R/qtlbim Software Demo

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what should software be like?

- intuitive
  - easy, visual (pull-down menus, GUI)
  - obvious names (typed commands, CLI)
- high throughput / production mode
  - easy to process many tasks
  - few steps requiring decisions
- adaptable to new needs
  - extensible (able to add new functionality)
  - easy to document

how does one build tools?

- no one solution for all situations
- use existing tools wherever possible
  - new tools take time and care to build!
  - downloaded databases must be updated regularly
  - need bridges (interfaces) between tools
- human component is key
  - need informatics expertise
  - need continual dialog with biologists
  - continually rethink, redesign software architecture

why build tools?

- common storage / maintenance of data
  - one well curated copy
  - central repository
  - reduce errors, ensure analysis on same data
- automate commonly used methods
  - biologist gets immediate feedback
  - statistician can focus on new methods
  - codify standard choices
- platform independent (Windows, Mac, Linux)

why use R?

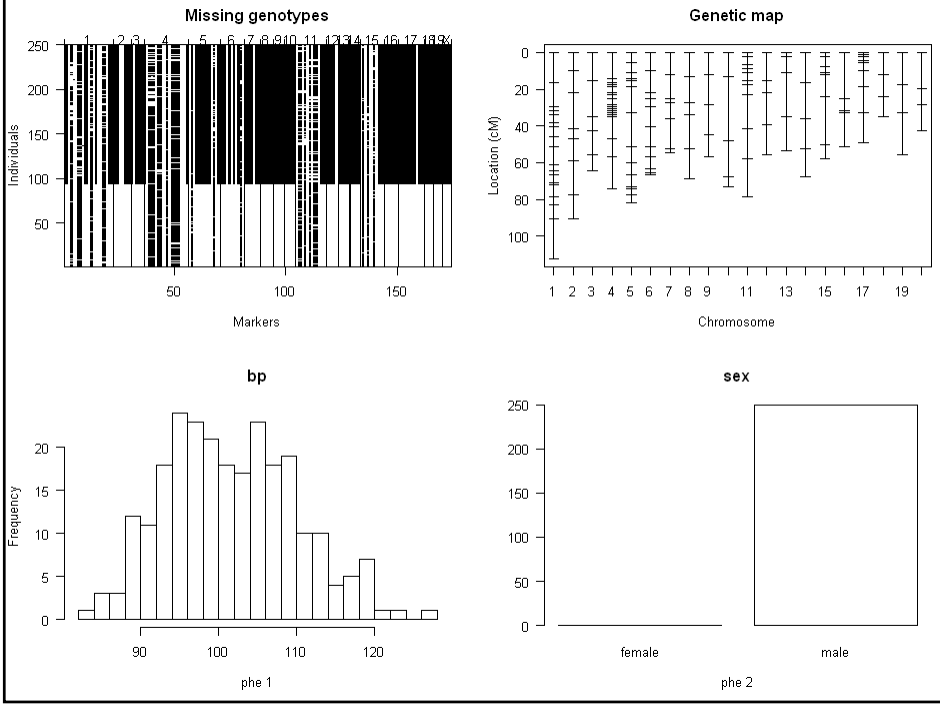
- language environment for data analysis
  - platform independent
  - used worldwide by statisticians
  - growing acceptance among biologists
  - extensible and easy to document new tools
- command line interface (CLI)
  - challenging for biologists used to GUI
  - copy and modify example scripts (rip & burn)
  - quickly redo analysis if (when) data changes
  - readily modify scripts for production mode

