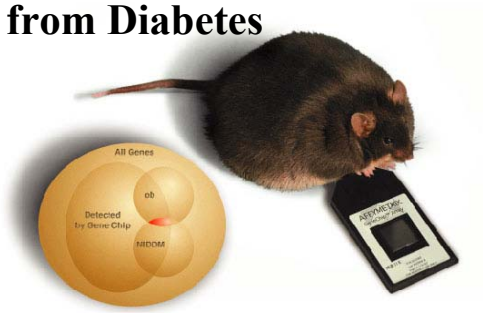
Gene Mapping for High Throughput Expression Profiles: Lessons from Diabetes



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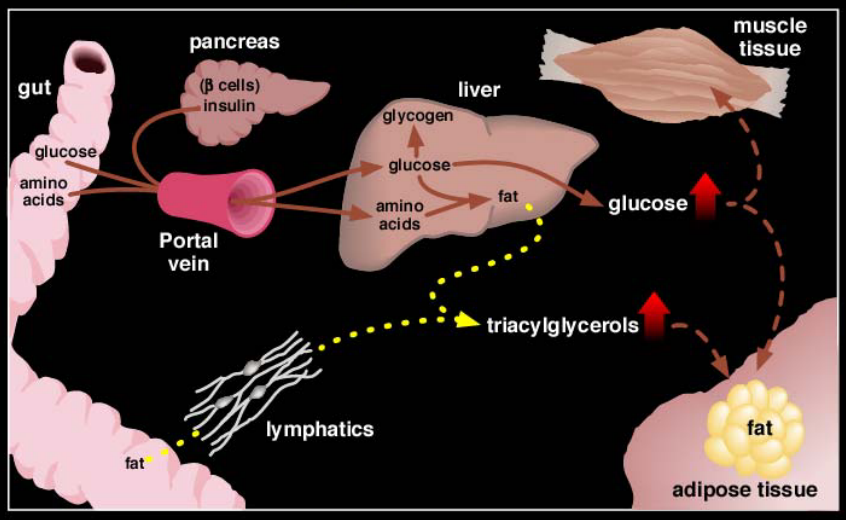
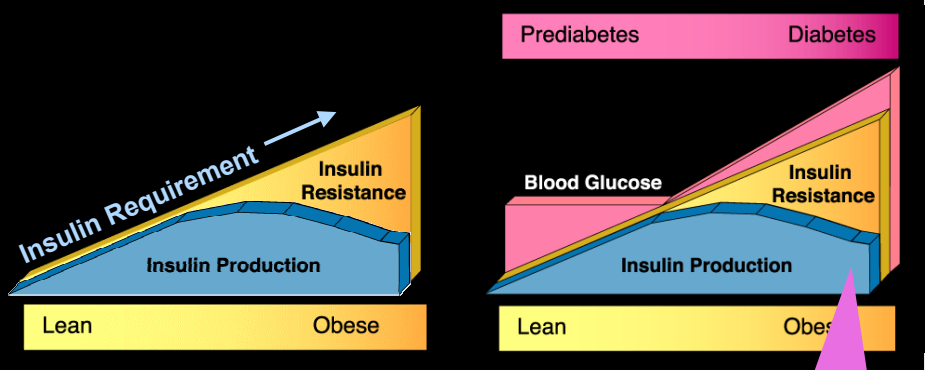
Outline

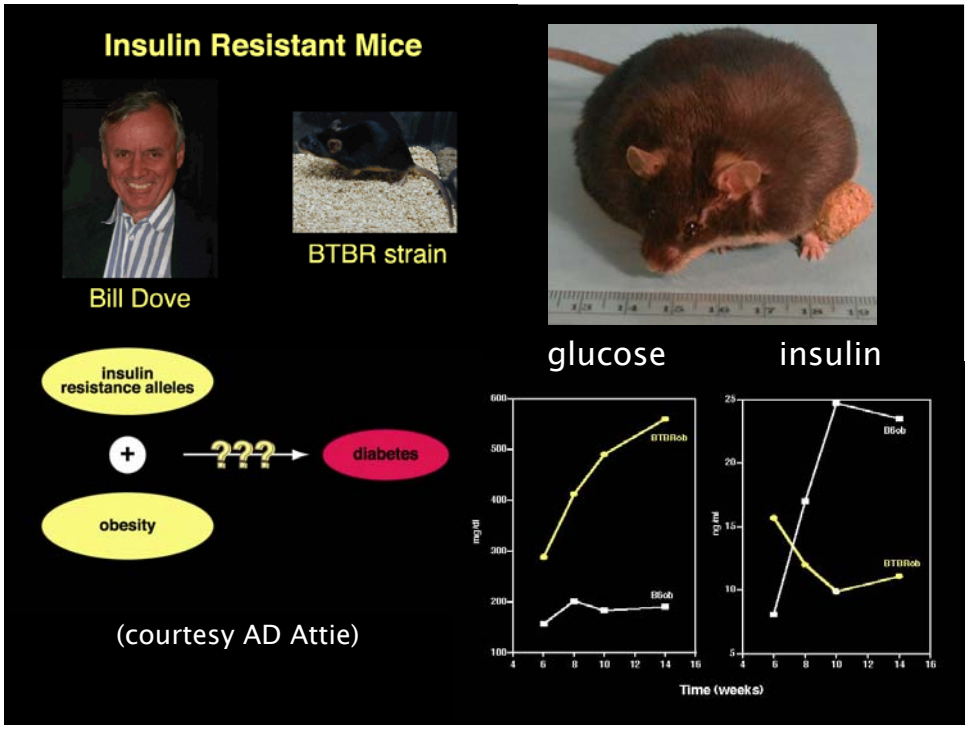
- why study diabetes in a mouse model?
- why map gene expression?
- what are QTL?
  - why multiple QTL?
  - how to select genetic architecture?
- how to map massive gene expression?
- preliminary results

