

Multivariate Statistics in Ecology and Quantitative Genetics

Quantitative Traits Loci (QTL) Mapping

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http://evol.bio.lmu.de/_statgen

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- QTL model assumptions

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- LOD score

- Interval mapping

More than one QTL

 K.W. Broman, S. Sen (2009) *A guide to QTL Mapping with R/qtI*.
Springer, New York.

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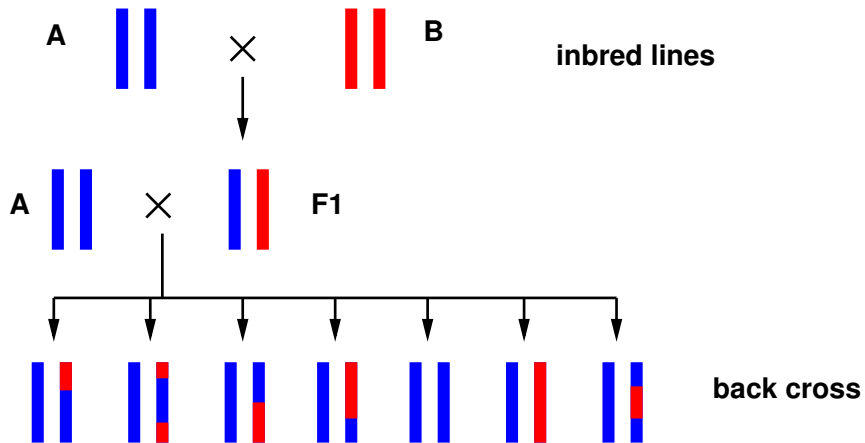
QTL model assumptions

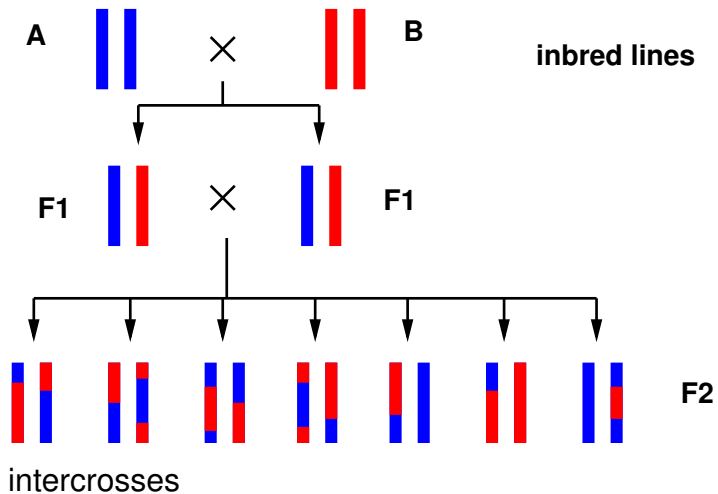
Single-QTL analysis

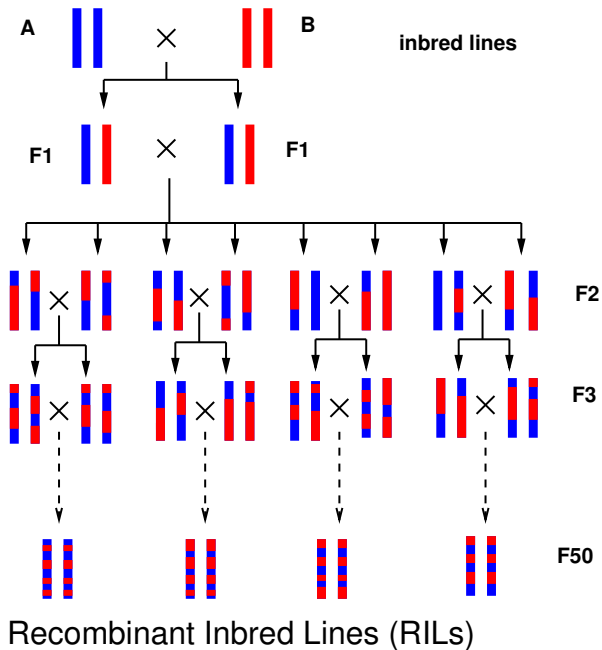
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Example dataset with backcrosses