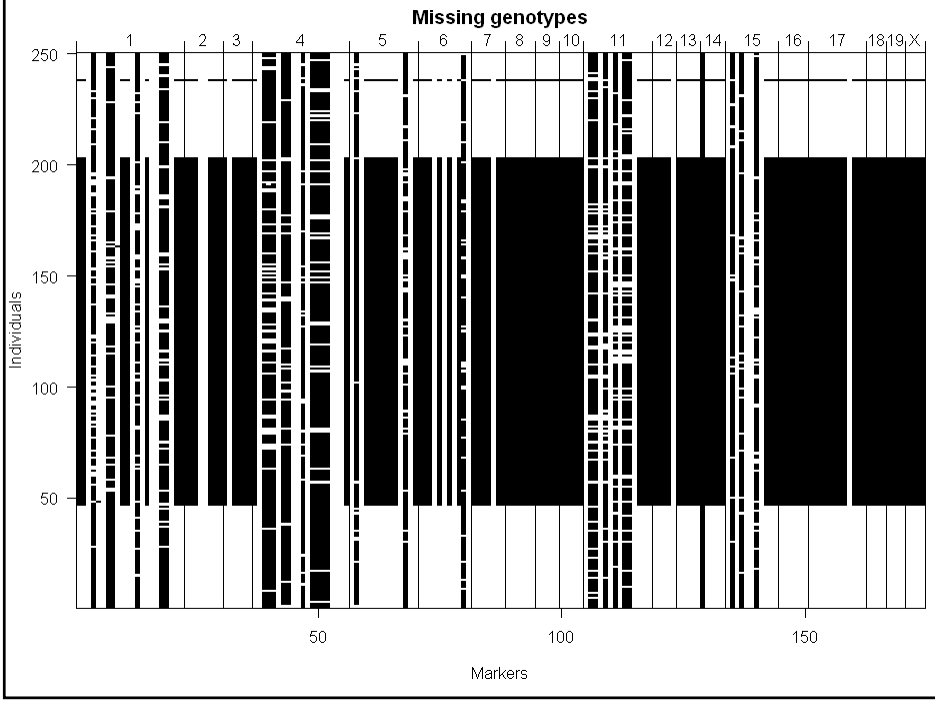
R/qtl & R/qtlbim Tutorials

- R statistical graphics & language system
- R/qtl tutorial
  - R/qtl web site: www.rqtl.org
  - Tutorial: www.rqtl.org/tutorials/rqtltour.pdf
  - R code: www.stat.wisc.edu/~yandell/qtlbim/rqtltour.R
- R/qtlbim tutorial
  - R/qtlbim web site: www.qtlbim.org
  - Tutorial and R code:
    - www.stat.wisc.edu/~yandell/qtlbim/rqtlbimtour.pdf
    - www.stat.wisc.edu/~yandell/qtlbim/rqtlbimtour.R

R/qtl tutorial (www.rqtl.org)

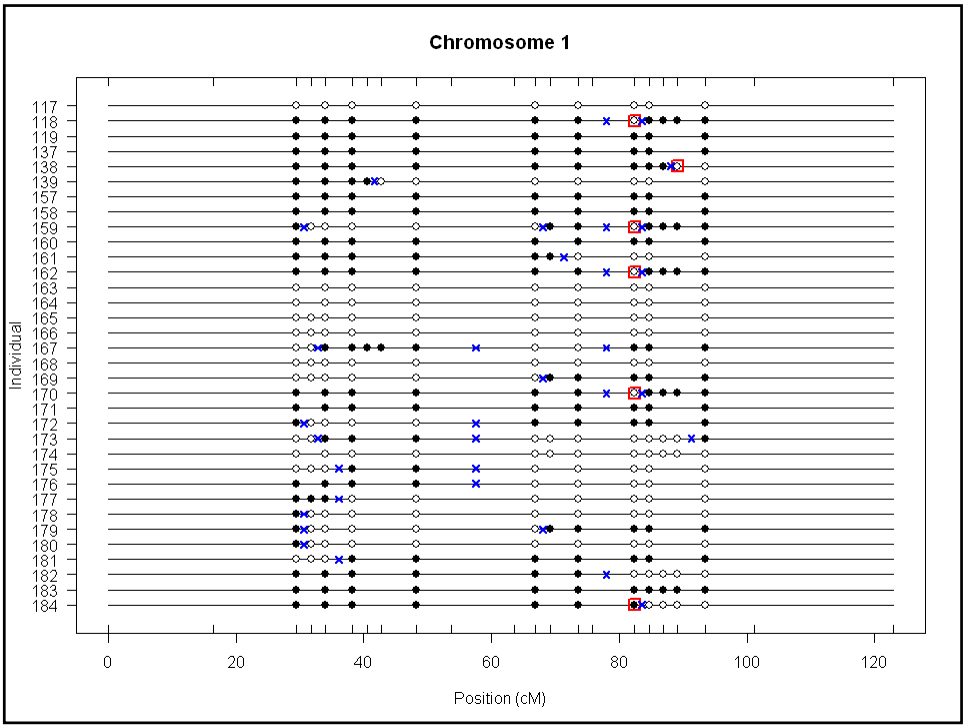
```
> library(qtl)
> data(hyper)
> summary(hyper)
    Backcross

    No. individuals:    250

    No. phenotypes:     2
    Percent phenotyped: 100 100

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

    Total markers:      174
    No. markers:        22 8 6 20 14 11 7 6 5 5 14 5 5 5 11 6 12 4 4 4
    Percent genotyped:  47.7
    Genotypes (%):      AA:50.2  AB:49.8
> plot(hyper)
> plot.missing(hyper, reorder = TRUE)
```



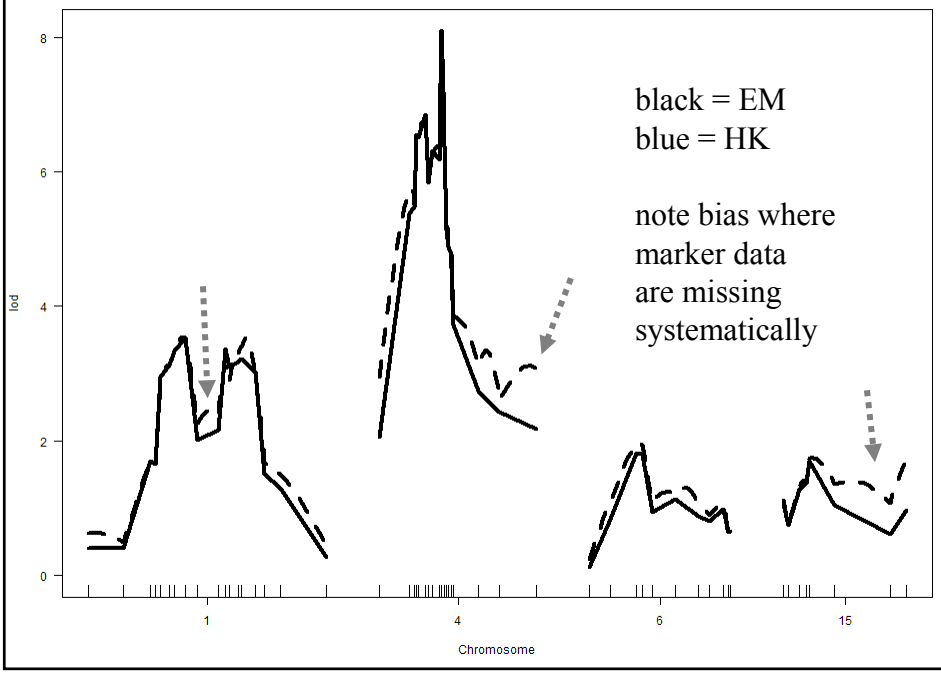
R/qtl: find genotyping errors

```
> hyper <- calc.errorlod(hyper, error.prob=0.01)
> top.errorlod(hyper)

   chr  id    marker errorlod
1    1 118   D1Mit14 8.372794
2    1 162   D1Mit14 8.372794
3    1 170   D1Mit14 8.372794
4    1 159   D1Mit14 8.350341
5    1  73   D1Mit14 6.165395
6    1  65   D1Mit14 6.165395
7    1  88   D1Mit14 6.165395
8    1 184   D1Mit14 6.151606
9    1 241   D1Mit14 6.151606
...
16   1 215  D1Mit267 5.822192
17   1 108  D1Mit267 5.822192
18   1 138  D1Mit267 5.822192
19   1 226  D1Mit267 5.822192
20   1 199  D1Mit267 5.819250
21   1  84  D1Mit267 5.808400

> plot.geno(hyper, chr=1, ind=c(117:119,137:139,157:184))
```