Type 2 Diabetes Mellitus





from Unger & Orci *FASEB J.* (2001) 15,312



studying diabetes in an F2

- segregating cross of inbred lines
  - B6.ob x BTBR.ob → F1 → F2
  - selected mice with ob/ob alleles at leptin gene (chr 6)
  - measured and mapped body weight, insulin, glucose at various ages (Stoehr et al. 2000 Diabetes)
  - sacrificed at 14 weeks, tissues preserved
- gene expression data
  - Affymetrix microarrays on parental strains, F1
    - key tissues: adipose, liver, muscle, β-cells
    - novel discoveries of differential expression (Nadler et al. 2000 PNAS; Lan et al. 2002 in review; Ntambi et al. 2002 PNAS)
  - RT-PCR on 108 F2 mice liver tissues
    - 15 genes, selected as important in diabetes pathways
    - SCD1, PEPCK, ACO, FAS, GPAT, PPARgamma, PPARalpha, G6Pase, PDI,…

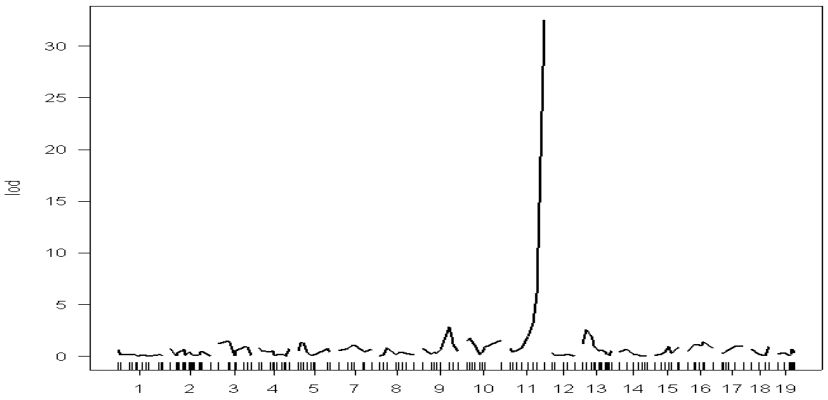
why map gene expression as a quantitative trait?

- *cis*- or *trans*-action?
  - does gene control its own expression?
  - evidence for both modes (Brem et al. 2002 Science)
- mechanics of gene expression mapping
  - measure gene expression in intercross (F2) population
  - map expression as quantitative trait (QTL technology)
  - adjust for multiple testing via false discovery rate
- research groups working on expression QTLs
  - review by Cheung and Spielman (2002 *Nat Gen Suppl*)
  - Kruglyak (Brem et al. 2002 *Science*)
  - Doerge et al. (Purdue); Jansen et al. (Waginingen)
  - Williams et al. (U KY); Lusis et al. (UCLA)
  - Dumas et al. (2000 *J Hypertension*)

What is a QTL?

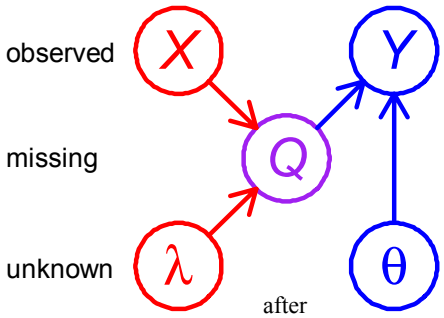
- QTL = quantitative trait locus (or loci)
  - trait = phenotype = characteristic of interest
  - quantitative = measured somehow
    - qualitative traits can often be directly mapped
    - quantitative traits not readily mapped
  - locus = location in genome affecting trait
    - gene or collection of tightly linked genes
    - some physical feature of genome

LOD map for PDI: *cis*-regulation



interval mapping basics

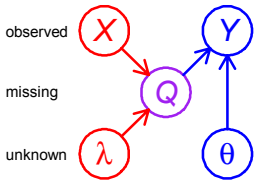
- observed measurements
  - Y = phenotypic trait
  - X = markers & linkage map
    - i = individual index 1,…,n
- missing data
  - missing marker data
  - Q = QT genotypes
    - alleles QQ, Qq, or qq at locus
- unknown genetic architecture
  - λ = QT locus (or loci)
  - θ = genetic action
  - m = number of QTL
- pr($Q|X,\lambda,m$) recombination model
  - grounded by linkage map, experimental cross
  - recombination yields multinomial for Q given X
- pr($Y|Q,\theta,m$) phenotype model
  - distribution shape (assumed normal here)
  - unknown parameters θ (could be non-parametric)



after
Sen Churchill (2001)

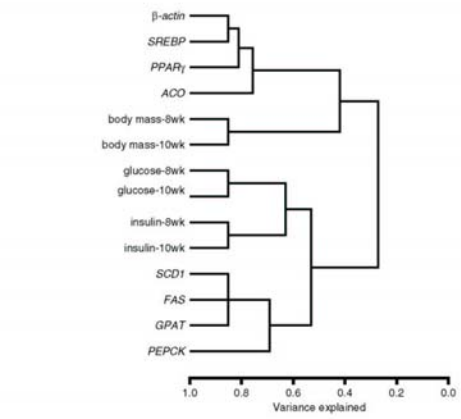
interval mapping details and interpretation

