Efficient and Robust Statistical Methods for Quantitative Trait Loci Analysis

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Goals

away from normality

- fewer assumptions
- extended phenotypes
- check robustness
- multiple crosses

how many QTL?

- inferring the number
- sampling all QT loci
- estimating heritability

Interval Mapping Basics

- known measurements
  - phenotypic trait Y
  - markers X (and linkage map)
- unknown quantities
  - QT locus (or loci) λ
  - QT genotypes Q
- known segregation model $P(Q|X,\lambda)$
  - based on recombination, map function
- unknown aspects of phenotype model $P(Y|Q)$
  - distribution shape (could be assumed normal)
  - parameters θ, σ^2, if used (could be non-parametric)

Interval Mapping Mixture (BC)

- what shape histogram does trait *Y* have?
  - shape $P(Y|\mathrm{Qq})$ with genotype Qq
  - shape $P(Y|\mathrm{QQ})$ with genotype QQ
- is the QTL at a given locus λ?
  - no QTL: $P(Y|\mathrm{Qq}) = P(Y|\mathrm{QQ})$
  - yes QTL: mixture if genotype unknown
- mixture of shapes across possible genotypes

$$P(Y|X,\lambda) = P(\mathrm{Qq}|X,\lambda)P(Y|\mathrm{QQ}) + P(\mathrm{QQ}|X,\lambda)P(Y|\mathrm{QQ})$$

$P(Y|X,\lambda)$ = sum over possible Q of $P(Q|X,\lambda)P(Y|Q)$

Interval Mapping Likelihood

- likelihood: basis for scanning the genome

  $L(\lambda|Y)$ = product of $P(Y_i|X_i,\lambda)$ over $i = 1,\ldots,n$

  $L(\lambda|Y)$ = product_i of sum_Q of $P(Q|X_i,\lambda)P(Y_i|Q)$

- problem: unknown phenotype model $P(Y|Q)$
  - parametric $P(Y|Q) = Normal(Q\theta, \sigma^2)$
  - semi-parametric $P(Y|Q) = f(Y)\exp(Q\beta)$
  - non-parametric $P(Y|Q) = F_Q(Y)$ unspecified

Limitations of Parametric Models

- measurements not normal
  - counts (*e.g.* number of tumors)
  - survival time (*e.g.* days to flowering)
- false positives due to miss-specified model
  - check model assumptions?
- want more robust estimates of effects
  - parametric: only center (mean), spread (SD)
  - shape of distribution may be important

Semi-parametric QTL

- phenotype model $P(Y|Q) = f(Y)\exp(Q\beta)$
- test for QTL at locus λ
  - $\beta = 0$ implies $P(Y|\mathrm{QQ}) = P(Y|\mathrm{Qq})$
- includes many standard phenotype models

  normal $P(Y|Q) = N(\mu_Q,\sigma^2)$

  Poisson $P(Y|Q) = \mathrm{Poisson}(\mu_Q)$

  exponential, binomial, …

  (exercise: verify these are special cases for BC)

Semi-parametric Empirical Likelihood

- phenotype model $P(Y|Q) = f(Y)\exp(Q\beta)$
- non-parametric empirical likelihood (Owen 1988)

$$L(\lambda,\beta,f|Y,X) = \text{product}_i\ [\text{sum}_Q\ P(Q|X_i,\lambda)f(Y_i)\exp(Q\beta)]$$
$$= \text{product}_i\ f(Y_i)\ [\text{sum}_Q\ P(Q|X_i,\lambda)\exp(Q\beta)]$$
$$= \text{product}_i\ f(Y_i)\ w(X_i|\lambda,\beta)$$

  - weights $w(X_i|\lambda,\beta)$ rely only on flanking markers
- "point mass" at each measured phenotype
  - subject to constraints to be a distribution

  $\text{sum}_i\ P(Y_i|Q) = 1$ for all possible genotypes Q
- profile likelihood: $L(\lambda|Y,X) = \max_{\beta,f} L(\lambda,\beta,f|Y,X)$

Semi-parametric Formal Tests

- clever trick: use partial empirical LOD
  - Zou, Fine, Yandell (2001 *Biometrika*)
  - $\mathrm{LOD}(\lambda) \approx \log_{10} L(\lambda|Y,X)$
- has same formal behavior as parametric LOD
  - single locus test: approximately χ^2 with 1 d.f.
  - genome-wide scan: can use same critical values
  - permutation test: possible with some work
- can estimate cumulative distributions
  - nice properties (converge to Gaussian processes)

Non-parametric Methods

- phenotype model $P(Y|Q) = F_Q(Y)$
- Kruglyak, Lander (1995)
  - formal rank-sum test, replacing Y by rank(Y)
  - claimed no estimator of QTL effects
- estimators are indeed possible
  - semi-parametric shift (Hodges-Lehmann)
    - Zou (2001) thesis
  - non-parametric cumulative distribution
    - Fine, Zou, Yandell (2001 in review)

Rank-Sum QTL Methods

- phenotype model $P(Y|Q) = F_Q(Y)$
- replace Y by rank(Y) and perform IM
  - extension of Wilcoxon rank-sum test
  - fully non-parametric
- Hodges-Lehmann estimator of shift β
  - most efficient if $P(Y|Q) = F(Y+Q\beta)$
  - find β that matches medians
    - problem: genotypes Q unknown
    - resolution: Haley-Knott (1992) regression scan

Non-Parametric QTL CDFs

- estimate non-parametric phenotype model
  - cumulative distributions $F_Q(y) = P(Y \leq y \,|Q)$
  - can use to check parametric model validity
- basic idea:

  $P(Y \leq y \,|X,\lambda) = \text{sum}_Q \, P(Q|X,\lambda)F_Q(y)$
  - depends on X only through flanking markers
  - few possible flanking marker genotypes
    - 4 for BC, 9 for F2, etc.

Finding NP QTL CDFs

- cumulative distributions $F_Q(y) = P(Y \leq y \,|Q)$
- $F = \{F_Q$, all possible QT genotypes $Q\}$
  - BC: $F = \{F_{QQ}=P(Y \leq y|QQ), F_{Qq}=P(Y \leq y|Qq)\}$
- find F to minimize over all phenotypes y

  $\text{sum}_i\, [I(Y_i \leq y) - \text{sum}_Q\, P(Q|X,\lambda)F_Q(y)]^2$
- looks complicated, but simple to implement

Non-parametric CDF Properties

- readily extended to censored data
  - time to flowering for non-vernalized plants
- nice large sample properties
  - estimates of $F(y) = \{F_Q(y)\}$ jointly normal
  - point-wise, experiment-wise confidence bands
- more robust to heavy tails and outliers
- can use to assess parametric assumptions

Combining Multiple Crosses

- combining inbred lines in search of QTL
  - most IM methods limited to single cross
  - animal model largely focuses on polygenes
  - individuals no longer independent given *Q*
- recent work in plant sciences
  - Bernardo (1994) Wright's relationship matrix *A*
  - Rebai *et al.* (1994) regression method
  - Xu Atchley (1995) IBD & *A* for QTLs & polygenes
  - Liu Zeng (2000) multiple inbred lines, fixed effect IM
  - Zou, Yandell, Fine (2001 *Genetics*) power, threshold

Thresholds for Multiple Crosses

- permutation test
  - Churchill Doerge (1994); Doerge Churchill (1996)
  - computationally intensive
  - difficult to compare different designs
- theoretical approximation
  - Lander Botstein (1989) Dupuis Siegmund (1999)
    - single cross, dense linkage map
  - Rebai *et al.* (1994, 1995) approximate extension
    - Piepho (2001) improved calculation of efficiency
  - Zou, Yandell, Fine (2001) extend original theory

Extension of Threshold Theory

- likelihood for multiple crosses of inbred lines with *m* founders
  - approximately χ^2 with *m* degrees of freedom
  - genome-wide threshold theory
    - extends naturally based on Ornstein-Uhlenbeck
  - threshold based on dense or sparse linkage map
- some calculations based on BC1, F2, BC2
  - Liu Zeng (2000) ECM method to estimate

$Y_{ij} \sim Normal(Q_{ij}\theta_j+\mu_j, \sigma_j^2)$, j = cross

Bayesian Interval Mapping for Inbred Lines

- return to single inbred cross
  - parametric phenotype model (normal)
- connection between likelihood and posterior
  - maximize *L*, sample from posterior
- how many QTL?
  - model selection: number of QTL as unknown
- learning from data

