Prototype QTL Strategy: Phenotype bp in Cross hyper

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1 Overview

This document analyzes trait bp for dataset hyper using the 1-D and 2-D Bayesian genome scan routines that build on Markov chain Monte Carlo (MCMC) samples from a posterior for the genetic architecture of a trait. Below the generic `cross` is actually the cross passed by the user in a call to `qb.sweave`. This entire document was created automatically by a function call in `R`. The function is not yet part of `R/qtlbim`. The actual call was

```
> library(qtlbim)

> qb.sweave(hyper, pheno.col = 1,
+  n.iter = 3000, n.draws = 64,
+  scan.type = "2logBF", hpd.level = 0.5,
+  threshold = c(upper = 2),
+  SweaveFile = "/tmp/Rinst2351176914/qtlbim/doc/hyperslide.Rnw",
+  SweaveExtra = "/tmp/Rinst2351176914/qtlbim/external/hyperpaperextra.Rnw",
+  PDFDir = "bpPDF",
+  remove.qb = TRUE)
```

At present, the `threshold` values are somewhat arbitrary, chosen for the hyper dataset to pick up apparently real QTL and previously detected epistasis.

This document automates a search for main and epistatic QTL. The main QTL positions are reliably estimated using `qb.scanone`. This pass also seems to capture the major chromosomes possibly involved in epistasis, although it does not provide very good estimates of positions of purely epistatic QTL within those chromosomes. Next we use `qb.scantwo` and to identify which pairs of QTL are epistatic, and to get initial estimates of their positions. We refine there positions with `qb.slice`. Along the way, we use generic `summary` and `plot` routines to view results.

Once we have reasonable estimates of QTL postions and effects, we use confirmatory ANOVA tools to refine the model. That is, we use R/qtl's simulation-based `fitqtl` followed by a stepwise backward fitting approach, using a new `step.fitqtl`, to confirm key QTL. That completes this automated analysis. It would be possible to add other, user-supplied refined analysis at the end of this document if desired.

2 Generating Samples

Here is a summary of the `cross` copy of the hyper object, followed by creation of 3000 MCMC samples.

```
> summary(cross)

    Backcross

    No. individuals:    250

    No. phenotypes:     1
```

```
    Percent phenotyped: 100

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

    Total markers:      170
    No. markers:        22 8 6 20 14 11 7 6 5 5 14 5 5 5 11 6 12 4 4
    Percent genotyped:  47.9
    Genotypes (%):      AA:50.1  AB:49.9
```

```
> cross <- qb.genoprob(cross,step=2)
> cross.qb <- qb.mcmc(cross, pheno.col = pheno.col
+   genoupdate=TRUE, n.iter = 3000, mydir = "bpPDF")
```

3 1-D Scans

Now a 1-D scan picks out the major effects. We could uxe `qb.scanone` directly. Instead, we use `qb.hpdone`, which gives us a profile scan as well as a scan of genotypic means.

```
> hpd.level
```

```
[1] 0.5
```

```
> cross.hpd <- qb.hpdone(cross.qb, hpd.level)
> sum.one <- summary(cross.hpd)
> sum.one
```

```
     chr n.qtl  pos lo.50% hi.50% 2logBF       A       H
<NA>   1 0.694 64.5   64.5   69.9  6.796 103.604  99.073
<NA>   4 3.460 29.5   25.1   31.7 11.347 104.561  98.026
<NA>   6 1.107 59.0   56.8   66.7  6.179  99.606 102.924
<NA>  15 0.341 17.5   17.5   17.5  6.032 101.940 100.692
```

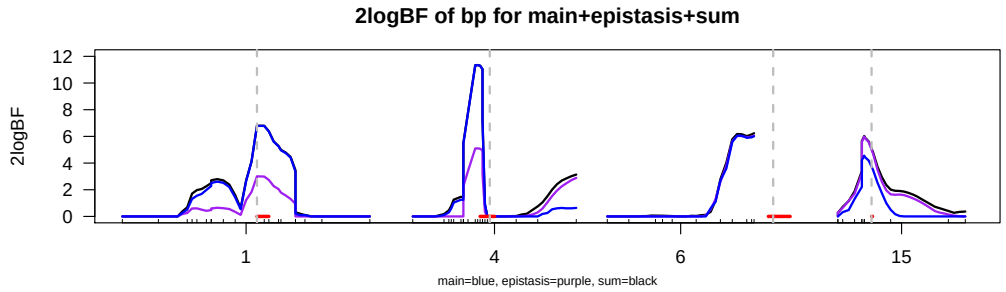
The `qb.hpdone` routine mostly automates the selection of peaks. We are still working on how to set reasonable thresholds, but for now a HPD level of 0.5 for the overall (`sum`) picks up the key features nicely for many phenotypes. The variable `hpd.level` was set in the call to `qb.sweave` that created this document. The new variables `chrs` and `pos` capture the chromosome numbers and main QTL positions, respectively.