- likelihood models relation of data to unknown architecture
  - $L(\lambda,\theta|m)$ $= \text{pr}(Y|X,\lambda,\theta,m)$
    $= \text{product}_i\ [\text{sum}_Q\ \text{pr}(Q|X_i,\lambda,m)\ \text{pr}(Y_i|Q,\theta,m)]$
  - complicated to evaluate: product of sum of products
- classical interval mapping: maximize LOD
  - $\text{LOD}(\lambda) = \max_\theta \log_{10} L(\lambda,\theta|Y,m)/L(\mu|Y)$
    - scan loci systematically across genome
  - threshold for testing presence vs. no QTL
    - theory for single QTL
      (Lander Botstein 1989; Dupuis Siegmund 1999 *Genetics*)
    - permutation tests for more general setting
      (Churchill Doerge 1994; Doerge Churchill 1996 *Genetics*)
- study genetic architecture
  - assess with Bayesian Information Criteria (BIC)



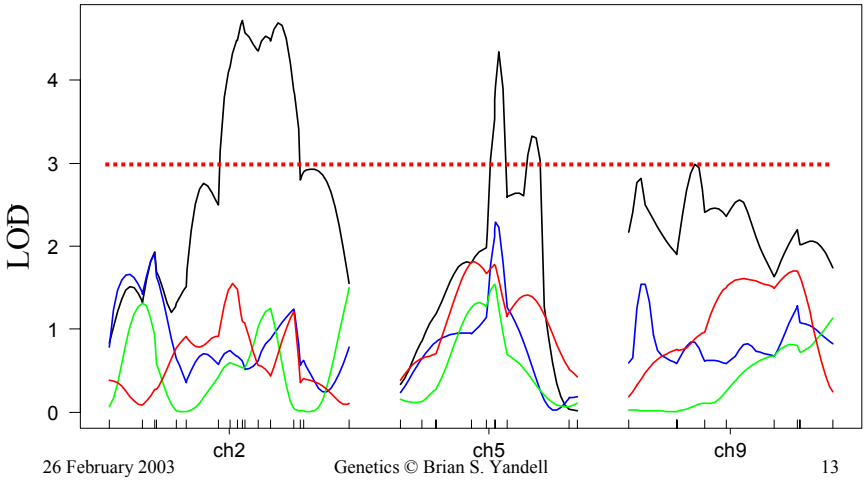
high throughput: which genes are the key players?

Lan et al., mapping mRNA, Figure 2

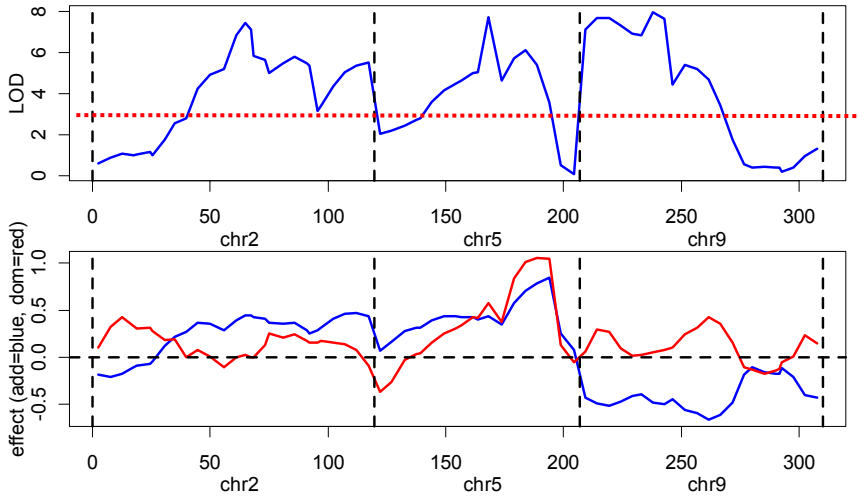


- one approach:
  clustering of expression
  seed by insulin, glucose
- advantage:
  subset relevant to trait
- disadvantage:
  still many genes to study

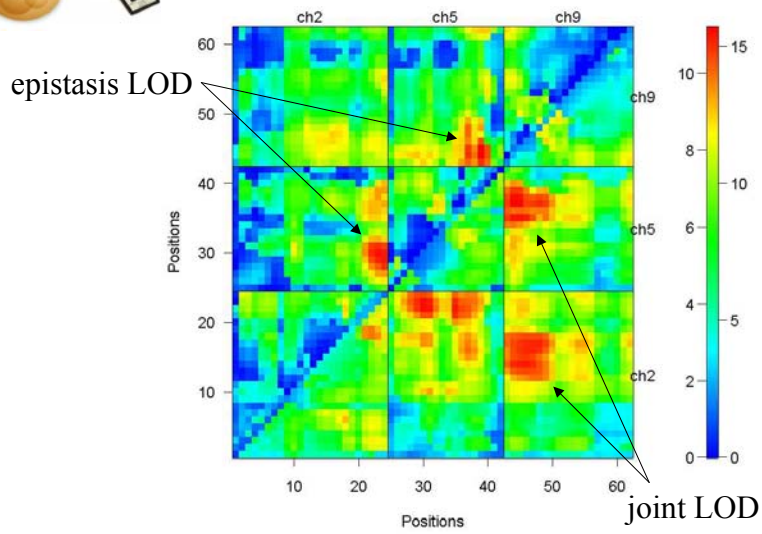
SCD1, FAS,GPAT, PEPCK: *trans*-regulation by multiple QTL?



Multiple Interval Mapping SCD1: multiple QTL plus epistasis!