Bayesian Interval Mapping

- recall likelihood for inbred lines

  $L(\lambda|Y) = \text{product}_i\ [\text{sum}_Q\ P(Q|X_i,\lambda)P(Y_i|Q,\theta)]$

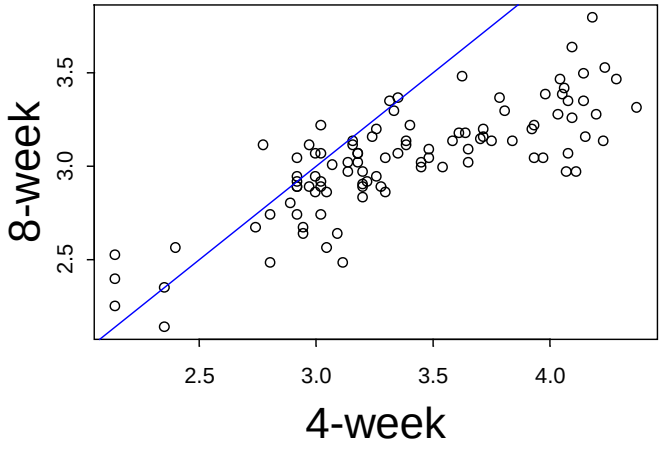
- Bayesian posterior idea
  - sample unknown data instead

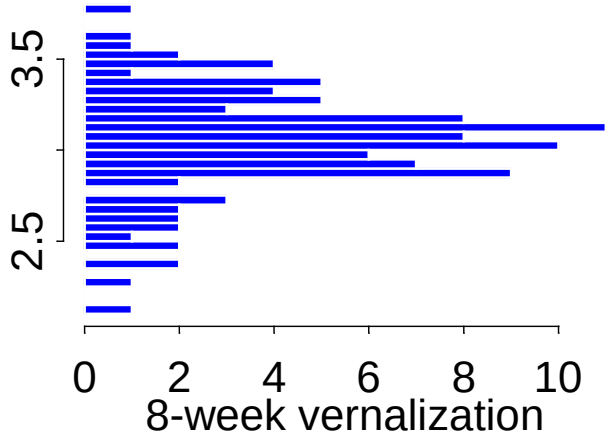
    $P(\lambda,Q,\theta|Y,X) = [\text{product}_i\ P(Q_i|X_i,\lambda)\ P(Y_i|Q_i,\theta)]P(\lambda,\theta|X)$
  - marginal summaries provide key information
    - loci: $P(\lambda|Y,X) = \text{sum}_{Q,\theta}\ P(\lambda,Q,\theta|Y,X)$
    - effects: $P(\theta|Y,X) = \text{sum}_{Q,\lambda}\ P(\lambda,Q,\theta|Y,X)$
  - Satagopan *et al.* (1996 *Genetics*)

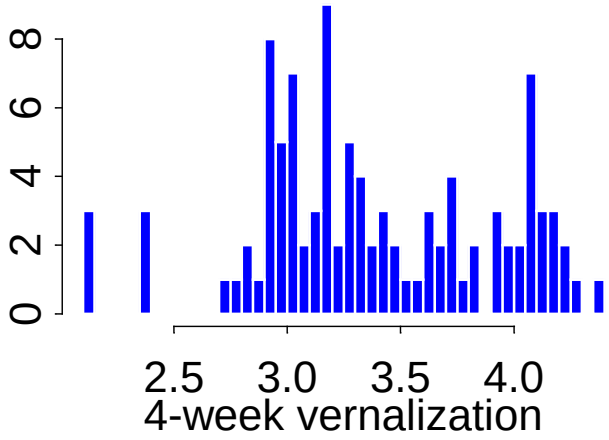
Brassica napus Data

- 4-week & 8-week vernalization effect
  - log(days to flower)
- genetic cross of
  - Stellar (annual canola)
  - Major (biennial rapeseed)
- 105 F1-derived double haploid (DH) lines
  - homozygous at every locus (*QQ* or *qq*)
- 10 molecular markers (RFLPs) on LG9
  - two QTLs inferred on LG9 (now chromosome N2)
  - corroborated by Butruille (1998)
  - exploiting synteny with *Arabidopsis thaliana*