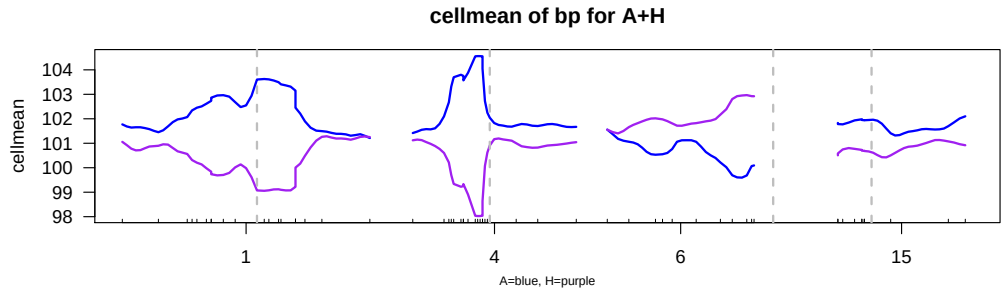
```
> chrs <- as.vector(sum.one[, "chr"])
> pos <- sum.one[, "pos"]
> pos
```

```
<NA> <NA> <NA> <NA>
64.5 29.5 59.0 17.5
```

The following two figures highlight the selected chromosomes. In the top panel, the blue curve represents the `2logBF` (twice log of Bayes factor) score for the `main` effect of a QTL at each locus, while the purple curve shows the score for any `epistasis` between a locus and any other loci. The black curve shows the combination of main and epistatic effects. The second panel shows the marginal means, which are roughly symmetric about the phenotype mean of 101.

```
> plot(cross.hpd)
```





4 2-D Scan

Now a look at a 2-D scan reveals the strength of epistasis. The summary suggests some other epistases, but some of this may be spurious [i.e. we will want to look further!]. We consider a subset of this summary above the `upper` threshold of 2.

```
> two <- qb.scantwo(cross.qb, chr = chrs, type = scan.type)
> sum.two <- summary(two, threshold = threshold, refine = TRUE)
> sum.two
```

```
      chr1 chr2 n.qtl l.pos1 l.pos2  lower u.pos1 u.pos2  upper
1:1      1    1 0.362   43.7   77.6  7.583 43.700 75.400  4.697
1:4      1    4 1.352   67.8   29.5 15.705 72.100 29.500  7.303
1:6      1    6 1.831   67.8   59.0 12.611 88.867  4.900  4.756
1:15     1   15 1.145   67.8   17.5 12.012 77.600 17.500  5.794
4:4      4    4 0.298   29.5   74.3 11.820  2.029 30.600  4.756
4:6      4    6 1.561   29.5   66.7 14.884 74.300 59.000  7.728
4:15     4   15 0.446   29.5   17.5 14.539 74.300 35.546  7.350
6:6      6    6 1.214   61.2   66.7  7.442 27.300 66.700  4.756
6:15     6   15 1.080   59.0   17.5 12.779 59.000 17.500 12.751
15:15   15   15 0.105   17.5   27.5  8.125 17.500 25.500  7.234
```

Now let's extract the genetic architecture implied by this evidence for epistasis. The loci pairs thar show epistasis are indexed to the vector of main QTL. In addition we see the pairs of chromosomes involved in epistasis. Some effort is made to merge nearby QTL estimated positions in `qb.arch`.

```
> cross.arch <- qb.arch(sum.two, chrs, pos)
> cross.arch
```

```
main QTL loci:
       1    2     3    4     5    6   7    8     9   10    11
chr  1.0  1.0  1.00 4.00  4.00  4.0 6.0  6.0  6.00 15.0 15.00
pos 43.7 72.4 88.87 2.03 29.87 74.3 4.9 27.3 60.92 19.1 35.55
```

```
Epistatic pairs by qtl, chr, pos:
  qtla qtlb chra chrb  posa  posb
1    1    2    1    1 43.70 72.40
2    2    5    1    4 72.40 29.87
3    3    7    1    6 88.87  4.90
4    2   10    1   15 72.40 19.10
5    4    5    4    4  2.03 29.87
6    6    9    4    6 74.30 60.92
7    6   11    4   15 74.30 35.55
8    8    9    6    6 27.30 60.92
9    9   10    6   15 60.92 19.10
Epistatic chromosomes by connected sets:
1,4,6,15
```

5 ANOVA Model Fit

Here we want to merge the 1-D `chrs` and `pos` with the 2-D epistatic pairs to determine the chromosomes and positions to include in an ANOVA fit. We equate QTL that are within, say 10cM of each other. After fitting a (very) full model, we use `step.fitqtl`, a newly written routine, to step-by-step reduce the model to key main effects and interactions, preserving hierarchy.