2-QTL scan for SCD1

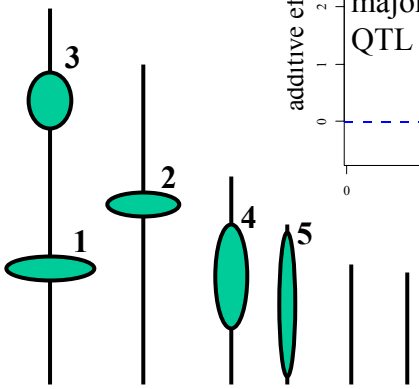


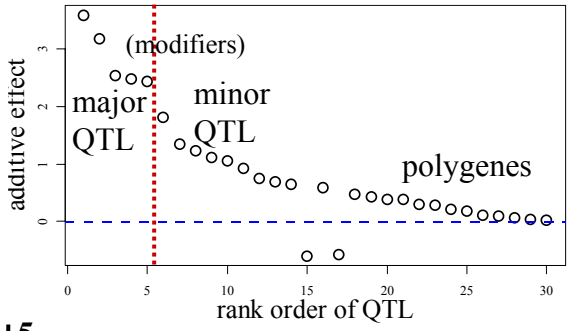
multiple QTL & gene expression

- does one locus affect expression of many genes?
  - is this a controlling locus?
  - is there coordinated expression across many genes?
- multiple QTL affecting gene expression?
  - multiple controlling loci for key pathways?
  - single QTL approach would be inadequate
- multiple QTL literature
  - multiple interval mapping (Kao, et al. 1999 *Genetics*; Zeng et al. 2000 *Genetics*; Broman Speed 2002 *JRSSB*)
  - Bayesian interval mapping (Satagopan et al. 1996 *Genetics*; Satagopan Yandell 1996; Stevens Fisch 1998 *Biometrics*; Silanpää Arjas 1998, 1999 *Genetics*; Sen Churchill 2001 *Genetics*; Gaffney 2001; Yi Xu 2002 *Genetics*)

Pareto diagram of QTL effects

major QTL on linkage map



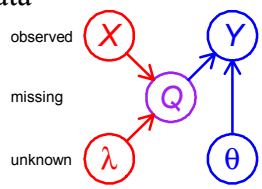


how many (detectable) QTL?

- many, many QTL may affect most any trait
  - how many QTL are detectable with these data?
    - limits to useful detection (Bernardo 2000)
    - depends on sample size, heritability, environmental variation
  - consider probability that a QTL is in the model
    - avoid sharp in/out dichotomy
    - major QTL usually selected, minor QTL sampled infrequently
- build m = number of QTL detected into QTL model
  - directly allow uncertainty in genetic architecture
  - model selection over number of QTL, architecture
  - use Bayes factors and model averaging
    - to identify "better" models

Bayesian interpretation

- consider likelihood of data augmented by QTL genotypes
  - pr($Y,Q|X,\lambda,\theta,m$) = product$_i$ pr($Q|X_i,\lambda,m$) pr($Y_i|Q,\theta,m$)
- reinterpret likelihood as posterior for architecture
  - pr($\lambda,Q,\theta,m|Y,X$) = [product$_i$ pr($Q_i|X_i,\lambda,m$) pr($Y_i|Q_i,\theta,m$)] [pr($\lambda,\theta|X,m$)pr(m)]
    = [augmented likelihood] x [prior]
- examine posterior of architecture given data
  - controlling loci λ and gene action θ
    - pr($\lambda,\theta|Y,X,m$) = sum$_Q$ pr($\lambda,Q,\theta|Y,X,m$) with m fixed
    - average over missing QTL genotypes
  - number of QTL m
    - pr($m|Y,X$) = sum$_{(\lambda,\theta)}$ pr($\lambda,\theta|Y,X,m$)pr(m)
    - average over possible m-QTL architectures



- assess using Bayes factors
  - extends Bayes Information Criterion to compare any 2 models

Bayes factors to assess models

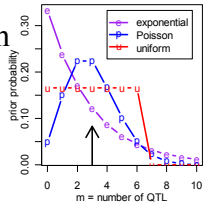
- Bayes factor: which model best supports the data?
  - ratio of posterior odds to prior odds
  - ratio of model likelihoods
- equivalent to *LR* statistic when
  - comparing two nested models
  - simple hypotheses (e.g. 1 vs 2 QTL)
- related to Bayes Information Criteria (BIC)
  - Schwartz introduced for model selection in general settings
  - penalty to balance model size (p = number of parameters)

$$BF = \frac{\text{pr}(m|Y,X)/\text{pr}(m+1|Y,X)}{\text{pr}(m)/\text{pr}(m+1)} = \frac{\text{pr}(Y|m,X)}{\text{pr}(Y|m+1,X)}$$

$$-2\log(BF) = -2\log(LR) - 2\log(n) = BIC$$

computing QTL Bayes factors

- easy to compute Bayes factors from samples
  - sample posterior using MCMC
  - posterior pr($m|Y,X$) is marginal histogram
  - posterior affected by prior pr(m)



- *BF* insensitive to shape of prior
  - geometric, Poisson, uniform
  - precision improves when prior mimics posterior
- *BF* sensitivity to prior variance on effects θ
  - prior variance should reflect data variability
  - resolved by using hyper-priors
    - automatic algorithm; no need for user tuning

multiple QTL phenotype model

- $Y = \mu + G_Q +$ environment
- partition genotypic effect into separate QTL effects