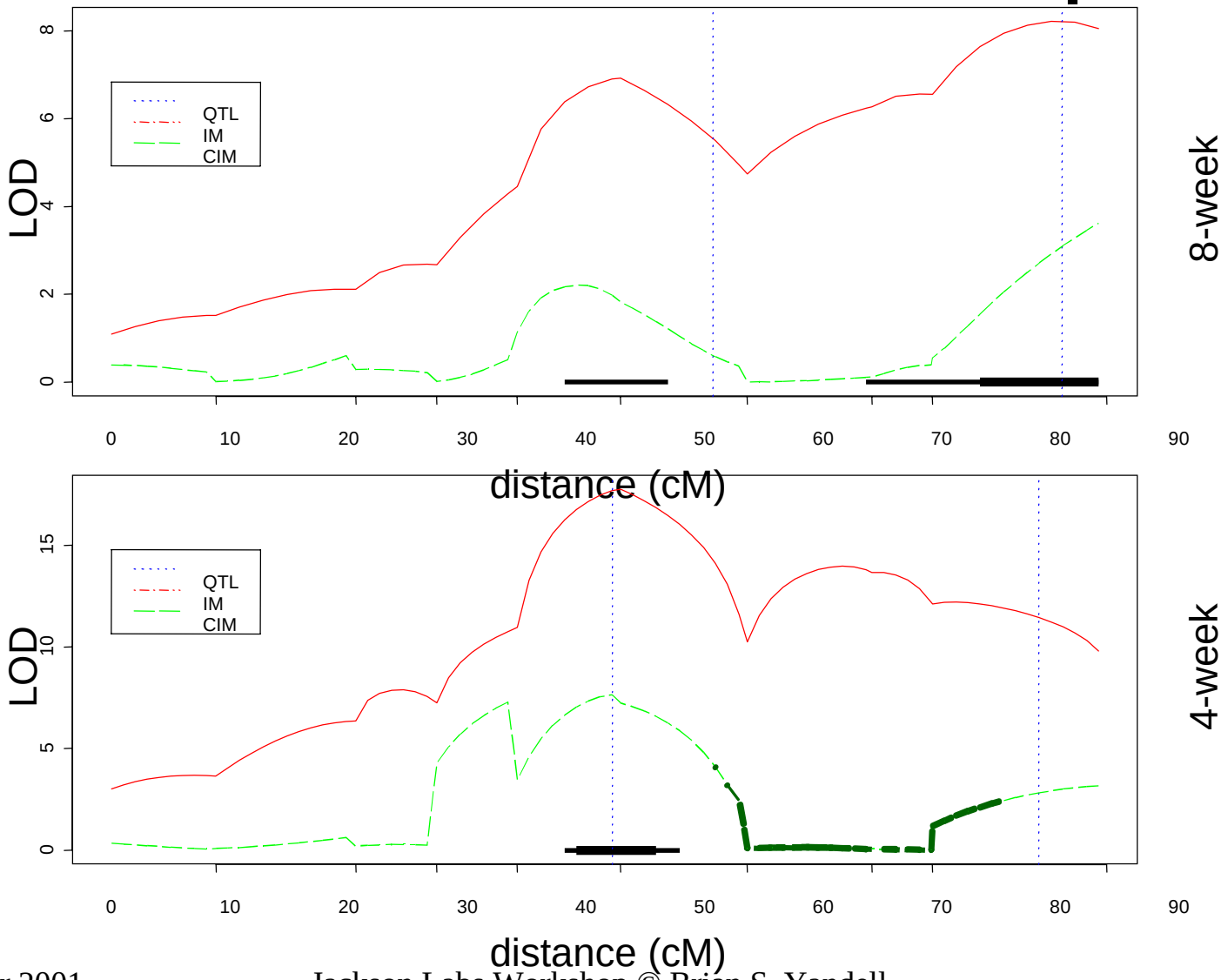
Brassica 4- & 8-week Data





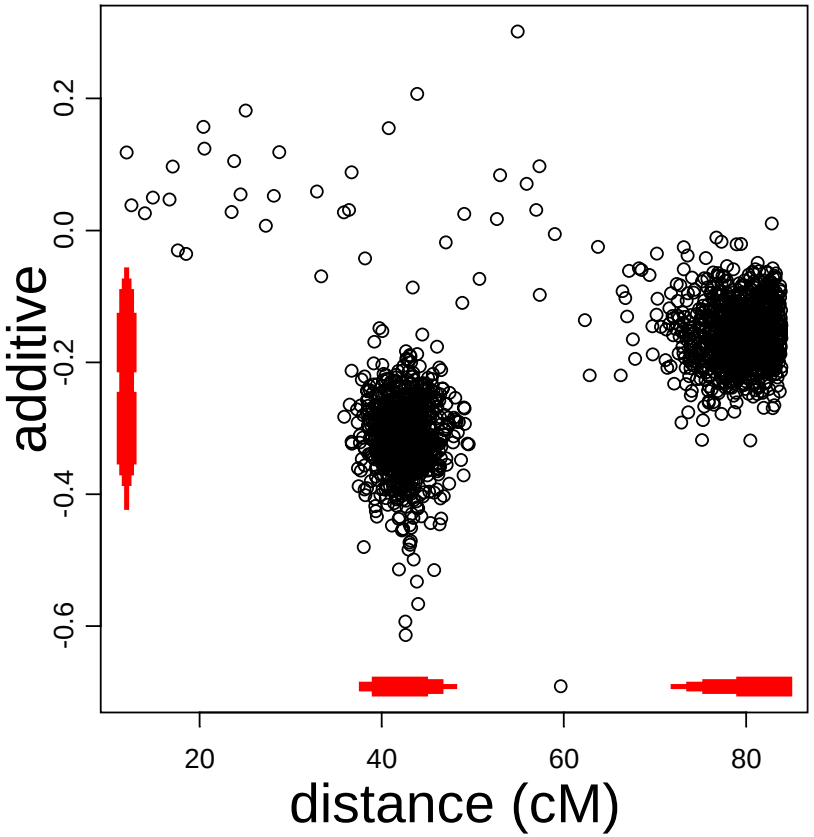


Brassica Data LOD Maps

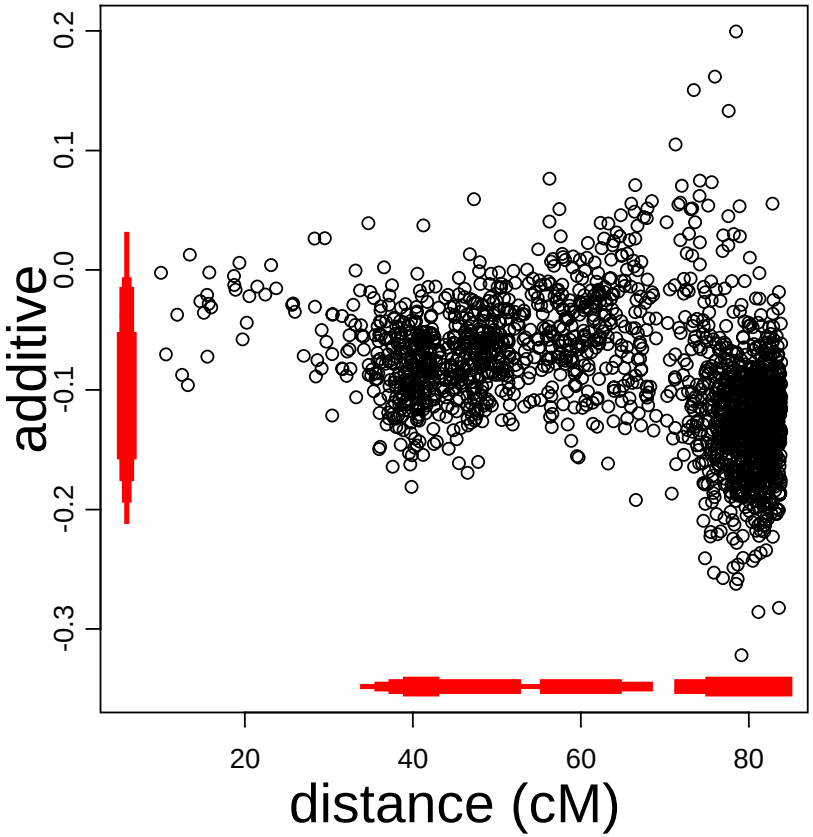


Brassica Sampled Summaries

4-week



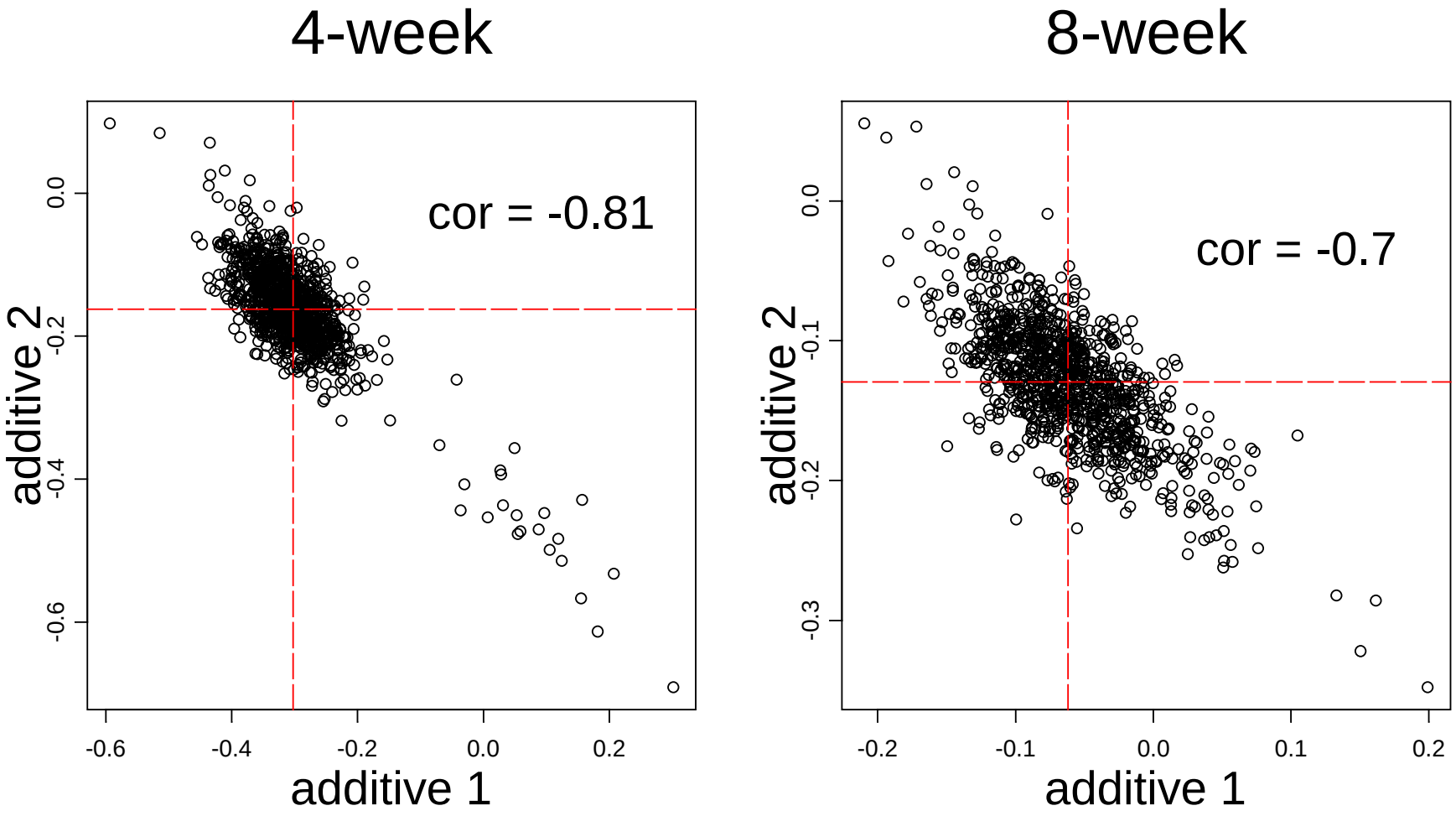
8-week



Collinearity of QTLs

- multiple QT genotypes are correlated
  - QTL linked on same chromosome
  - difficult to distinguish if close
- estimates of QT effects are correlated
  - poor identifiability of effects parameters
  - correlations give clue of how much to trust
- which QTL to go after in breeding?
  - largest effect?
  - may be biased by nearby QTL

Brassica effect Correlations



How many (detectable) QTL?