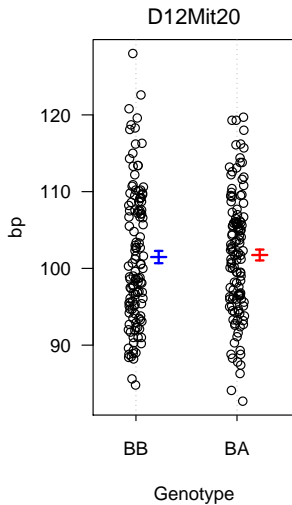
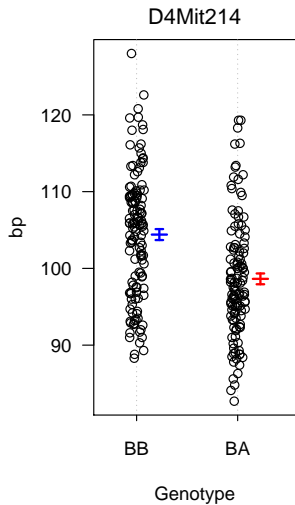
```
> library(qtl)
> data(hyper)
> summary(hyper)
  Backcross

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 (%):       BB:50.2 BA:49.8
```



```
par(mfrow=c(1,2))  
plot.pxs(hyper,"D4Mit214")  
plot.pxs(hyper,"D12Mit20")  
par(mfrow=c(1,1))
```

Assume that p sites have an influence on the quantitative trait y of interest and denote an individual's genotype at these sites by $\mathbf{g} = (g_1, g_2, \dots, g_p)$

$$\mu_{\mathbf{g}} := \mathbb{E}(y|\mathbf{g})$$

$$\sigma_{\mathbf{g}}^2 := \text{var}(y|\mathbf{g})$$

we assume: $y|\mathbf{g} \sim \mathcal{N}(\mu_{\mathbf{g}}, \sigma_{\mathbf{g}}^2)$

additive model:
$$\mu_{\mathbf{g}} = \mu + \sum_{j=1}^p z_j \cdot \Delta_j,$$

whereas z_j is 0 or 1 according to the genotype of g_j , and Δ_j is the effect of the QTL at position j .

In a strict sense, *epistasis* means that the effect of a mutation can be masked by a mutation at a different loci.

However, in the context of QTL mapping, the word epistasis is often used to express that there is a non-additive interaction between two loci.

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However, in the context of QTL mapping, the word epistasis is often used to express that there is a non-additive interaction between two loci.

Main problem: We do not know where the QTLs are. We only have genetic markers to determine for several sites whether the stem from A or B.

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Assume a backcross experiment with n F2 individuals
Let $y = (y_1, \dots, y_n)$ be their phenotypes for the trait of interest.

Null hypothesis H_0 : no QTL

Residual sum of squares under H_0 :

$$\text{RSS}_0 = \sum_{k=1}^n (y_k - \bar{y})^2$$

Very simple alternative H_1 : single QTL at marker position i

$$y|g_i \sim \mathcal{N}(\mu_{g_i}, \sigma^2)$$

Likelihood function:

$$\begin{aligned} L_1(\mu_{AA}, \mu_{AB}, \sigma^2) &= \Pr(y|\text{QTL marker}, \mu_{AA}, \mu_{AB}, \sigma^2) \\ &= \prod_{k=1}^n \phi(y_k; \mu_{g_{ik}}, \sigma^2), \end{aligned}$$

whereas ϕ is the density of the normal distribution and g_{ik} is the genotype of individual k at marker position i .

The maximal likelihood under H_1 is $RSS_1^{-n/2}$, with

$$RSS_1 = \sum_{k=1}^n (y_k - \widehat{\mu}_{g_{ik}})^2,$$

where $\mu_{g_{ik}}$ is the mean trait value over all individuals that have type g_{ik} at marker position i .

The LOD score is the \log_{10} of the likelihood ratio of H_1 and H_0 :

$$LOD = \frac{n}{2} \log_{10} \left(\frac{RSS_0}{RSS_1} \right)$$

The LOD score is traditionally used in QTL mapping. However, it is equivalent to the classical anova F -statistic:

$$F = \frac{(RSS_0 - RSS_1)/df}{RSS_1/(n - df - 1)} = (10^{2 \cdot \text{LOD}/n} - 1) \cdot \frac{n - df - 1}{df}$$

$$\text{LOD} = \frac{n}{2} \log_{10} \left(\frac{F \cdot df}{n - df + 1} + 1 \right)$$

So, if the marker positions are our candidates for the QTLs we just perform anovas.