  G_Q = main QTL effects + epistatic interactions

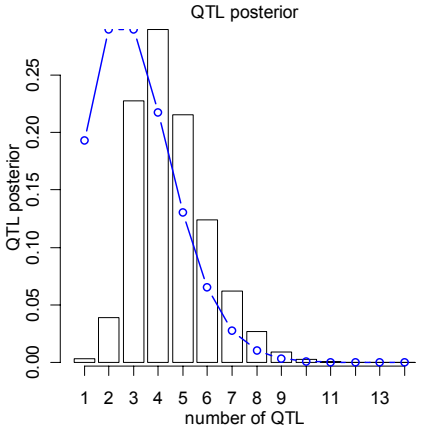
  $G_Q = \theta_{1Q} + \ldots + \theta_{mQ} + \theta_{12Q} + \ldots$
- priors on mean and effects

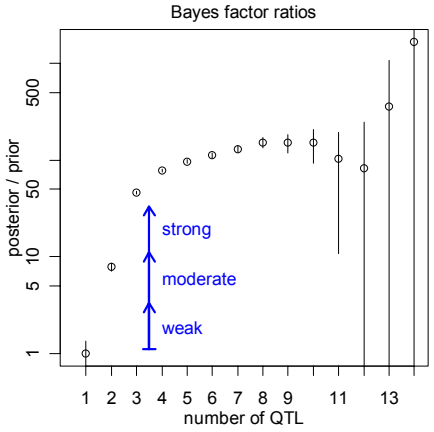
  $G_Q \sim N(0, h^2 s^2)$ model independent genotypic prior

  $\theta_{jQ} \sim N(0, \kappa_1 s^2/m.)$ additive effects (down-weighted)

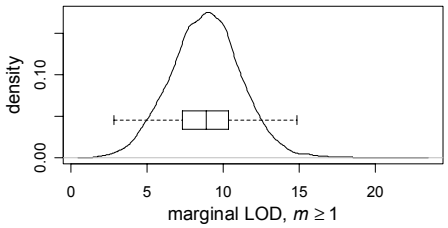
  $\theta_{j2Q} \sim N(0, \kappa_2 s^2/m.)$ epistatic interactions (down-weighted)
- hyper-parameters (to reduce sensitivity of Bayes factors to prior)
  - s^2 = total sample variance
  - $m. = m + m_2$ = number of QTL effects and interactions
  - $h^2 = (m\kappa_1 + m_2\kappa_2)/m.$ = unknown heritability, $h^2/2 \sim$ Beta(a,b)

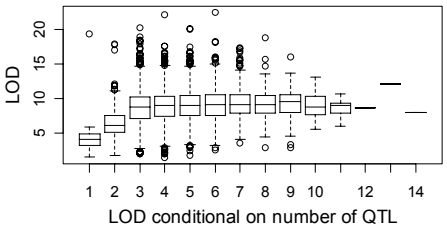
Bayesian model assessment: number of QTL for SCD1