- build m = number of QTL into model

  $P(\lambda,Q,\theta|Y,X,m) = P(Q|X,\lambda,m)\ P(Y|Q,\theta,m)P(\lambda,\theta|X,m)$

  – prior on number of QTL
    - Poisson or exponential seem to work best
    - uniform can push posterior to more complicated model
- model selection
  – Bayes factors (Jaya Satagopan talk)
  – sample m as part of a bigger model
- many, many QTL affect most any trait
  – how many detectable with these data?
  – limits to useful detection (Bernardo 2000)

Sampling the Number of QTL

- almost analogous to stepwise regression

  $P(Y_i|Q_i,\theta)$: $\quad Y_i = \mu + Q_{i1}\alpha_1 + \ldots + Q_{im}\alpha_m + e_i$

  – but regressors (QT genotypes) are unknown
  – linked loci = collinear regressors = correlated effects
- use reversible jump MCMC to change *m*
  – bookkeeping helps in comparing models
  – adjust to change of variables between models
  – Green (1995); Richardson Green (1997)
  – other approaches out there these days…

Model Selection in Regression

- consider known regressors (X = markers)
  - models with 1 or 2 regressors
- jump between models
  - centering regressors simplifies calculations

$$m = 1 : Y_i = \mu + \alpha(X_{i1} - \overline{X}_1) + e_i$$

$$m = 2 : Y_i = \mu + \alpha_1(X_{i1} - \overline{X}_1) + \alpha_2(X_{i1} - \overline{X}_1) + e_i$$

Regressor Slope Estimators

recall least squares estimators of slopes

note relation of α_1 in model 2 to α in model 1

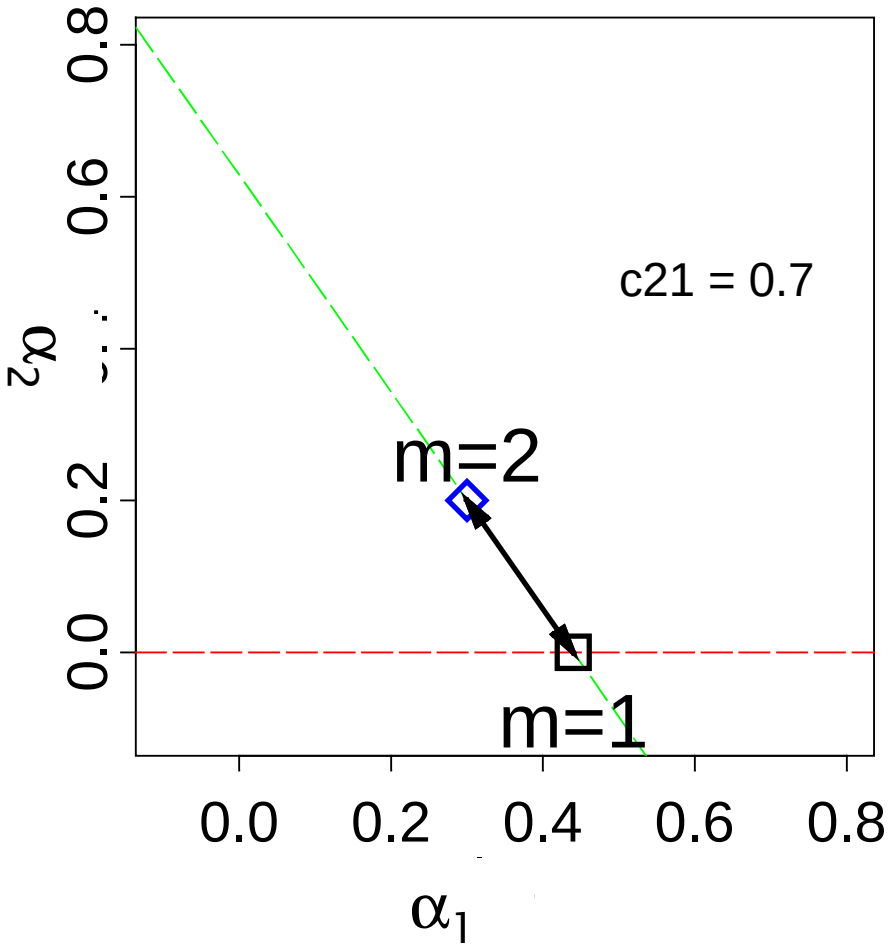
$$m=1: \quad \hat{\alpha}=\frac{c_{1Y}\, s_Y}{s_1}, \quad c_{1Y}=\mathrm{corr}(X_1,Y), s_Y=\mathrm{SD}(Y),...$$

$$m=2: \quad \hat{\alpha}_1=\frac{(c_{1Y}-c_{12}c_{2Y})s_Y}{s_1}=\hat{\alpha}-\frac{c_{12}c_{2Y}s_Y}{s_1}$$

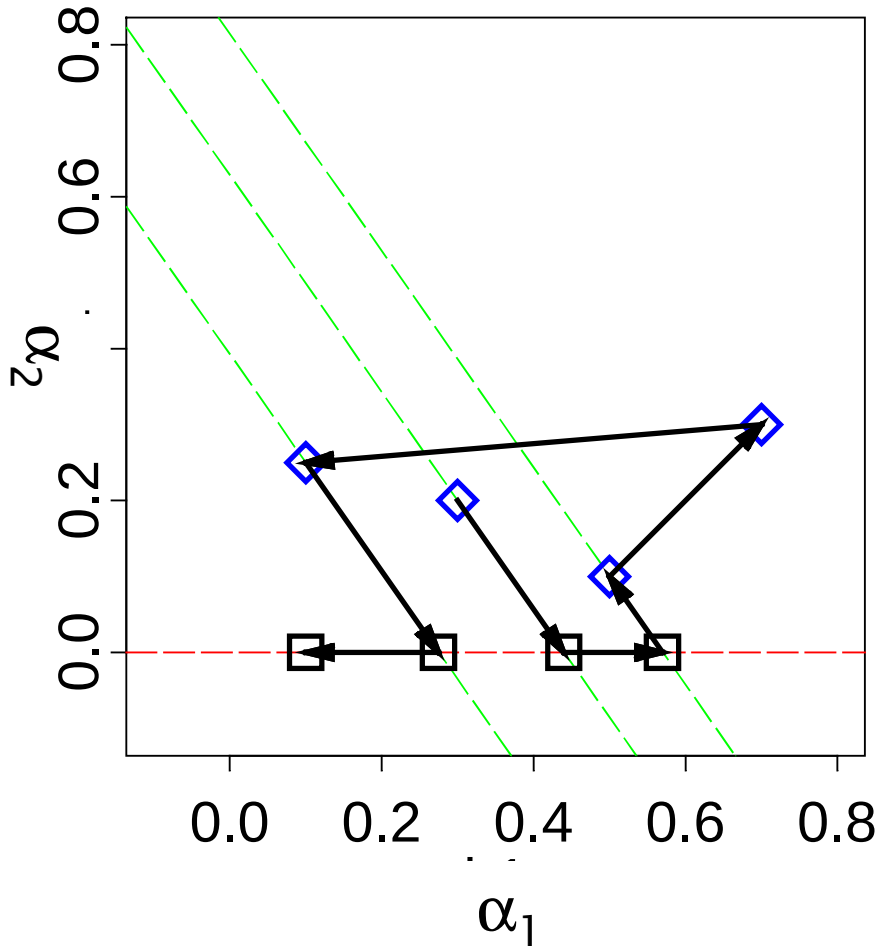
$$m=2: \quad \hat{\alpha}_2=\frac{(c_{2Y}-c_{12}c_{1Y})s_Y}{s_2}$$