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- ▶ The QTLs may be between the marker positions, and their genotypes can only be estimated from the markers.

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- ▶ Let M_k be the multipoint marker genotype of individual k and $g_{\ell k}$ its QTL genotype at candidate position ℓ , and

$$p_{kj} := \Pr(g_{\ell k} = j | M_k).$$

(Computation uses recombination rates.)

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$$p_{kj} := \Pr(g_{\ell k} = j | M_k).$$

(Computation uses recombination rates.)

- ▶ Probability density of an individual's phenotype (at candidate locus ℓ) is a mixture of normal distribution densities:

$$\sum_j p_{kj} \cdot \phi(y_k; \mu_j, \sigma^2)$$

EM algorithm for ML-estimation of μ_j and σ

Start with initial estimates $\mu_j^{(0)}$ and $\sigma^{(0)}$ and iterate the following steps for $s = 1, \dots, N$:

E-step

$$\begin{aligned} w_{kj}^{(s)} &:= \Pr(g_{\ell k} = j | M_k, y_k, \mu_j^{(s-1)}, \sigma^{(s-1)}) \\ &= \frac{p_{kj} \phi(y_k; \mu_j^{(s-1)}, \sigma^{(s-1)})}{\sum_h p_{kh} \phi(y_k; \mu_h^{(s-1)}, \sigma^{(s-1)})} \end{aligned}$$

M-step

$$\begin{aligned} \mu_j^{(s)} &:= \sum_k w_{kj}^{(s)} y_i / \sum_h w_{hj}^{(s)} \\ \sigma^{(s)} &:= \sqrt{\sum_{kj} w_{kj}^{(s)} (y_k - \mu_{g_{kj}}^{(s)})^2 / n} \end{aligned}$$

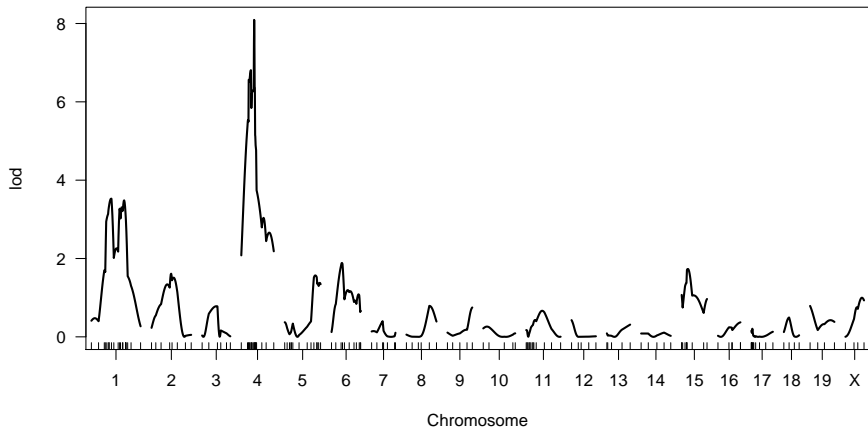
The aim of the EM algorithm is that $\mu_j^{(s)}$ and $\sigma^{(s)}$ converge against the ML estimators $\hat{\mu}$ and $\hat{\sigma}$.

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Then, the LOD score can be computed:

$$\text{LOD} = \log_{10} \left(\frac{\prod_i \sum_j p_{ij} \phi(y_i; \hat{\mu}_j, \hat{\sigma}^2)}{\prod_i \phi(y_i; \hat{\mu}_0, \hat{\sigma}_0^2)} \right)$$

```
## calculate p_{kj}
hyper <- calc.genoprob(hyper,step=1,error.prob=0.001)
out.em <- scanone(hyper,method="em")
plot(out.em)
```



Sometimes EM can be very slow.