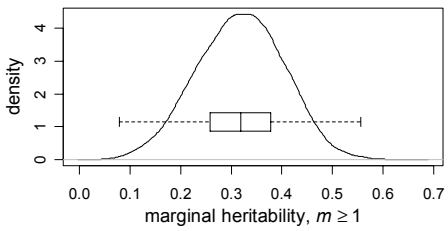


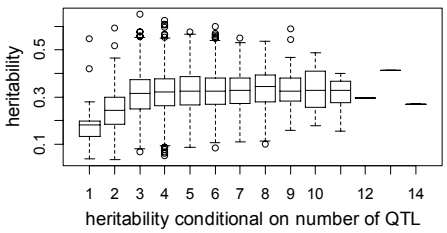


Bayesian LOD and h^2 for SCD1

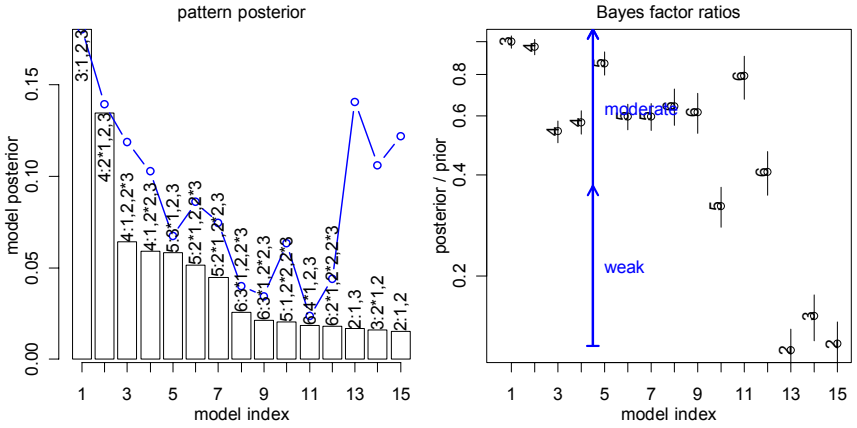






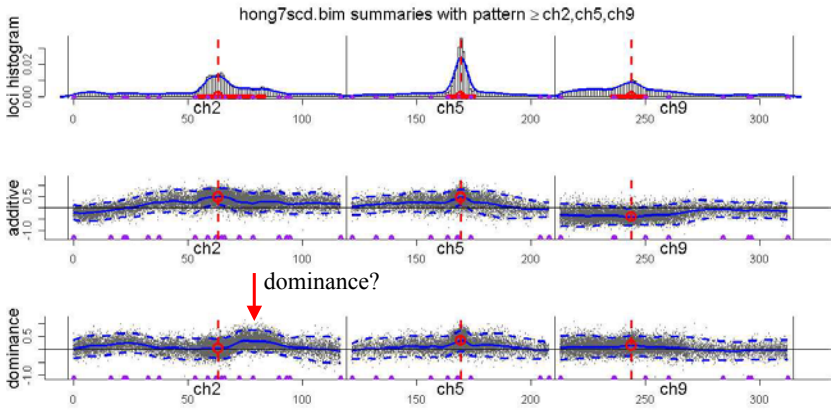


Bayesian model assessment: chromosome QTL pattern for SCD1

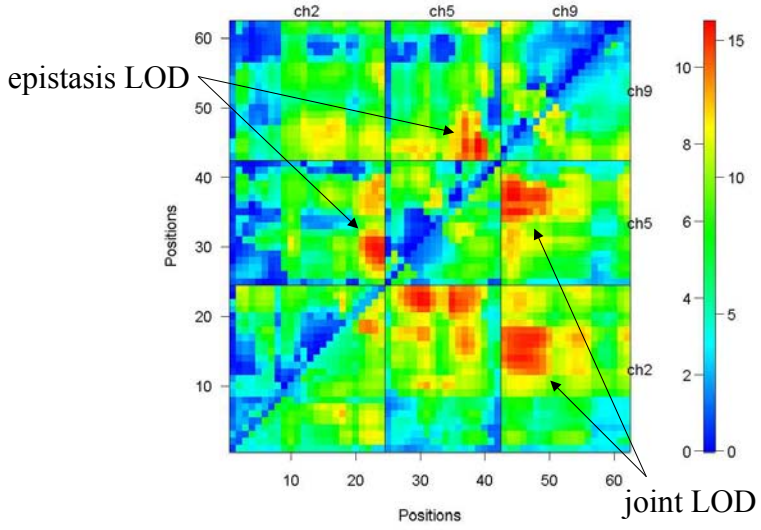


trans-acting QTL for SCD1

(no epistasis yet: see Yi Xu 2002)



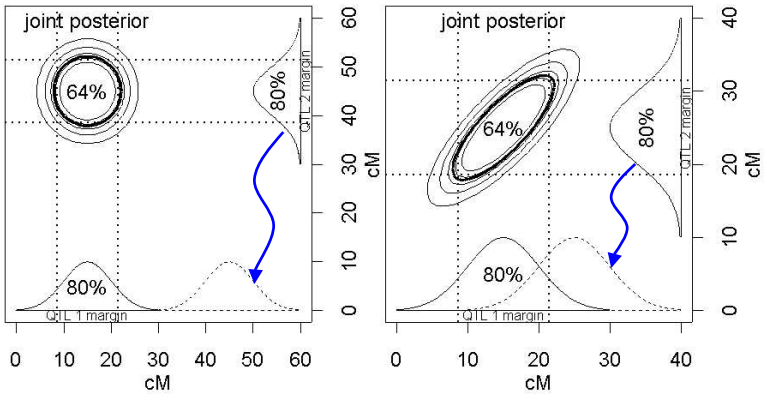
2-D scan: assumes only 2 QTL!



1-D and 2-D marginals

pr(QTL at λ | *Y,X, m*)

unlinked loci linked loci



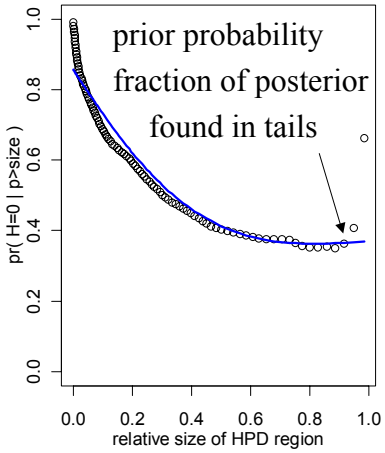
false detection rates and thresholds

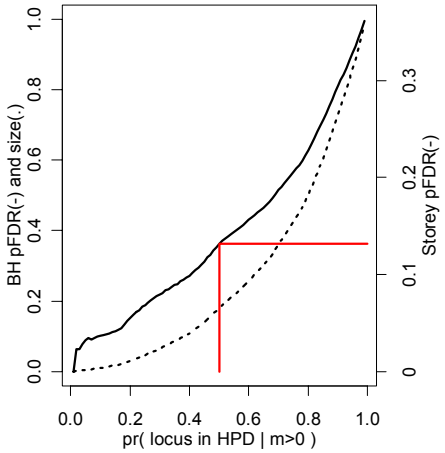
- multiple comparisons: test QTL across genome
  - size = pr(LOD(λ) > threshold | no QTL at λ)
  - threshold guards against a single false detection
    - very conservative on genome-wide basis
  - difficult to extend to multiple QTL
- positive false discovery rate (Storey 2001)
  - pFDR = pr(no QTL at λ | LOD(λ) > threshold)
  - Bayesian posterior HPD region based on threshold
    - $\Lambda = \{\lambda \mid \text{LOD}(\lambda) > \text{threshold}\} \approx \{\lambda \mid \text{pr}(\lambda \mid Y,X,m) \text{ large}\}$
  - extends naturally to multiple QTL

