagnostics. While we might just use the R command `rm` or `remove` to remove the internal object, it is wise to instead use the `R/qtlbim` command `qb.remove` to remove internal and external files. Recall that `qb.mcmc` creates an external directory with a unique name, containing tens of Mb of MCMC samples. The proper way to remove the object `qbSim` is as follows:

```r
qb.remove(qbSim)
```

[If you use `remove.qb` on `qbHyper`, you will also remove it from your installed library, so be careful!]

It is possible to recover an orphaned external `qb` directory, say if an R session is corrupted or the internal `qb` object is inadvertently removed. See the manual page on `qb.recover` for further information.