The following uses R/qtl tools `calc.genoprob`, `sim.geno` and `makeqtl`, plus the new `step.fitqtl`, which calls `fitqtl` multiple times.

```
> cross.sub <- subset(cross, chr = cross.arch$qtl$chr)
> n.draws

[1] 64

> cross.sub <- sim.geno(cross.sub, n.draws = n.draws, step = 2,
+     error = 0.01)
> qtl <- makeqtl(cross.sub, cross.arch$qtl$chr, cross.arch$qtl$pos)
```

Now we run stepwise backward elimination, preserving hierarchy.

```
> cross.step <- step.fitqtl(cross.sub, qtl, pheno.col, cross.arch)

  drop                  LOD     p
1 Chr4@2.03:Chr4@29.87 -0.0135 1.000
2 Chr6@27.3:Chr6@60.92  0.1030 0.509
3 Chr6@27.3             0.1290 0.459
4 Chr4@2.03             0.1690 0.396
5 Chr1@43.7:Chr1@72.4   0.2950 0.261
6 Chr1@72.4:Chr15@19.1  0.3270 0.236
7 Chr1@72.4:Chr4@29.87  0.7050 0.081

> summary(cross.step$fit)

Full model result
----------------------------------

       df        SS        MS      LOD     %var Pvalue(Chi2) Pvalue(F)
Model  13  7820.110 601.54691 31.72824 44.25909            0         0
Error 236  9848.827  41.73232
Total 249 17668.936
```

```
Drop one QTL at a time ANOVA table:
---------------------------------
                       df Type III SS      LOD     %var F value Pvalue(F)
Chr1@43.7               1     312.760    1.697    1.770   7.494  0.006660 **
Chr1@72.4               1     365.954    1.981    2.071   8.769  0.003377 **
Chr1@88.87              2     266.939    1.452    1.511   3.198  0.042611 *
Chr4@29.87              1    2177.490   10.844   12.324  52.178  6.95e-12 ***
Chr4@74.3               3    1064.637    5.572    6.025   8.504  2.19e-05 ***
Chr6@4.9                2     290.401    1.578    1.644   3.479  0.032419 *
Chr6@60.92              3    1932.361    9.726   10.936  15.435  3.35e-09 ***
Chr15@19.1              2    1464.936    7.528    8.291  17.552  7.83e-08 ***
Chr15@35.55             2     582.125    3.117    3.295   6.975  0.001141 **
Chr1@88.87:Chr6@4.9     1     246.387    1.341    1.394   5.904  0.015854 *
Chr4@74.3:Chr6@60.92    1     324.445    1.760    1.836   7.774  0.005731 **
Chr4@74.3:Chr15@35.55   1     553.170    2.967    3.131  13.255  0.000334 ***
Chr6@60.92:Chr15@19.1   1    1314.184    6.800    7.438  31.491  5.60e-08 ***
```

The final model may be more complicated than a model found 'by hand' using standard R/qtl tools. Hopefully that model is a subset of the automatically found model.

6 2-D Epistasis Plots

Should there be any evidence for epistasis that is confirmed by ANOVA, it can be useful to view 2-D plots similar to `scantwo`, but now using the marginal 2logBF. Here is the reduced, final genetic architecture:

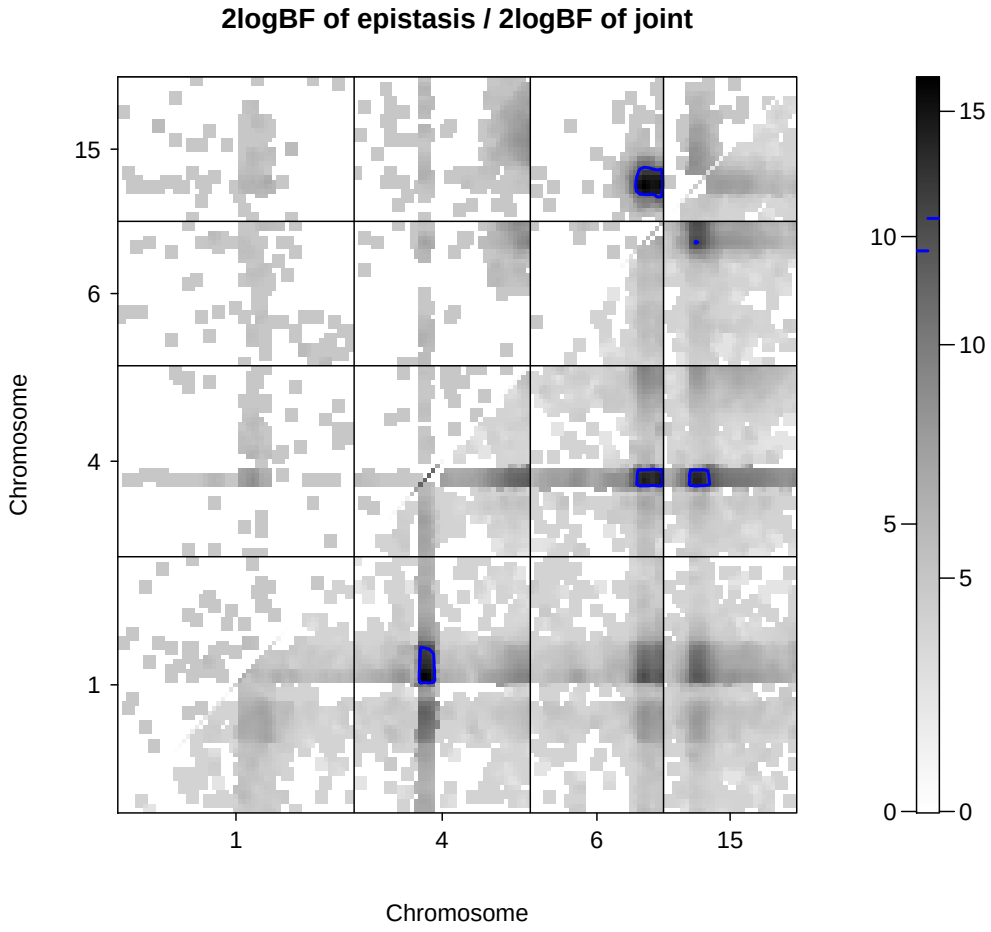
```
> cross.arch <- cross.step$arch
> cross.arch

main QTL loci:
       1    2     3     5    6   7     9   10    11
chr  1.0  1.0  1.00  4.00  4.0 6.0  6.00 15.0 15.00
pos 43.7 72.4 88.87 29.87 74.3 4.9 60.92 19.1 35.55

Epistatic pairs by qtl, chr, pos:
  q1 q2 chra chrb  posa  posb
1  3  7    1    6 88.87  4.90
2  6  9    4    6 74.30 60.92
3  6 11    4   15 74.30 35.55
4  9 10    6   15 60.92 19.10
Epistatic chromosomes by connected sets:
1,4,6,15
```

Here are the plots by clique (if any).