pFDR and QTL posterior

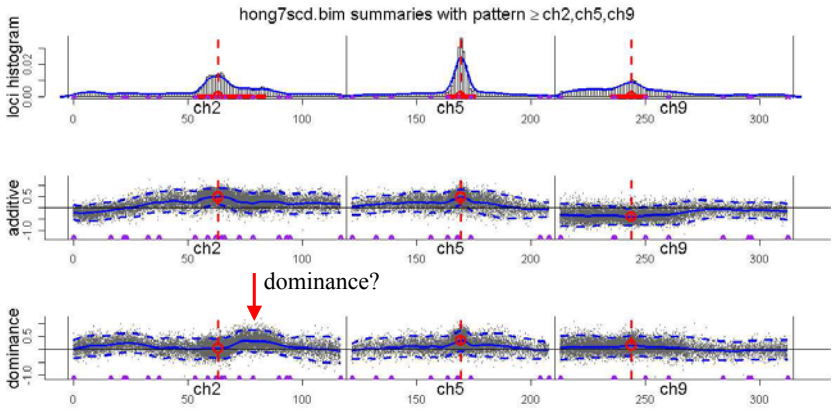
- positive false detection rate
  - pFDR = pr(no QTL at λ | Y,X, λ in Λ)
  - pFDR = $\dfrac{\text{pr}(H=0)*\text{size}}{\text{pr}(m=0)*\text{size}+\text{pr}(m>0)*\text{power}}$
  - power = posterior = pr(QTL in Λ | $Y,X, m>0$)
  - size = (length of Λ) / (length of genome)
- extends to other model comparisons
  - m = 1 vs. m = 2 or more QTL
  - pattern = ch1,ch2,ch3 vs. pattern > 2*ch1,ch2,ch3

pFDR for SCD1 analysis





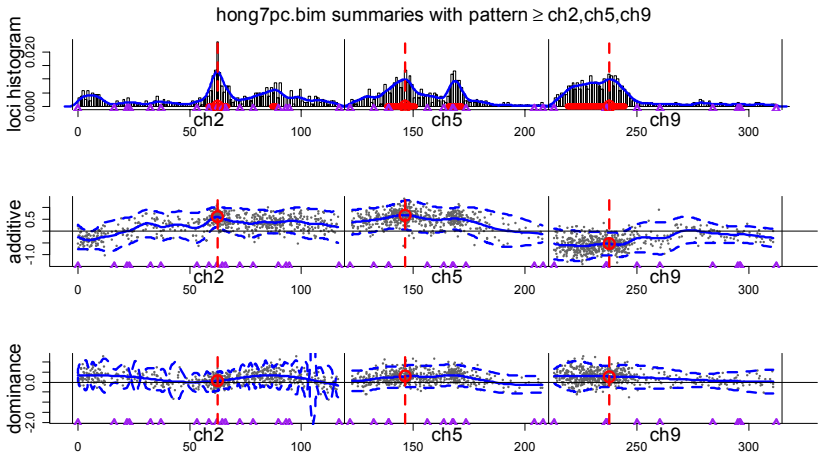
trans-acting QTL for SCD1



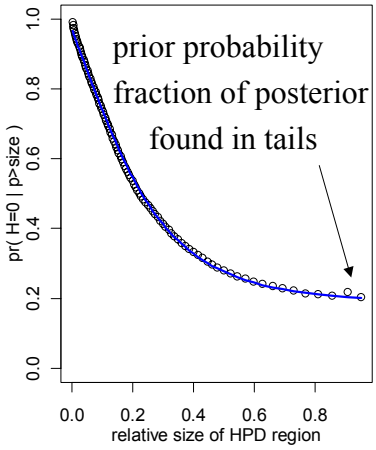
high throughput dilemma

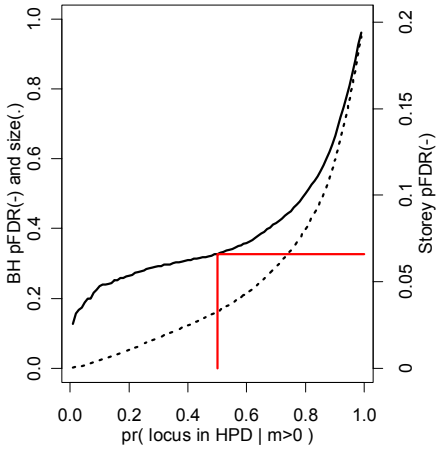
- want to focus on gene expression network
  - ideally capture pathway in a few dimensions
  - allow for complicated genetic architecture
- may have multiple controlling loci
  - could affect many genes in coordinated fashion
  - could show evidence of epistasis
  - quick assessment via interval mapping may be misleading
- mapping principle component as quantitative trait
  - multiple interval mapping with epistatic interactions
  - Liu et al. (1996 *Genetics*); Zeng et al. (2000 *Genetics*) Mahler et al. (2002 *Genomics*)
  - elicit biochemical pathways (Henderson et al. Hoeschele 2001; Ong Page 2002)

mapping first PC as a trait



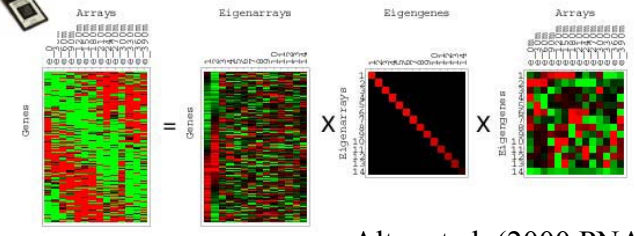
pFDR for PC1 analysis





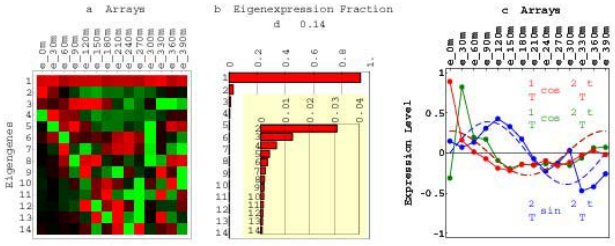
mapping controlling loci via PC

- Y = expression data from chips for F2 population
  - principle components (singular value decomposition)
    - $Y = UDV^T$
    - V has eigen-genes as rows, individuals as columns
  - Hilsenbeck et al. (1999); Alter et al. (2000); West et al. (2000)
- V = combined expression of coordinated genes
  - map V_1, V_2 as quantitative traits
  - identify mRNA with strong correlation: coordinated expression?