Geometry of Reversible Jump

Move Between Models

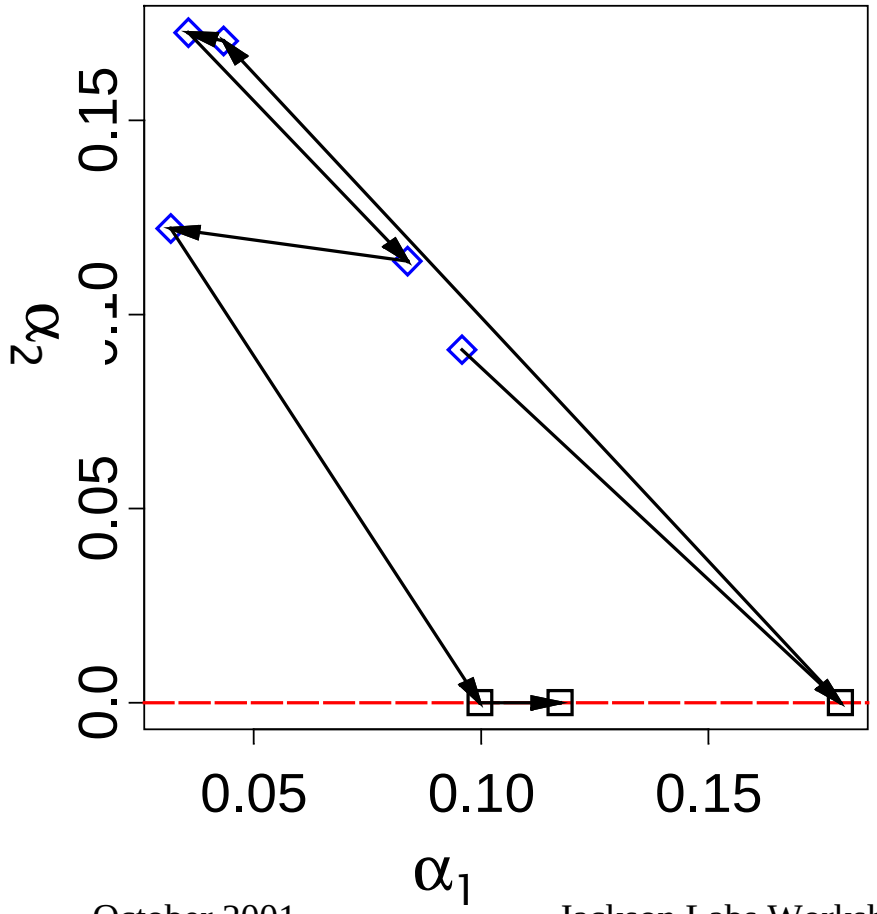


Reversible Jump Sequence

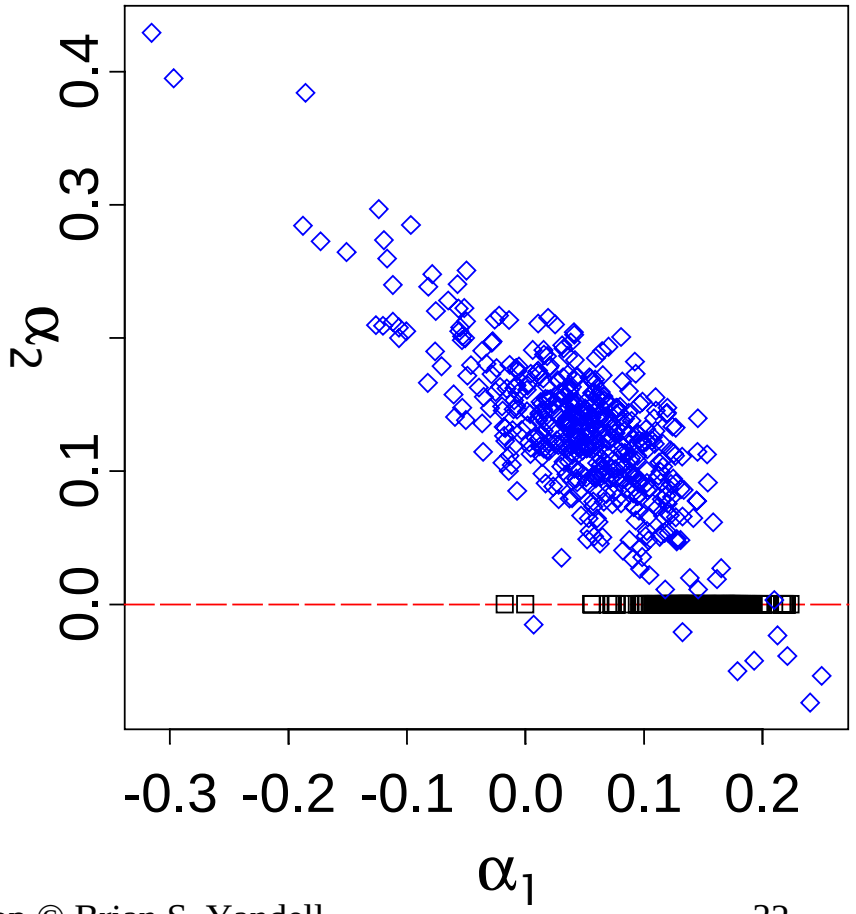


QT additive Reversible Jump

a short sequence



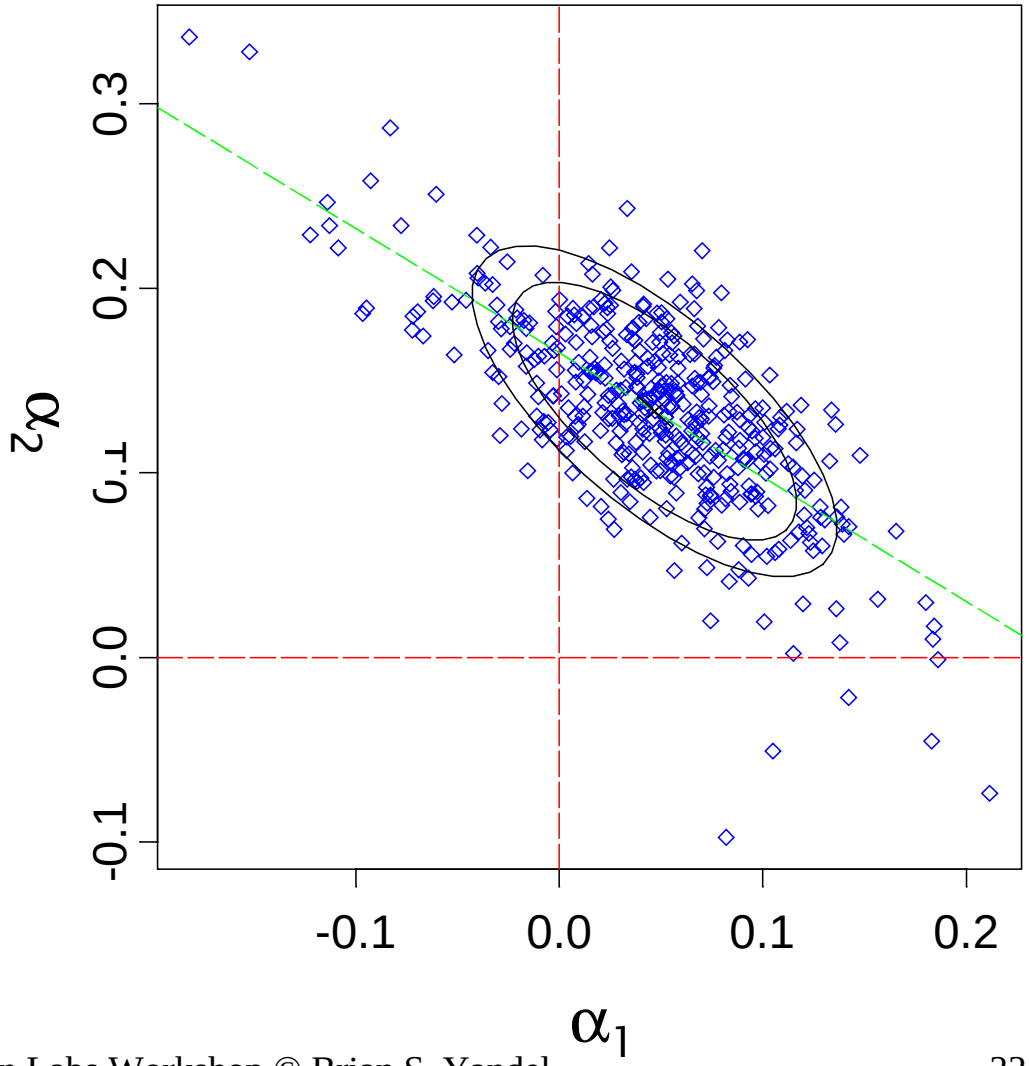
first 1000 with m<3



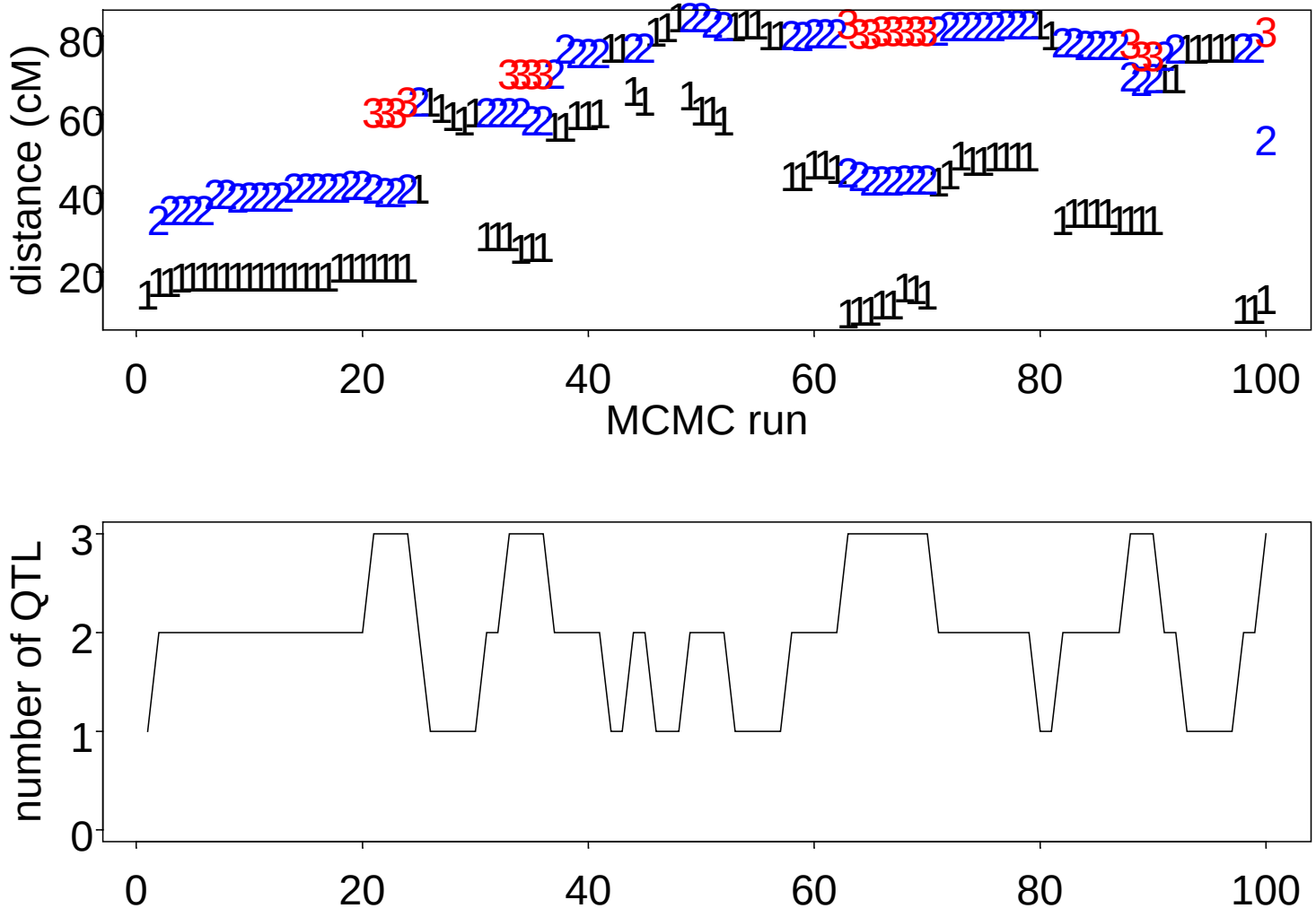
Credible Set for additive