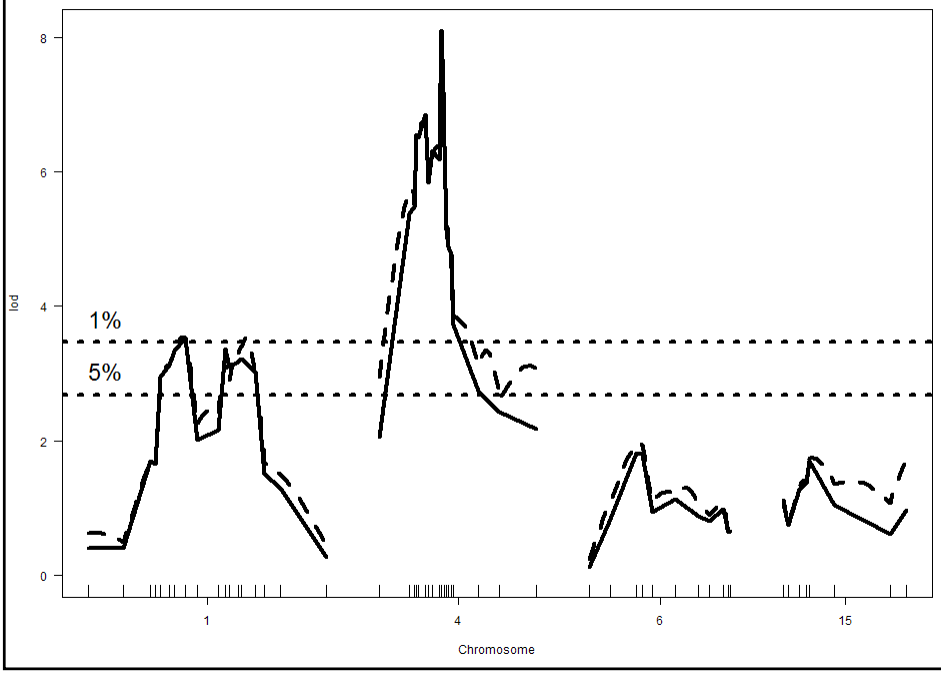
R/qtl: 1 QTL interval mapping

```
> hyper <- calc.genoprob(hyper, step=1,
   error.prob=0.01)
> out.em <- scanone(hyper)
> out.hk <- scanone(hyper, method="hk")
> summary(out.em, threshold=3)
          chr  pos  lod
c1.loc45    1 48.3 3.52
D4Mit164    4 29.5 8.02

> summary(out.hk, threshold=3)
          chr  pos  lod
c1.loc45    1 48.3 3.55
D4Mit164    4 29.5 8.09

> plot(out.em, chr = c(1,4,6,15))
> plot(out.hk, chr = c(1,4,6,15), add = TRUE, lty = 2)
```



R/qtl: permutation threshold

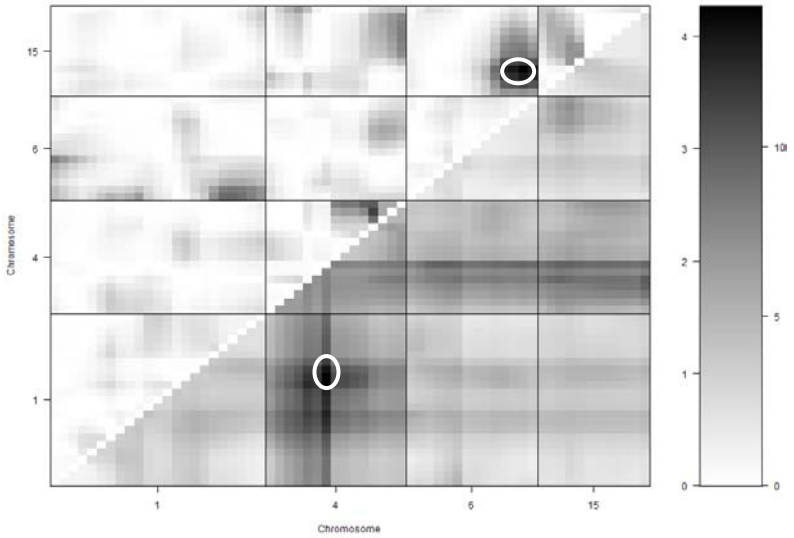
```
> operm.hk <- scanone(hyper, method="hk",
  n.perm=1000)
Doing permutation in batch mode ...
> summary(operm.hk, alpha=c(0.01,0.05))
LOD thresholds (1000 permutations)
    lod
1% 3.79
5% 2.78

> summary(out.hk, perms=operm.hk, alpha=0.05,
  pvalues=TRUE)
  chr  pos  lod  pval
1   1 48.3 3.55 0.015
2   4 29.5 8.09 0.000
```