Haley-Knott (HK) regression is a fast approximation:

For each point on the grid calculate $p_{kj} = \Pr(g_i = j|M)$ and estimate μ_j and σ by fitting a linear model

$$y_k|M_k \sim \mathcal{N} \left(\sum_j p_{kj} \mu_j, \sigma^2 \right)$$

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Haley-Knott (HK) regression is a fast approximation:

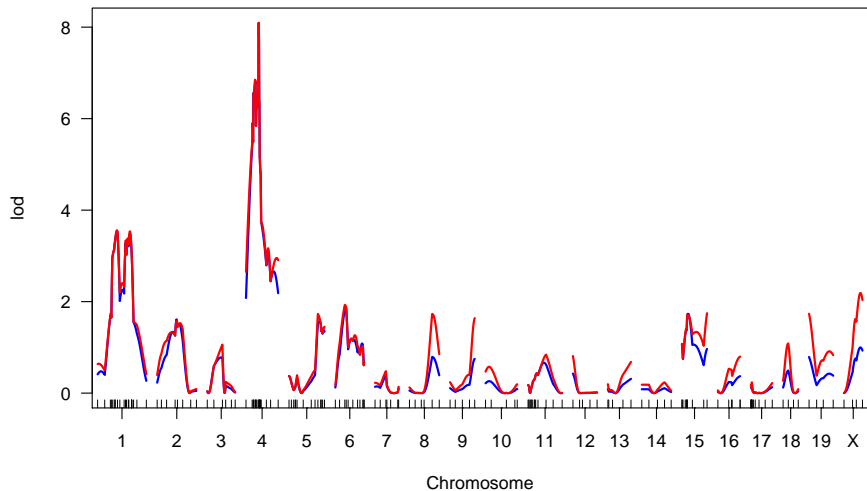
For each point on the grid calculate $p_{kj} = \Pr(g_i = j|M)$ and estimate μ_j and σ by fitting a linear model

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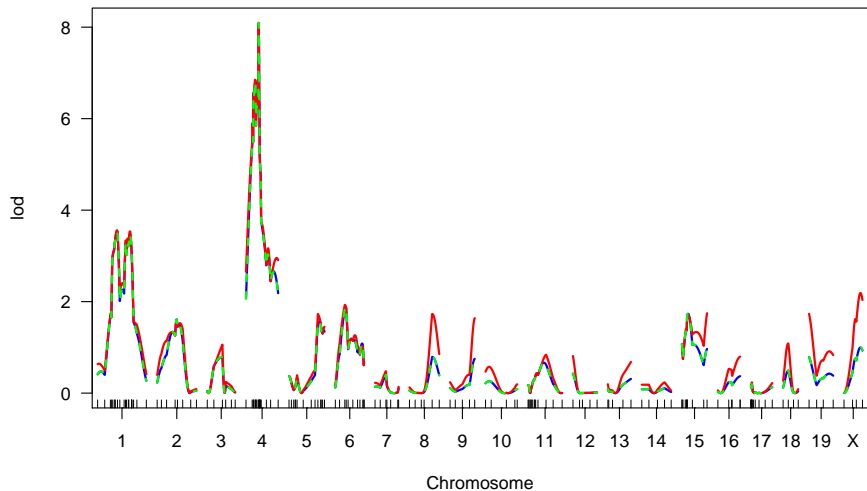
Extended Haley-Knott (EHK) regression: Takes into account that p_{kj} and μ_j have an influence on the variance:

$$y_k|M_k \sim \mathcal{N} \left(\sum_j p_{kj} \mu_j, \sum_j p_{kj} \left(\mu_j - \sum_h p_{kh} \mu_h \right)^2 + \sigma^2 \right)$$

```
out.hk <- scanone(hyper,method="hk")  
plot(out.em,out.hk,col=c("blue","red"))
```



```
out.ehk <- scanone(hyper,method="ehk")  
plot(out.em,out.hk,out.ehk,col=c("blue","red","green"),lty=c(1,1,2))
```

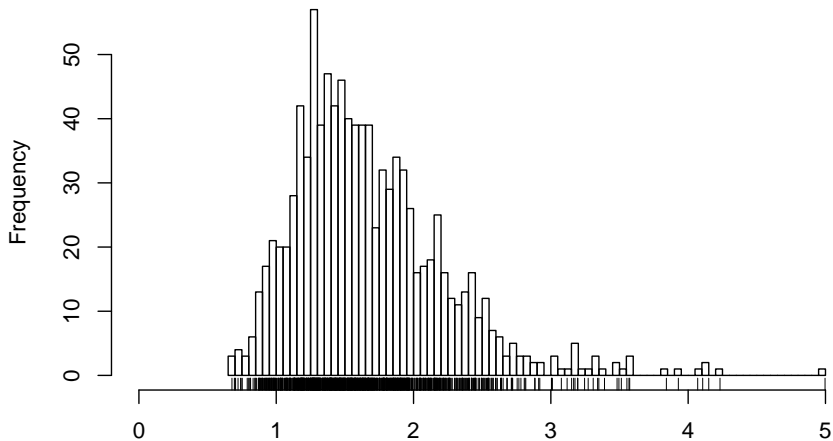


Which LOD scores are significant?