90% & 95% sets based on normal

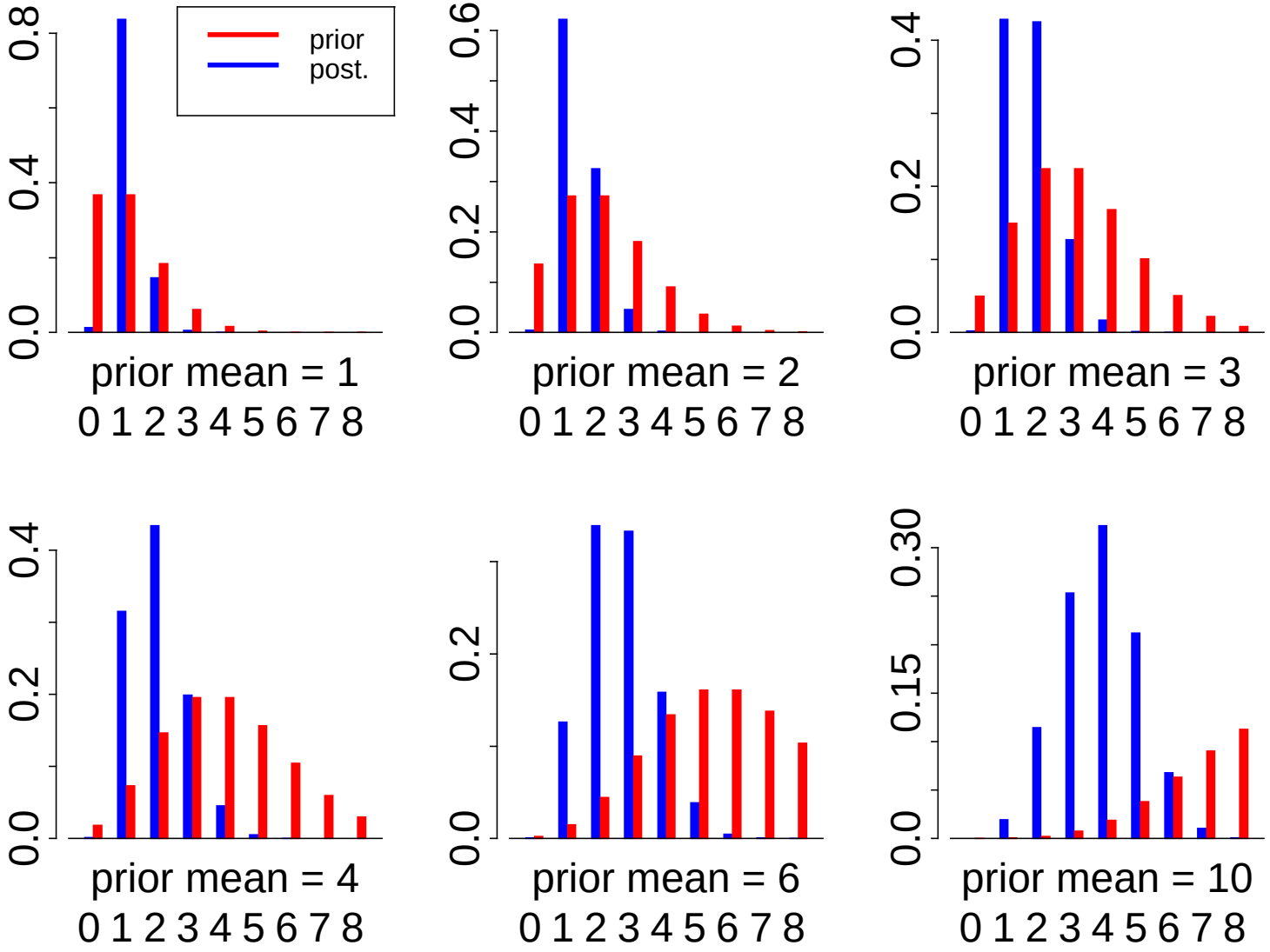
regression line corresponds to slope of updates



Jumping QTL number & loci



#QTL for *Brassica* 8-week



Bayes Factor Sensitivity

- Bayes factors computed from RJ-MCMC
  - posterior $P(m|Y,X)$ affected by prior $P(m)$
  - *BF* insensitive to prior