R/qtl: 2 QTL scan

```
> hyper <- calc.genoprob(hyper, step=5, error.prob=0.01)
>
> out2.hk <- scantwo(hyper, method="hk")
 --Running scanone
 --Running scantwo
 (1,1)
 (1,2)
...
 (19,19)
 (19,X)
 (X,X)
> summary(out2.hk, thresholds=c(6.0, 4.7, 4.4, 4.7, 2.6))

        pos1f pos2f lod.full lod.fv1 lod.int     pos1a pos2a lod.add lod.av1
c1 :c4   68.3  30.0    14.13    6.51   0.225      68.3  30.0   13.90   6.288
c2 :c19  47.7   0.0     6.71    5.01   3.458      52.7   0.0    3.25   1.552
c3 :c3   37.2  42.2     6.10    5.08   0.226      37.2  42.2    5.87   4.853
c6 :c15  60.0  20.5     7.17    5.22   3.237      25.0  20.5    3.93   1.984
c9 :c18  67.0  37.2     6.31    4.79   4.083      67.0  12.2    2.23   0.708
c12:c19   1.1  40.0     6.48    4.79   4.090       1.1   0.0    2.39   0.697

> plot(out2.hk, chr=c(1,4,6,15))
```

upper triangle/left scale: epistasis LOD
lower triangle/right scale: 2-QTL LOD



R/qtl: ANOVA imputation at QTL

```
> hyper <- sim.geno(hyper, step=2, n.draws=16, error.prob=0.01)
> qtl <- makeqtl(hyper, chr = c(1, 1, 4, 6, 15), pos = c(50, 76, 30, 70, 20))

> my.formula <- y ~ Q1 + Q2 + Q3 + Q4 + Q5 + Q4:Q5
> out.fitqtl <- fitqtl(hyper, pheno.col = 1, qtl, formula = my.formula)
> summary(out.fitqtl)

Full model result
----------------------------------
Model formula is:  y ~ Q1 + Q2 + Q3 + Q4 + Q5 + Q4:Q5

       df        SS        MS      LOD     %var Pvalue(Chi2) Pvalue(F)
Model   6  5789.089 964.84822 21.54994 32.76422            0         0
Error 243 11879.847  48.88826
Total 249 17668.936

Drop one QTL at a time ANOVA table:
----------------------------------
                 df Type III SS      LOD     %var F value Pvalue(F)
Chr1@50           1     297.149    1.341    1.682   6.078   0.01438 *
Chr1@76           1     520.664    2.329    2.947  10.650   0.00126 **
Chr4@30           1    2842.089   11.644   16.085  58.134  5.50e-13 ***
Chr6@70           2    1435.721    6.194    8.126  14.684  9.55e-07 ***
Chr15@20          2    1083.842    4.740    6.134  11.085  2.47e-05 ***
Chr6@70:Chr15@20  1     955.268    4.199    5.406  19.540  1.49e-05 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

selected R/qtl publications

www.stat.wisc.edu/~yandell/statgen

- www.rqtl.org
- tutorials and code at web site
  - www.rqtl.org/tutorials
- Broman et al. (2003 *Bioinformatics*)
  - R/qtl introduction
- Broman (2001 *Lab Animal*)
  - nice overview of QTL issues

R/qtlbim (www.qtlbim.org)

- cross-compatible with R/qtl
- model selection for genetic architecture
  - epistasis, fixed & random covariates, GxE
  - samples multiple genetic architectures
  - examines summaries over nested models
- extensive graphics

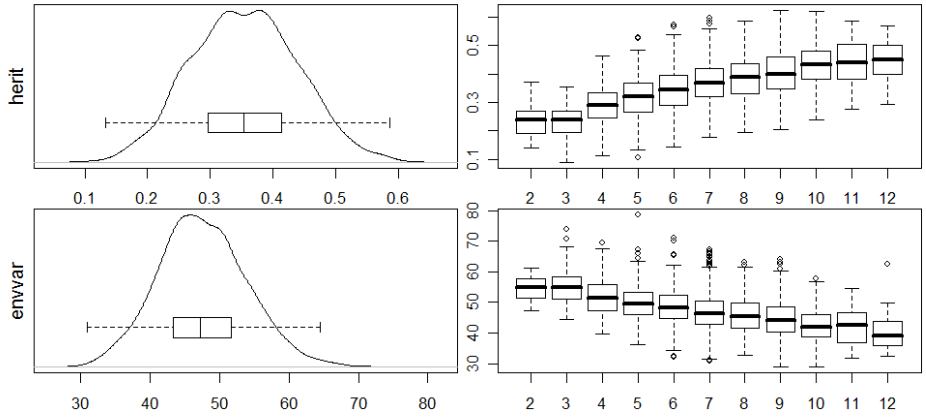
R/qtlbim: tutorial
(www.stat.wisc.edu/~yandell/qtlbim)

```
> data(hyper)
## Drop X chromosome (for now).
> hyper <- subset(hyper, chr=1:19)
> hyper <- qb.genoprob(hyper, step=2)
## This is the time-consuming step:
> qbHyper <- qb.mcmc(hyper, pheno.col = 1)
## Here we get stored samples.
> data(qbHyper)
> summary(qbHyper)
```


R/qtlbim: initial summaries

```
> 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.