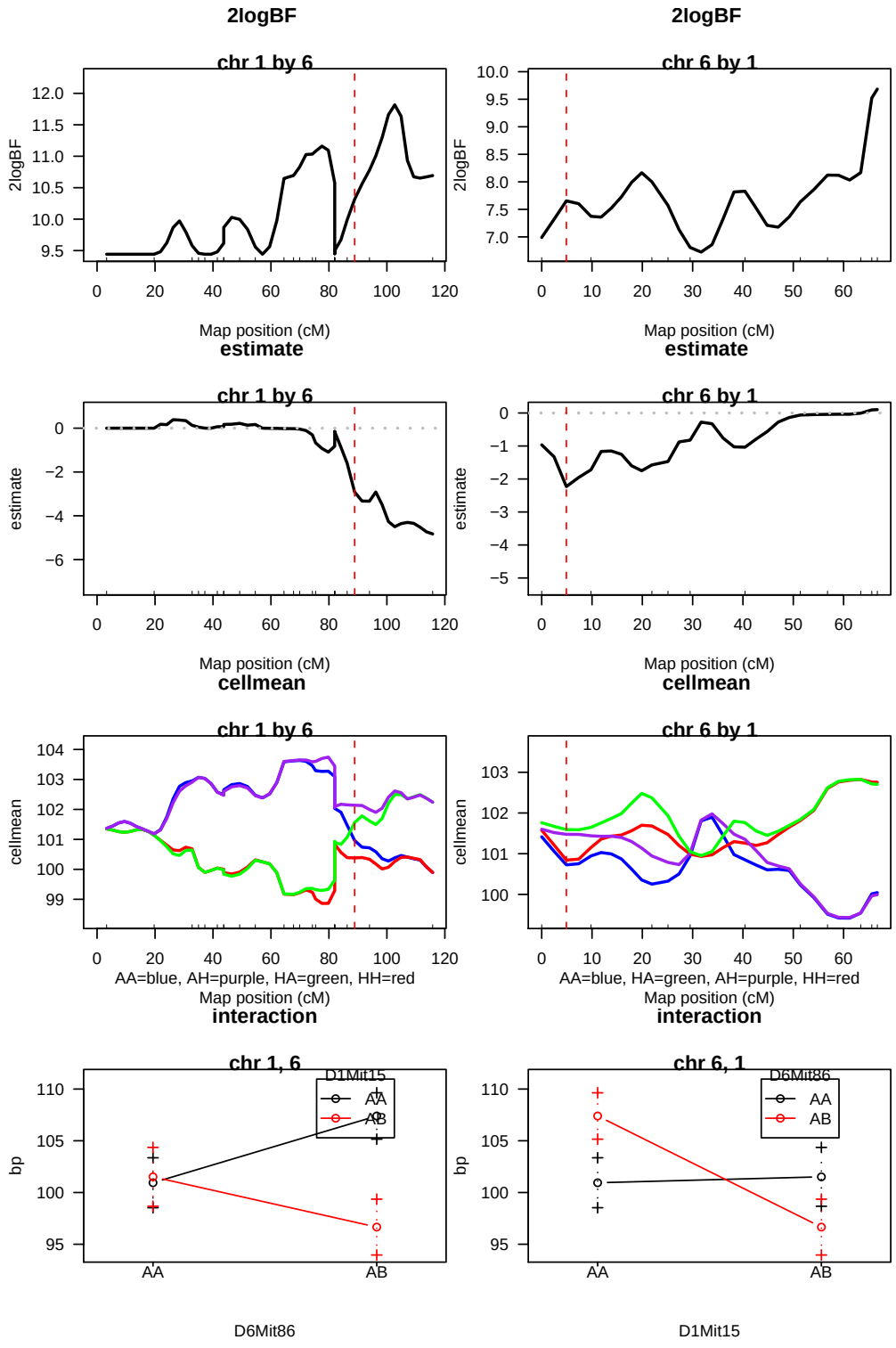
```
> for(i in names(cross.arch$chr.by.set))
+   plot(two, chr = cross.arch$chr.by.set[[i]], smooth = 3,
+     col = "gray", contour = 3)
```