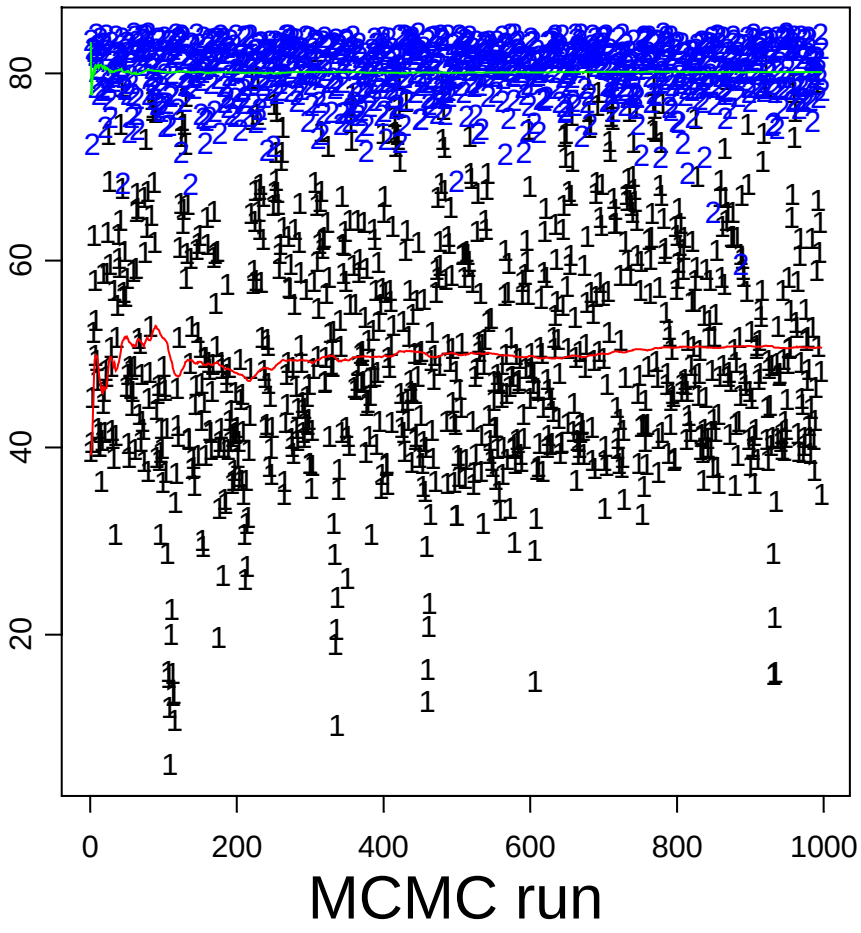
$$BF_{m,m+1} = \frac{P(m|Y,X)/P(m)}{P(m+1|Y,X)/P(m+1)}$$

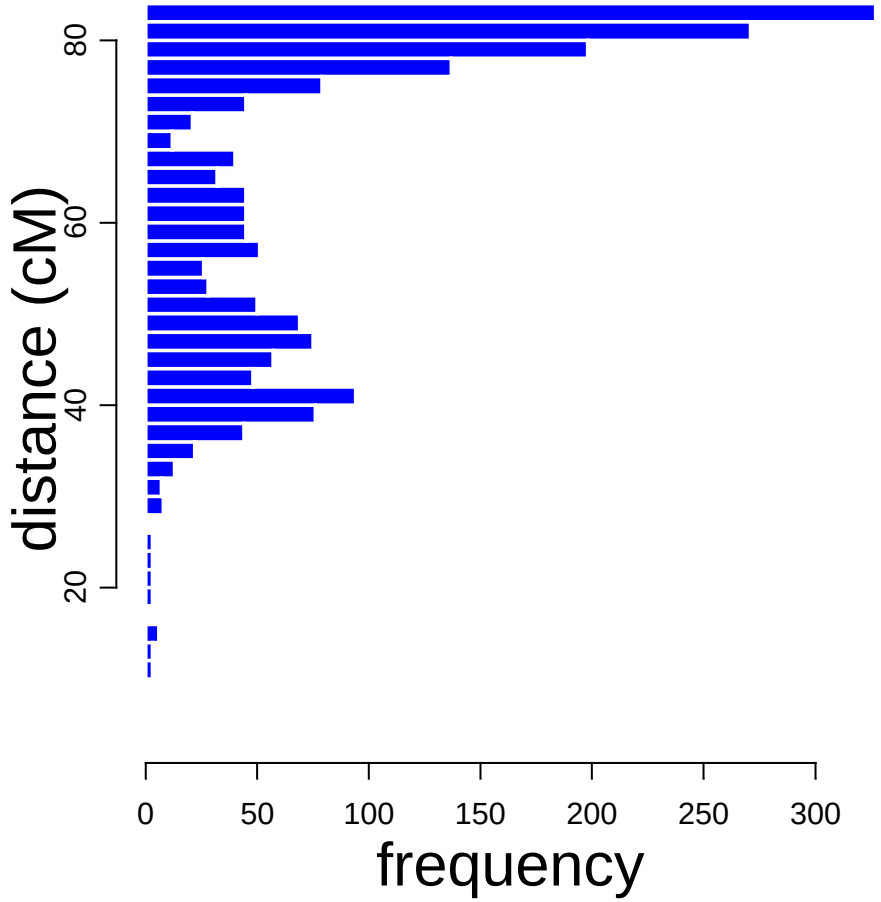
    - exponential, Poisson, uniform
  - *BF* sensitivity to prior variance on effects θ
    - prior variance should reflect data variability
    - resolved by using hyperpriors
      - automatic algorithm; no need for tuning by user

How To Infer Loci?

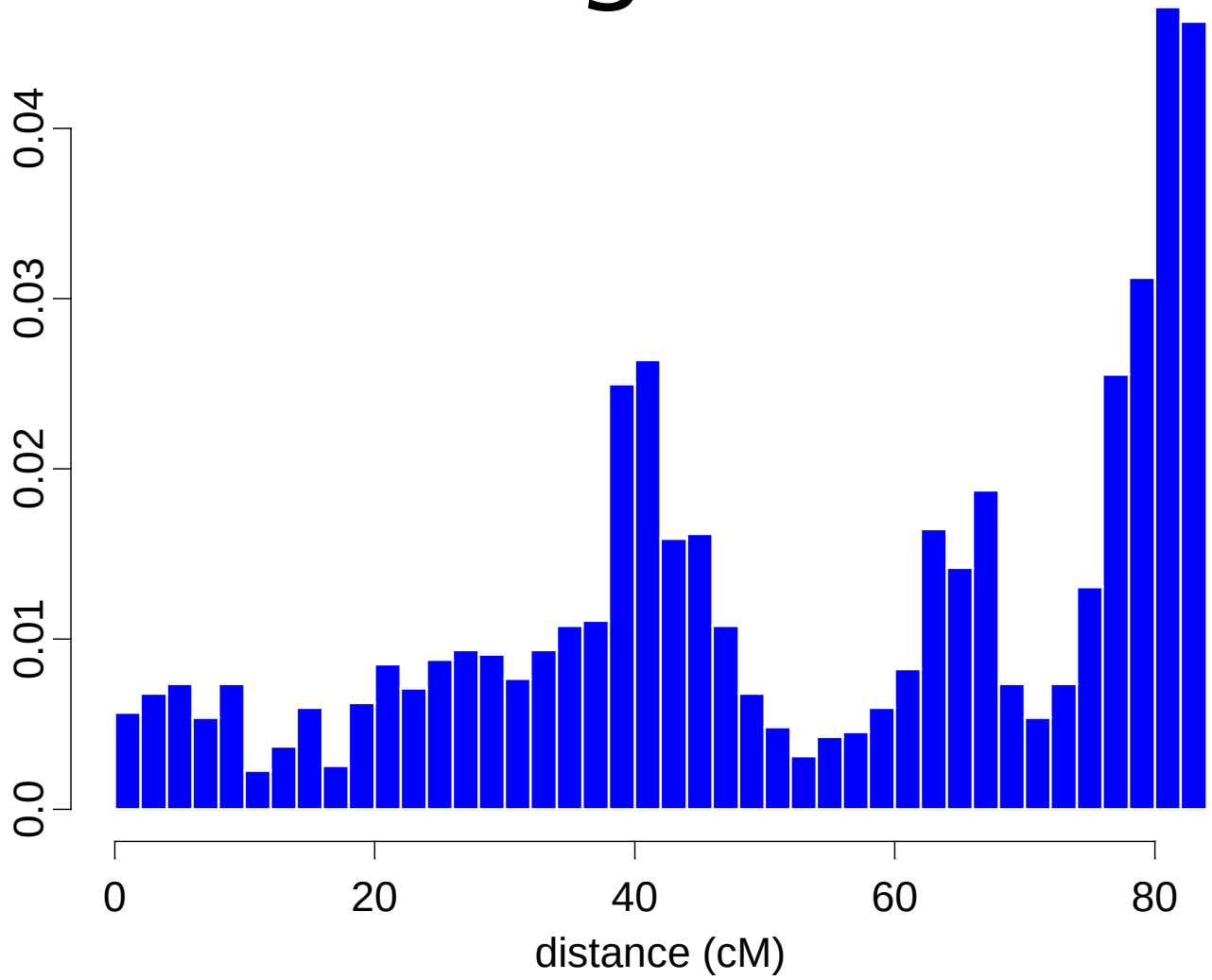
- if *m* is known, use fixed MCMC
  - histogram of loci
  - issue of bump hunting
- combining loci estimates in RJ-MCMC
  - some steps are from wrong model
    - too few loci (bias)
    - too many loci (variance/identifiability)
  - condition on number of loci
    - subsets of Markov chain