Diagnostic summaries:
          nqtl    mean envvar varadd  varaa    var
Min.     2.000  97.42  28.07  5.112  0.000  5.112
1st Qu.  5.000 101.00  44.33 17.010  1.639 20.180
Median   7.000 101.30  48.57 20.060  4.580 25.160
Mean     6.543 101.30  48.80 20.310  5.321 25.630
3rd Qu.  8.000 101.70  53.11 23.480  7.862 30.370
Max.    13.000 103.90  74.03 51.730 34.940 65.220

Percentages for number of QTL detected:
 2  3  4  5  6  7  8  9 10 11 12 13
 2  3  9 14 21 19 17 10  4  1  0  0

Percentages for number of epistatic pairs detected:
pairs
 1  2  3  4  5  6
29 31 23 11  5  1

Percentages for common epistatic pairs:
 6.15  4.15   4.6   1.7 15.15   1.4   1.6   4.9  1.15  1.17   1.5  5.11   1.2  7.15   1.1
   63    18    10     6     6     5     4     4     3     3     2     2     2     2

> plot(qb.diag(qbHyper, items = c("herit", "envvar")))
```

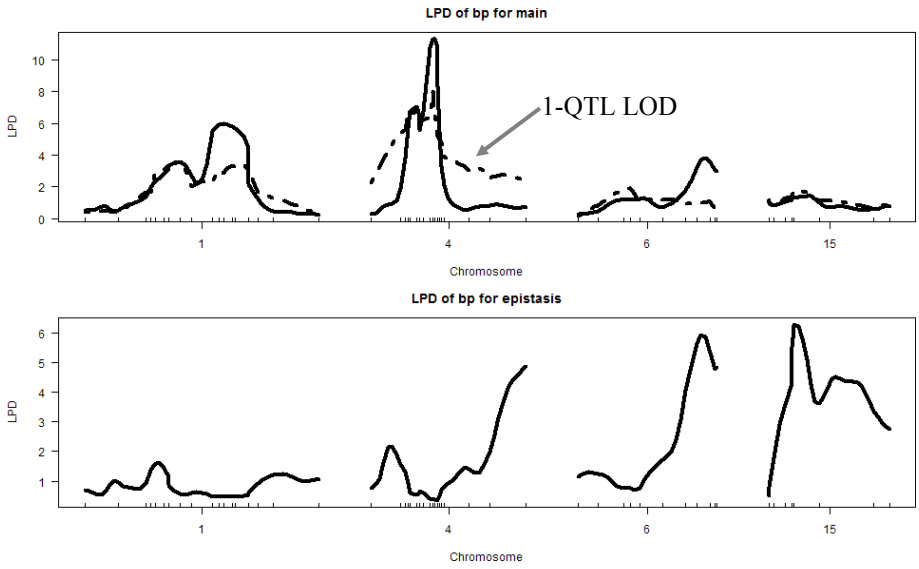
diagnostic summaries



R/qtlbim: 1-D (*not* 1-QTL!) scan

```
> one <- qb.scanone(qbHyper, chr = c(1,4,6,15), type =
   "LPD")
> summary(one)

LPD of bp for main,epistasis,sum

    n.qtl  pos m.pos e.pos  main epistasis   sum
c1  1.331 64.5  64.5  67.8  6.10     0.442  6.27
c4  1.377 29.5  29.5  29.5 11.49     0.375 11.61
c6  0.838 59.0  59.0  59.0  3.99     6.265  9.60
c15 0.961 17.5  17.5  17.5  1.30     6.325  7.28

> plot(one, scan = "main")
> plot(out.em, chr=c(1,4,6,15), add = TRUE, lty = 2)
> plot(one, scan = "epistasis")
```

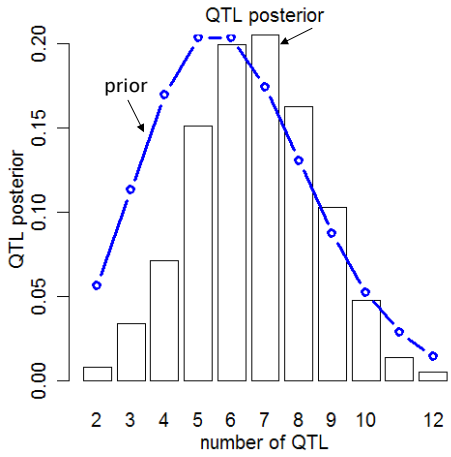
1-QTL LOD vs. marginal LPD

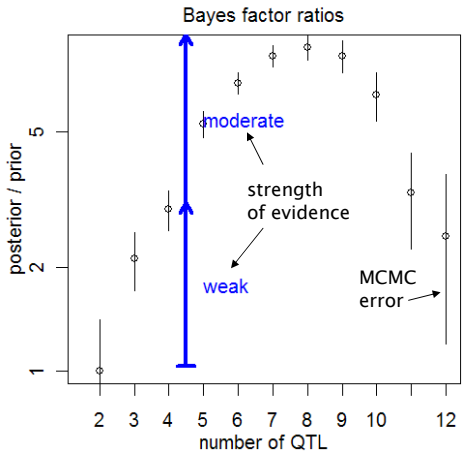


most probable patterns