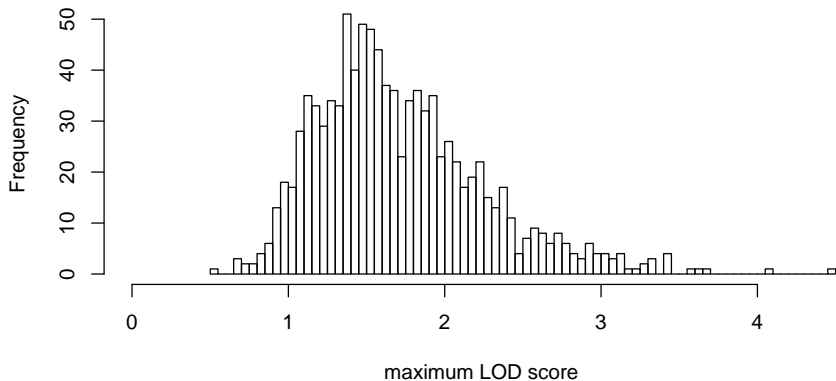
Which LOD scores are significant?

Assess this by a permutation test: shuffle the phenotype column.

```
## next command will take time  
out.hk.perm <- scanone(hyper,method="hk",n.perm=1000)  
plot(out.hk)
```



```
## this will take even longer:  
out.perm <- scanone(hyper,n.perm=1000)  
plot(out.perm)
```



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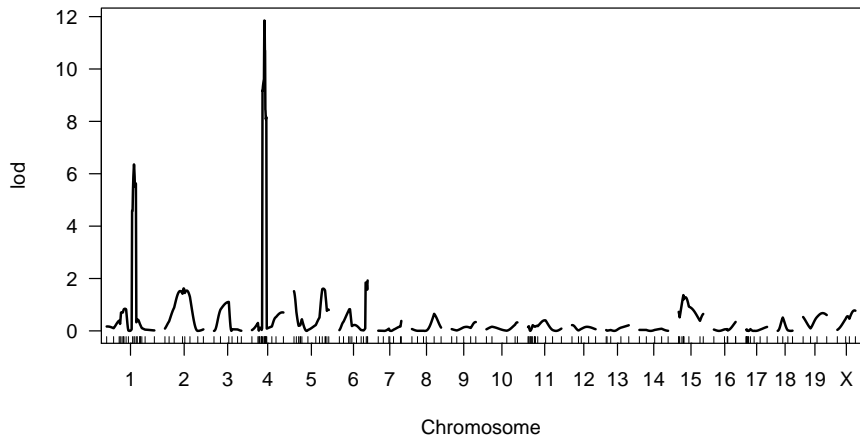
LOD score

Interval mapping

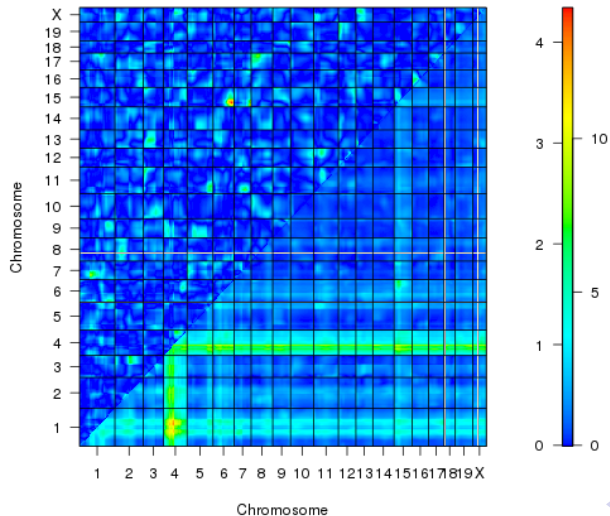
More than one QTL

- Composite Interval Mapping** While searching for a QTL in one interval use other markers as proxies for nearby QTLs. Thus, markers are used as covariates. Specify maximal number of covariates and how far they should be away from the interval under examination.
- two-QTL models** search for interacting pairs of QTLs. Same methods like in 1-QTL model: EM, HK, EHK
- multiple QTLs** When candidate loci are found, fit regression models allowing for interactions and do variable selection.