Brassica 8-week Data `locus` MCMC with $m=2$

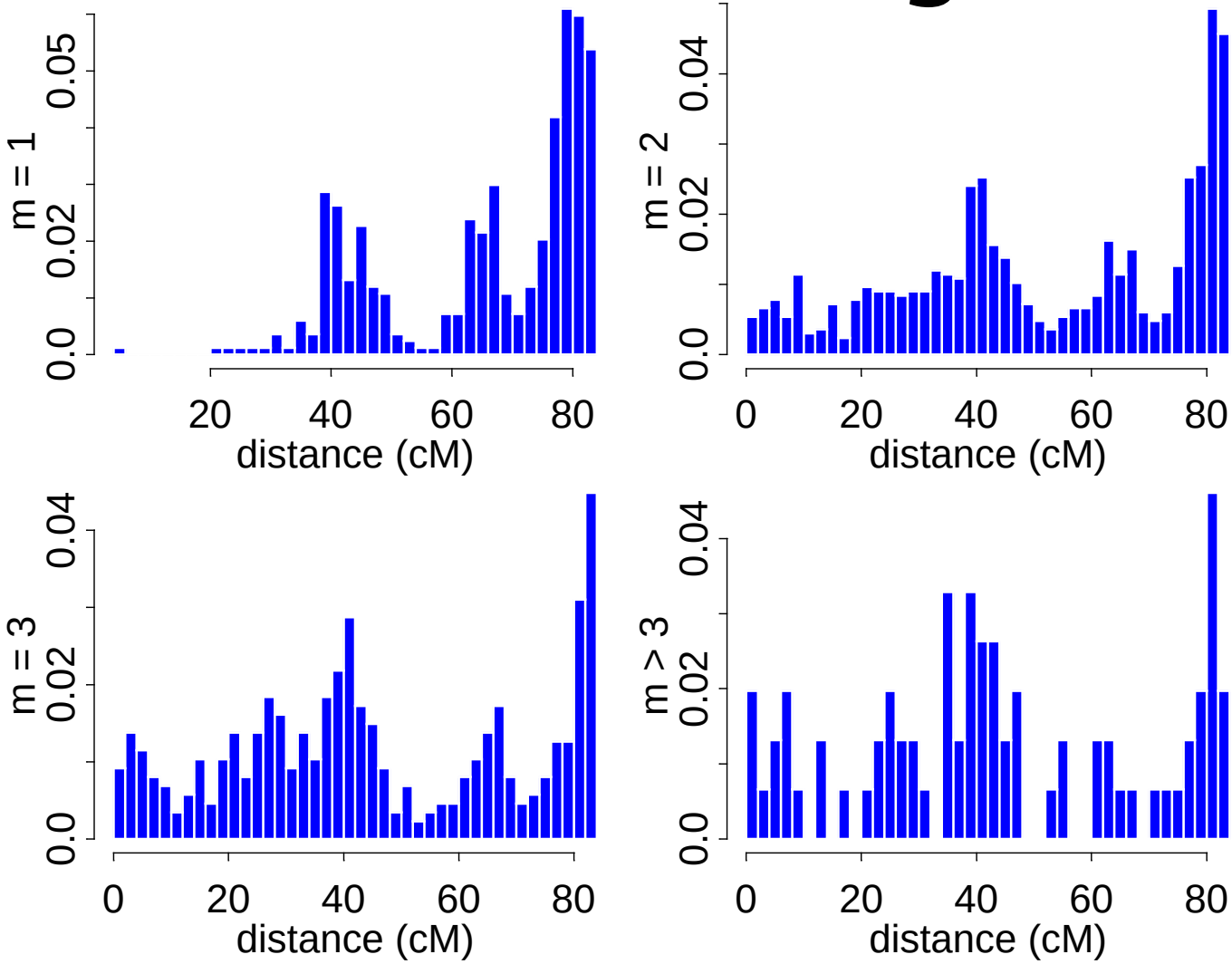




Raw Histogram of loci



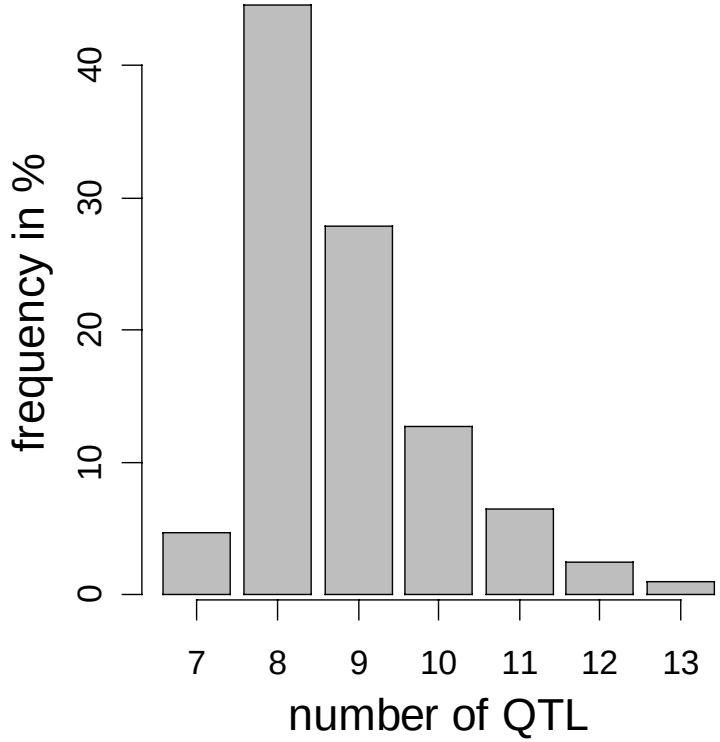
Conditional Histograms



A Complicated Example

- simulated 200 individuals (Stephens, Fisch 1998)
- 8 QTL, heritability = 50%; detected 3 QTL
- increase heritability to 97% to detect all 8

QTL	chr	loci	effect
1	**1**	**11**	**–3**
2	**1**	**50**	**–5**
3	**3**	**62**	**+2**
4	**6**	**107**	**–3**
5	**6**	**152**	**+3**
6	**8**	**32**	**–4**
7	**8**	**54**	**+1**
8	**9**	**195**	**+2**



Best Loci Model by Chromosome

- notice which chromosomes have persistent loci
- best pattern found 42% of the time

Chromosome

m	**1**	2	**3**	4	5	**6**	7	**8**	**9**	10	**Count of 8000**
8	**2**	**0**	**1**	**0**	**0**	**2**	**0**	**2**	**1**	**0**	3371
9	3	0	1	0	0	2	0	2	1	0	751
7	2	0	1	0	0	2	0	1	1	0	377
9	2	0	1	0	0	2	0	2	1	0	218
9	2	0	1	0	0	3	0	2	1	0	218
9	2	0	1	0	0	2	0	2	2	0	198

Reanalysis of other *Brassica* data

- *Brassica napus*
  - 19 chromosomes, 480 markers
  - infer 4 QTL (2 linked, 2 unlinked)
- *Brassica rapa*
  - 9 chromosomes
  - infer 4 QTL with added *FLC* marker
    - 2 tightly linked in repulsion
- Ferreira *et al.* (1994), Kole *et al.* (1997, 2001)

Computational Issues

- more complicated when $m > 2$
  - avoid matrix inverses: Cholesky decomposition
  - multivariate updates: all effects, all loci at once
- improvements in sampling efficiency
  - pre-burnin to overshoot *m*, burnin to wash out
  - occasional long distance loci update
- bump hunting to sort out loci
- Gaffney (2001 PhD thesis)

Bayesian IM Software

- General MCMC software
  - U Bristol links
    - http://www.stats.bris.ac.uk/MCMC/pages/links.html
  - BUGS (Bayesian inference Using Gibbs Sampling)
    - http://www.mrc-bsu.cam.ac.uk/bugs/
- Our MCMC software for QTLs
  - our C code using LAPACK
    - ftp://ftp.stat.wisc.edu/pub/yandell/revjump.tar.gz
  - our QTL Cart module
    - Bmapqtl 3rd party module (Windows available)
    - R post processing

QTL References

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