```
> summary(qb.BayesFactor(qbHyper, item = "pattern"))

                   nqtl posterior    prior    bf  bfse
1,4,6,15,6:15         5   0.03400 2.71e-05 24.30 2.360
1,4,6,6,15,6:15       6   0.00467 5.22e-06 17.40 4.630
1,1,4,6,15,6:15       6   0.00600 9.05e-06 12.80 3.020
1,1,4,5,6,15,6:15     7   0.00267 4.11e-06 12.60 4.450
1,4,6,15,15,6:15      6   0.00300 4.96e-06 11.70 3.910
1,4,4,6,15,6:15       6   0.00300 5.81e-06 10.00 3.330
1,2,4,6,15,6:15       6   0.00767 1.54e-05  9.66 2.010
1,4,5,6,15,6:15       6   0.00500 1.28e-05  7.56 1.950
1,2,4,5,6,15,6:15     7   0.00267 6.98e-06  7.41 2.620
1,4                   2   0.01430 1.51e-04  1.84 0.279
1,1,2,4               4   0.00300 3.66e-05  1.59 0.529
1,2,4                 3   0.00733 1.03e-04  1.38 0.294
1,1,4                 3   0.00400 6.05e-05  1.28 0.370
1,4,19                3   0.00300 5.82e-05  1.00 0.333

> plot(qb.BayesFactor(qbHyper, item = "nqtl"))
```

hyper: number of QTL
posterior, prior, Bayes factors



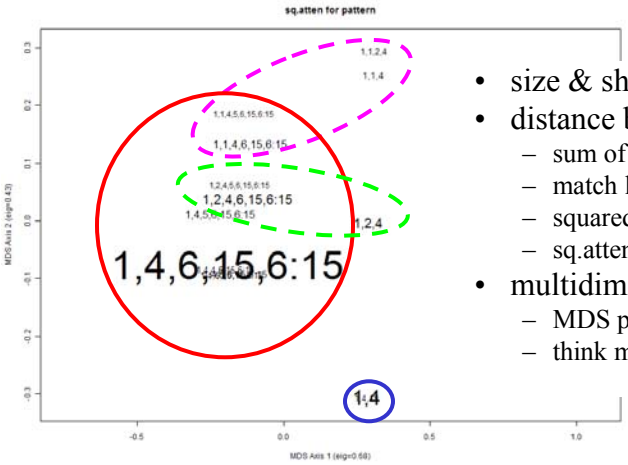


what is best estimate of QTL?

- find most probable pattern
  - 1,4,6,15,6:15 has posterior of 3.4%
- estimate locus across all nested patterns
  - Exact pattern seen ~100/3000 samples
  - Nested pattern seen ~2000/3000 samples
- estimate 95% confidence interval using quantiles

```
> best <- qb.best(qbHyper)
> summary(best)$best

    chrom locus locus.LCL locus.UCL     n.qtl
247     1  69.9  24.44875   95.7985 0.8026667
245     4  29.5  14.20000   74.3000 0.8800000
248     6  59.0  13.83333   66.7000 0.7096667
246    15  19.5  13.10000   55.7000 0.8450000

> plot(best)
```

what patterns are “near” the best?

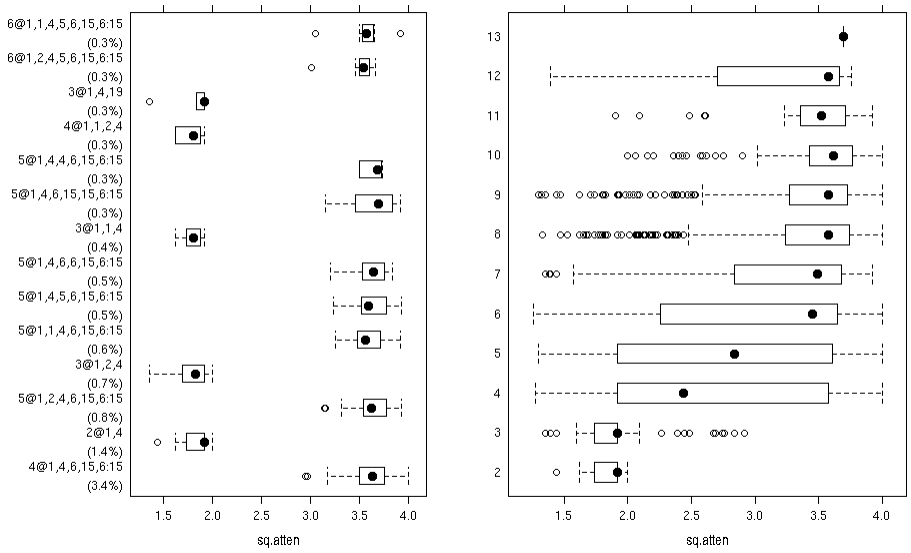


- size & shade ~ posterior
- distance between patterns
  - sum of squared attenuation
  - match loci between patterns
  - squared attenuation = $(1-2r)^2$
  - sq.atten in scale of LOD & LPD
- multidimensional scaling
  - MDS projects distance onto 2-D
  - think mileage between cities

how close are other patterns?