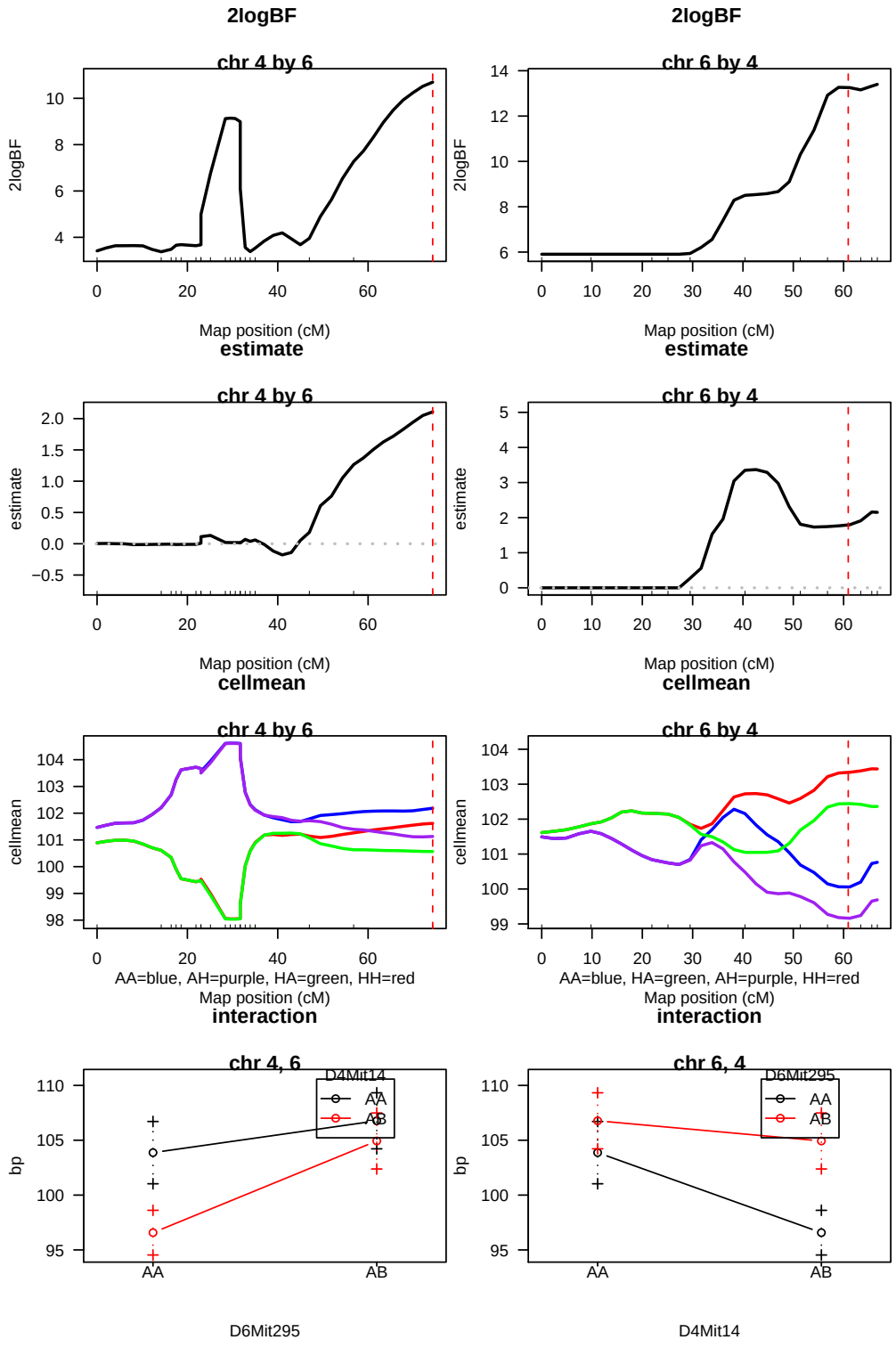


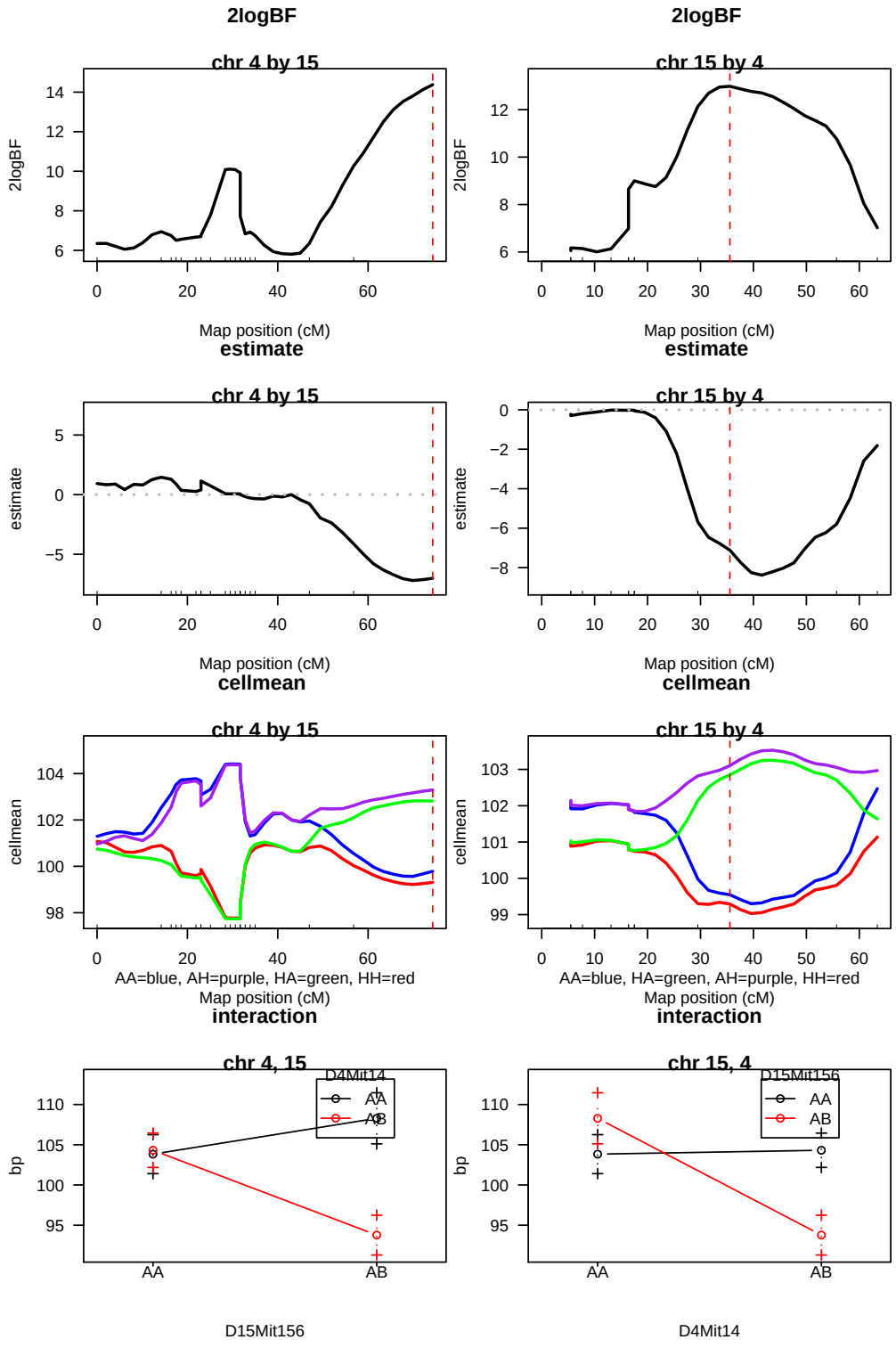
7 1-D Epistasis Slices

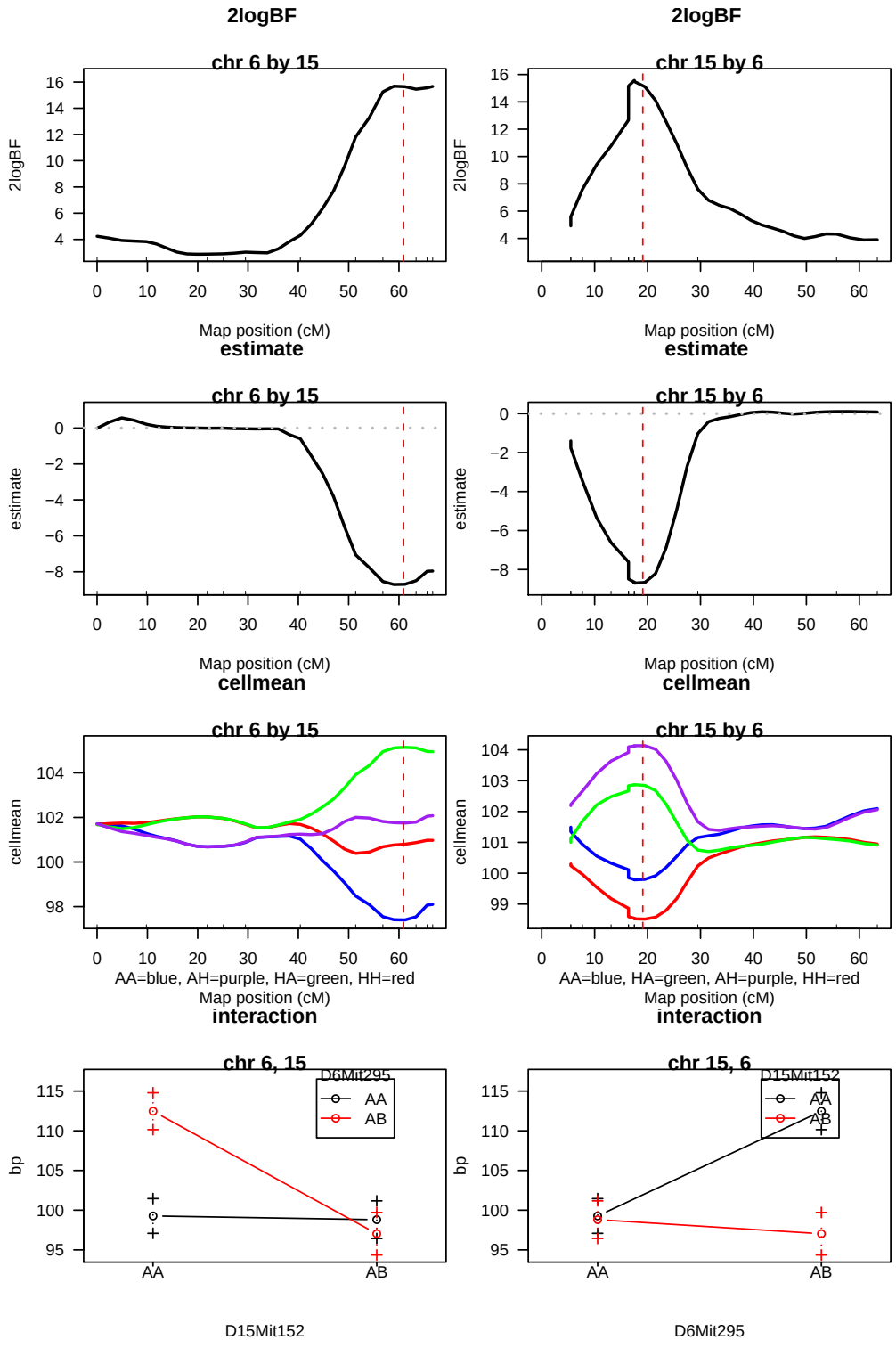
We then examine 1-D slices through the 2-D surface for epistatic pairs in the reduced model, to focus on epistasis for those identified pairs. We show 1-D slices of the LOD and the epistatic effects. In addition, we show interaction plots at the nearest markers.

```
> if(!is.null(cross.arch$pair.by.chr)) {
+  for(i in seq(nrow(cross.arch$pair.by.chr$chr))) {
+    chri <- cross.arch$pair.by.chr$chr[i,]
+    posi <- cross.arch$pair.by.chr$pos[i,]
+    plot(qb.slicetwo(cross.qb, chri, posi, scan.type), byrow = FALSE)
+  }
+}
```