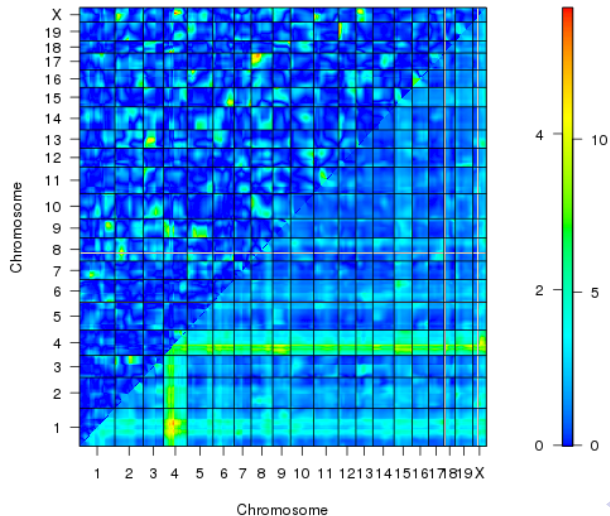
```
out.cim <- cim(hyper)
plot(out.cim)
```



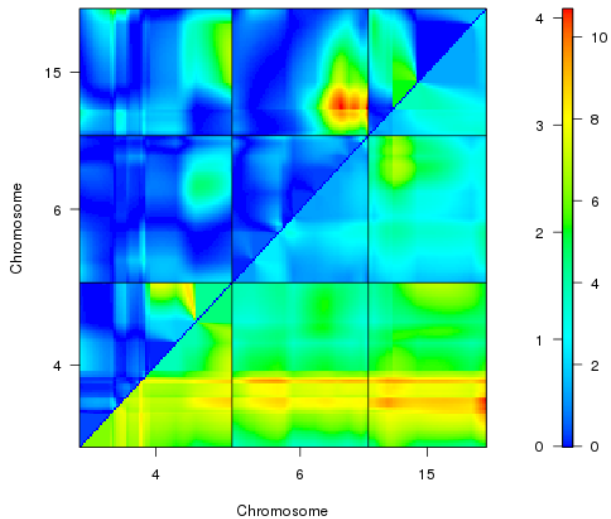
```
out2 <- scantwo(hyper) ## takes quite long  
plot(out2)
```




```
out2.hk <- scantwo(hyper,method="hk") ## much faster  
plot(out2.hk)
```



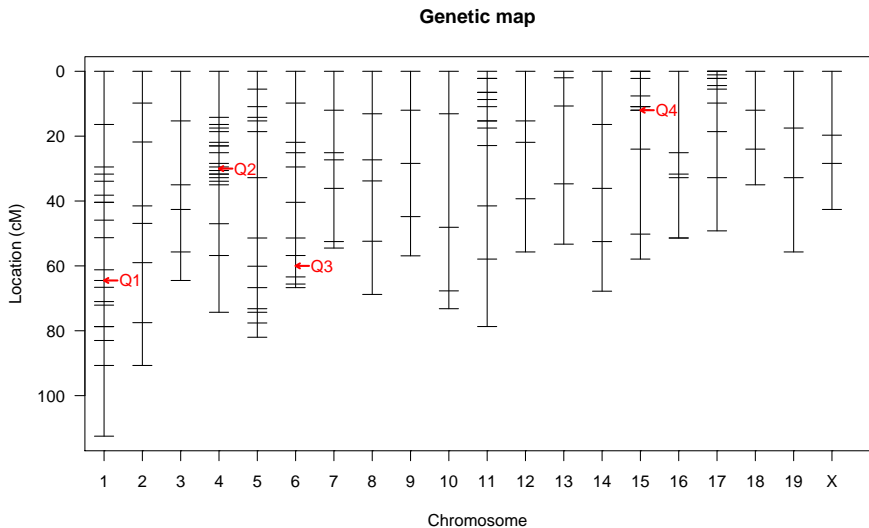
```
plot(out2.hk, chr=c(4,6,15))
```



```
> hyper <- sim.geno(hyper,step=2,n.draws=128,err=0.001)
> qtl <- makeqtl(hyper,chr=c(1,4,6,15),pos=c(68.3,30,60,18))
> qtl
QTL object containing imputed genotypes, with 128 imputations
```