```
> target <- qb.best(qbHyper)$model[[1]]
> summary(qb.close(qbHyper, target))

 score by sample number of qtl
    Min. 1st Qu. Median  Mean 3rd Qu.  Max.
2  1.437   1.735  1.919 1.834   1.919 2.000
3  1.351   1.735  1.916 1.900   1.919 2.916
4  1.270   1.916  2.437 2.648   3.574 4.000
5  1.295   1.919  2.835 2.798   3.611 4.000
6  1.257   2.254  3.451 3.029   3.648 4.000
...
13 3.694   3.694  3.694 3.694   3.694 3.694

 score by sample chromosome pattern
                    Percent  Min. 1st Qu. Median  Mean 3rd Qu.  Max.
4@1,4,6,15,6:15         3.4 2.946   3.500  3.630 3.613   3.758 4.000
2@1,4                   1.4 1.437   1.735  1.919 1.832   1.919 2.000
5@1,2,4,6,15,6:15       0.8 3.137   3.536  3.622 3.611   3.777 3.923
3@1,2,4                 0.7 1.351   1.700  1.821 1.808   1.919 2.000
5@1,1,4,6,15,6:15       0.6 3.257   3.484  3.563 3.575   3.698 3.916
5@1,4,5,6,15,6:15       0.5 3.237   3.515  3.595 3.622   3.777 3.923
5@1,4,6,6,15,6:15       0.5 3.203   3.541  3.646 3.631   3.757 3.835
...

> plot(close)
> plot(close, category = "nqtl")
```

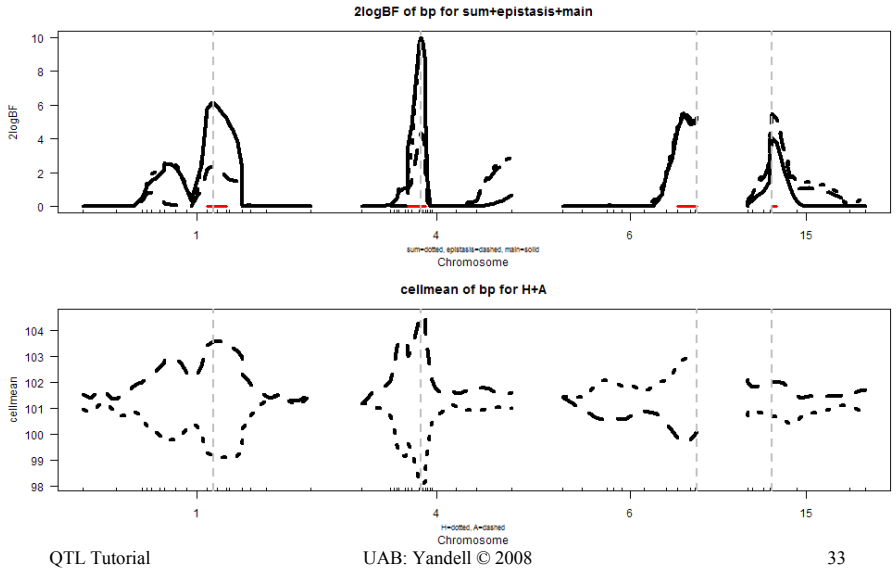
how close are other patterns?



R/qtlbim: automated QTL selection

```
> hpd <- qb.hpdone(qbHyper, profile = "2logBF")
> summary(hpd)

   chr n.qtl  pos lo.50% hi.50% 2logBF       A       H
1    1 0.829 64.5   64.5   72.1  6.692 103.611  99.090
4    4 3.228 29.5   25.1   31.7 11.169 104.584  98.020
6    6 1.033 59.0   56.8   66.7  6.054  99.637 102.965
15  15 0.159 17.5   17.5   17.5  5.837 101.972 100.702

> plot(hpd)
```

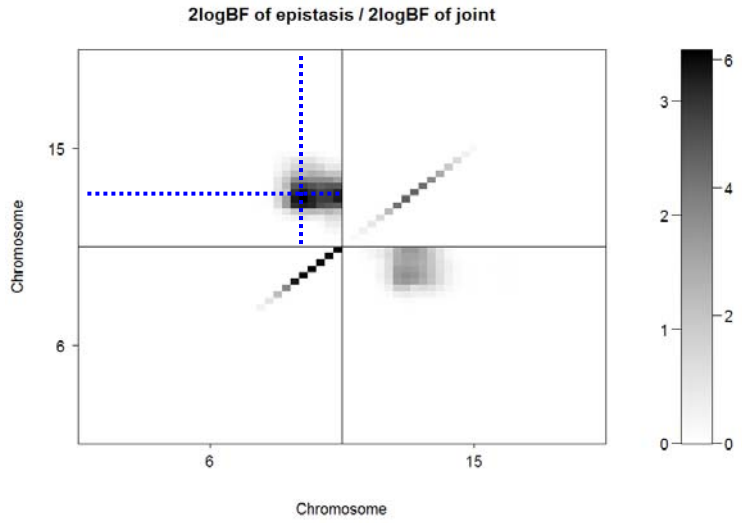
2log(BF) scan with 50% HPD region



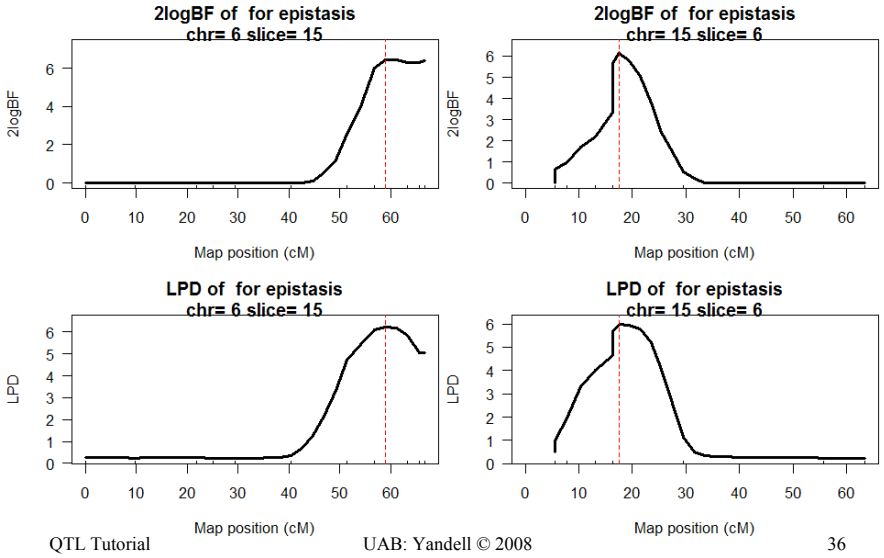
R/qtlbim: 2-D (*not* 2-QTL) scans