2logBF
chr 1 by 6
2logBF
Map position (cM)
2logBF
chr 6 by 1
2logBF
Map position (cM)
estimate
chr 1 by 6
estimate
Map position (cM)
estimate
chr 6 by 1
estimate
Map position (cM)
cellmean
chr 1 by 6
cellmean
AA=blue, AH=purple, HA=green, HH=red
Map position (cM)
cellmean
chr 6 by 1
cellmean
AA=blue, HA=green, AH=purple, HH=red
Map position (cM)
interaction
chr 1, 6
D1Mit15
AA
AB
bp
AA
AB
D6Mit86
interaction
chr 6, 1
D6Mit86
AA
AB
bp
AA
AB
D1Mit15


2logBF
chr 4 by 6
2logBF
Map position (cM)
2logBF
chr 6 by 4
Map position (cM)
estimate
chr 4 by 6
estimate
Map position (cM)
estimate
chr 6 by 4
Map position (cM)
cellmean
chr 4 by 6
cellmean
AA=blue, AH=purple, HA=green, HH=red
Map position (cM)
cellmean
chr 6 by 4
AA=blue, HA=green, AH=purple, HH=red
Map position (cM)
interaction
chr 4, 6
D4Mit14
AA
AB
bp
D6Mit295
interaction
chr 6, 4
D6Mit295
AA
AB
bp
D4Mit14


2logBF
chr 4 by 15
2logBF
Map position (cM)
2logBF
chr 15 by 4
2logBF
Map position (cM)
estimate
chr 4 by 15
estimate
Map position (cM)
estimate
chr 15 by 4
estimate
Map position (cM)
cellmean
chr 4 by 15
cellmean
AA=blue, AH=purple, HA=green, HH=red
Map position (cM)
cellmean
chr 15 by 4
cellmean
AA=blue, HA=green, AH=purple, HH=red
Map position (cM)
interaction
chr 4, 15
D4Mit14
AA
AB
bp
AA
AB
D15Mit156
interaction
chr 15, 4
D15Mit156
AA
AB
bp
AA
AB
D4Mit14



8 User Customized Section

We know from previous work that there are main QTLs on chromosomes 1 and 4, and epistatic pairs involving 6 and 15, and 7 and 15. Here we pick the nested model that contains these QTL.

```
> arch3 <- qb.arch(cross.step, main = c(1, 4), epistasis = data.frame(q1 = c(6,
+     7), q2 = rep(15, 2)))
> arch3
> cross.step2 <- step.fitqtl(cross.sub, qtl, pheno.col, arch3)

> summary(cross.step2$fit)
```

Now we do a formal comparison of this reduced model with the fuller model we automatically uncovered. It appears that the fuller model is a much better fit.

```
> anova(cross.step, cross.step2)
```

9 Cleaning Up

That completes the template. Now the penultimate task is to remove the objects created by `R/qtlbim`, if this is desired by the user.

```
> remove.qb

[1] FALSE

> if (remove.qb) {
+     qb.remove(cross.qb)
+     rm(cross, cross.sub, pheno.col, threshold, n.iter, n.draws,
+         remove.qb)
+ }
```

Finally, externally rename file hyperslide.tex to bp.tex and run pdflatex twice on it. Use a free Acrobat reader to view.

```
> file.rename("hyperslide.tex", "bp.tex")
> invisible(system("pdflatex bp.tex",intern=TRUE))
>  invisible(system("pdflatex bp.tex",intern=TRUE))
```