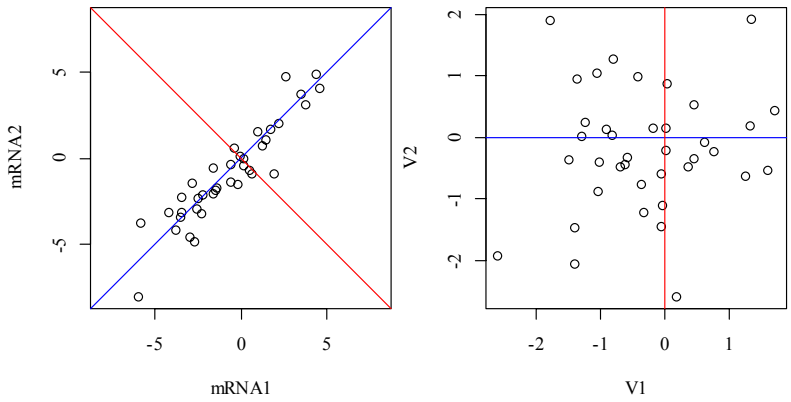


$Y = UDV^{\mathrm{T}}$

Alter et al. (2000 PNAS)
yeast cell cycle

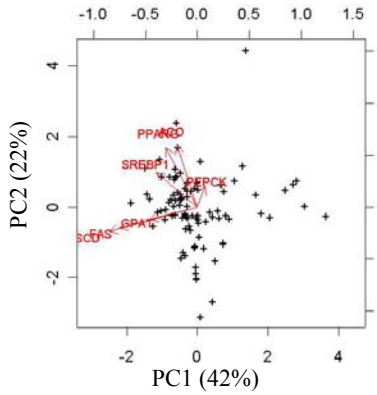


PC simply rotates & rescales to find major axes of variation

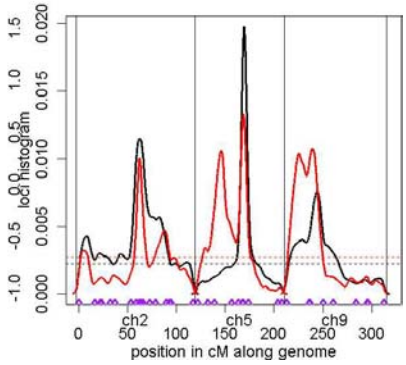


multivariate screen for gene expressing mapping

principal components



PC1(red) and SCD(black)



Relation of Composite Phenotypes to Individual mRNA Expressions

(after West et al. 2000)

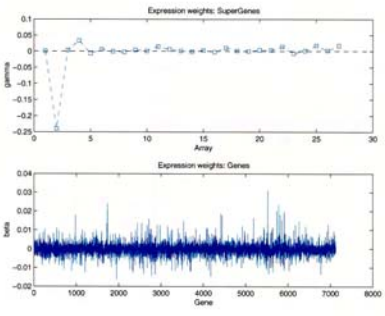


Figure 6: Summary of binary regression fit: Regression parameters

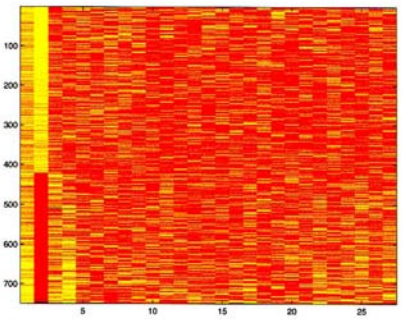


Figure 9: Factor loadings **A** for top 750 genes

SVD Pros and Cons

- advantages
  - superphenotypes V_1, V_2, ... are orthogonal
  - may only need a few
    - how fast do eigen-values D drop?
  - can dramatically increase power to detect QTL
- disadvantages
  - less efficient if many large eigen-values
  - may be difficult to interpret some superphenotypes
  - PCs may not reflect genetic differential expression
    - could iterate on putative QTL to improve discrimination

Ongoing & Future Work

- fine mapping via congenic lines
  - ongoing for physiological traits
  - candidate genes emerging
- new F2 population focusing on islets
  - expression mapping on a large scale (100-200 mice)
  - development of new methodology (Jin, Yang, Lan)
- model selection for genetic architecture
  - fast computation for multiple QTL (Yi, Gaffney)
  - high throughput model assessment

Summary

- mouse model for diabetes
  - studying pathways via gene expression
  - massive number of phenotypes: expression arrays
- model selection for multiple QTL
  - Bayes factors for model assessment
  - posteriors can reveal subtle hints of QTL
  - multiple trait mapping…
- dimension reduction to elicit pathways
  - study genetic architecture of "supergenes"
  - unravel correlation with individual mRNA
- connection to false discovery rate
  - whole genome evaluation
  - calibrate posterior region with pFDR

Collaborators

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software

- www.stat.wisc.edu/~yandell/qtl/software/Bmapqtl
  - module using QtlCart format
  - compiled in C for Windows/NT
  - extensions in progress
  - R post-processing graphics
    - library(bim) is cross-compatible with library(qtl)
- Bayes factor and reversible jump MCMC computation
- enhances MCMCQTL and revjump software
  - initially designed by JM Satagopan (1996)
  - major revision and extension by PJ Gaffney (2001)
    - whole genome
    - multivariate update of effects; long range position updates
    - substantial improvements in speed, efficiency
    - pre-burnin: initial prior number of QTL very large