```
> two <- qb.scantwo(qbHyper, chr = c(6,15),
  type = "2logBF")
> plot(two)

> plot(two, chr = 6, slice = 15)
> plot(two, chr = 15, slice = 6)

> two.lpd <- qb.scantwo(qbHyper, chr = c(6,15),
  type = "LPD")
> plot(two.lpd, chr = 6, slice = 15)
> plot(two.lpd, chr = 15, slice = 6)
```

2-D plot of 2logBF: chr 6 & 15

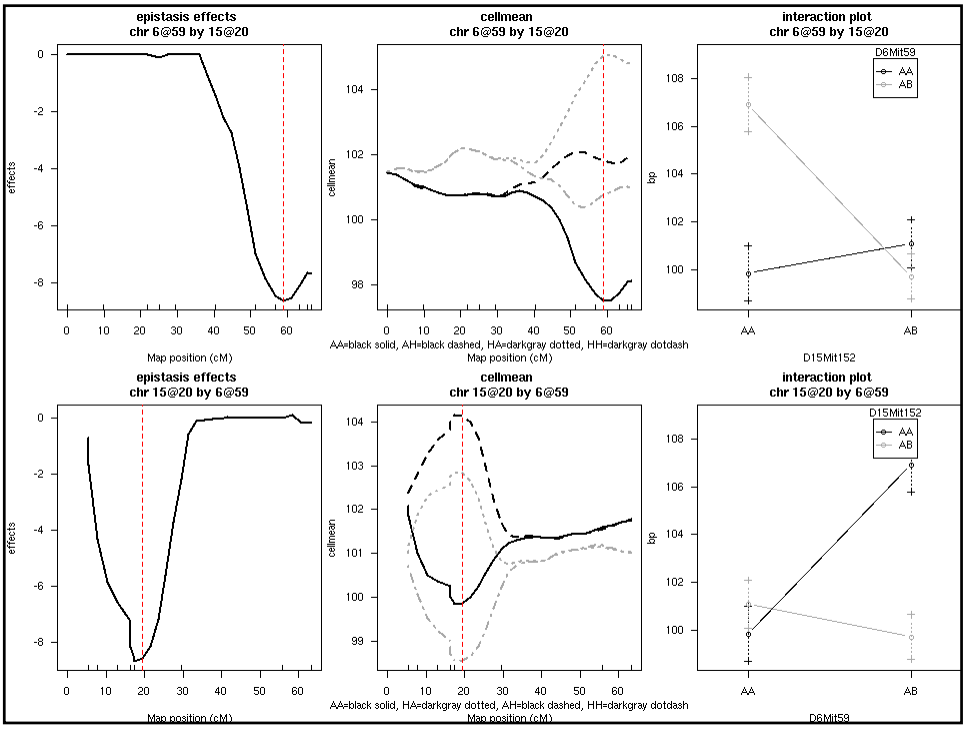


1-D Slices of 2-D scans: chr 6 & 15



R/qtlbim: slice of epistasis

```
> slice <- qb.slicetwo(qbHyper, c(6,15), c(59,19.5))
> summary(slice)

2logBF of bp for epistasis

    n.qtl  pos m.pos e.pos epistasis slice
c6  0.838 59.0  59.0  66.7      15.8  18.1
c15 0.961 17.5  17.5  17.5      15.5  60.6

cellmean of bp for AA,HA,AH,HH

    n.qtl  pos m.pos   AA  HA  AH    HH slice
c6  0.838 59.0  59.0 97.4 105 102 100.8  18.1
c15 0.961 17.5  17.5 99.8 103 104  98.5  60.6

estimate of bp for epistasis

    n.qtl  pos m.pos e.pos epistasis slice
c6  0.838 59.0  59.0  66.7     -7.86  18.1
c15 0.961 17.5  17.5  17.5     -8.72  60.6

> plot(slice, figs = c("effects", "cellmean", "effectplot"))
```

selected publications

www.stat.wisc.edu/~yandell/statgen

- www.qtlbim.org
- vignettes in R/qtlbim package
- Yandell, Bradbury (2007) *Plant Map* book chapter
  - overview/comparison of QTL methods
- Yandell et al. (2007 *Bioinformatics*)
  - R/qtlbim introduction
- Yi et al. (2005 *Genetics,* 2007 *Genetics*)
  - methodology of R/qtlbim