| | name | chr | pos | n.gen |
|----|---------|-----|------|-------|
| Q1 | 1@67.8 | 1 | 67.8 | 2 |
| Q2 | 4@30.0 | 4 | 30.0 | 2 |
| Q3 | 6@60.0 | 6 | 60.0 | 2 |
| Q4 | 15@17.5 | 15 | 17.5 | 2 |

```
plot(qt1)
```



```
> out.fq <- fitqtl(hyper, qtl=qtl, formula= y~(Q1+Q2+Q3+Q4)^2)
> summary(out.fq)
```

```
fitqtl summary
```

```
Method: multiple imputation
Model: normal phenotype
Number of observations : 250
```

```
Full model result
```

```
-----
Model formula: y ~ Q1 + Q2 + Q3 + Q4 + Q1:Q2 + Q1:Q3 + Q1:Q4 + Q2:Q3 + Q2:Q4 +
                Q3:Q4
```

| | df | SS | MS | LOD | %var | Pvalue(Chi2) | Pvalue(F) |
|-------|-----|-----------|-----------|----------|----------|--------------|-----------|
| Model | 10 | 6113.512 | 611.35116 | 23.05306 | 34.60034 | 0 | 0 |
| Error | 239 | 11555.425 | 48.34906 | | | | |
| Total | 249 | 17668.936 | | | | | |

```
Drop one QTL at a time ANOVA table:
```

```
-----
```

| | df | Type III SS | LOD | %var | F value | Pvalue(Chi2) | Pvalue(F) |
|---------|----|-------------|---------|---------|---------|--------------|--------------|
| 1@67.8 | 4 | 1548.22 | 6.8258 | 8.7624 | 8.0054 | 0.000 | 4.51e-06 *** |
| 4@30.0 | 4 | 3184.90 | 13.2152 | 18.0254 | 16.4683 | 0.000 | 6.23e-12 *** |
| 6@60.0 | 4 | 1671.00 | 7.3321 | 9.4573 | 8.6403 | 0.000 | 1.58e-06 *** |
| 15@17.5 | 4 | 1504.34 | 6.6437 | 8.5140 | 7.7785 | 0.000 | 6.57e-06 *** |

```
> out.fq <- fitqtl(hyper, qtl=qtl, formula= y~(Q1+Q2+Q3+Q4)^2)
> summary(out.fq)
```

```
.
.
.
```

Drop one QTL at a time ANOVA table:

```
-----
```

| | df | Type III SS | LOD | %var | F value | Pvalue(Chi2) | Pvalue(F) | |
|----------------|----|-------------|---------|---------|---------|--------------|-----------|-----|
| 1@67.8 | 4 | 1548.22 | 6.8258 | 8.7624 | 8.0054 | 0.000 | 4.51e-06 | *** |
| 4@30.0 | 4 | 3184.90 | 13.2152 | 18.0254 | 16.4683 | 0.000 | 6.23e-12 | *** |
| 6@60.0 | 4 | 1671.00 | 7.3321 | 9.4573 | 8.6403 | 0.000 | 1.58e-06 | *** |
| 15@17.5 | 4 | 1504.34 | 6.6437 | 8.5140 | 7.7785 | 0.000 | 6.57e-06 | *** |
| 1@67.8:4@30.0 | 1 | 79.45 | 0.3720 | 0.4496 | 1.6432 | 0.191 | 0.201 | |
| 1@67.8:6@60.0 | 1 | 50.96 | 0.2389 | 0.2884 | 1.0540 | 0.294 | 0.306 | |
| 1@67.8:15@17.5 | 1 | 57.42 | 0.2691 | 0.3250 | 1.1877 | 0.266 | 0.277 | |
| 4@30.0:6@60.0 | 1 | 54.02 | 0.2532 | 0.3057 | 1.1172 | 0.280 | 0.292 | |
| 4@30.0:15@17.5 | 1 | 29.70 | 0.1393 | 0.1681 | 0.6143 | 0.423 | 0.434 | |
| 6@60.0:15@17.5 | 1 | 1071.15 | 4.8124 | 6.0623 | 22.1544 | 0.000 | 4.26e-06 | *** |

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

- ▶ Candidate loci and interactions found by scanone and scantwo can then be used in multiple QTL analysis.
- ▶ Then, p-values from multiple QTL analysis are not reliable because not multiple-testing corrected. Massive multiple-testing problem is caused by preselection by scanone and scantwo.
- ▶ If two QTL are close to each other with only few marker loci inbetween, scanone may falsely indicate strong evidence for one QTL between the two.