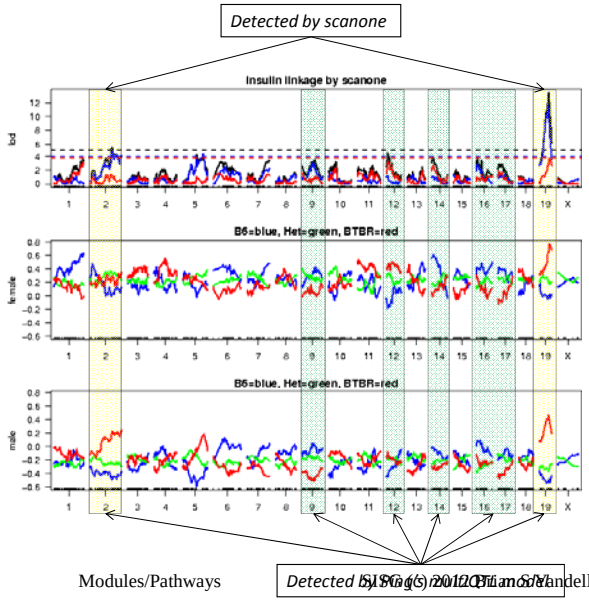
Expression Modules

Brian S. Yandell (with slides from
Steve Horvath, UCLA, and
Mark Keller, UW-Madison)

Weighted models for insulin

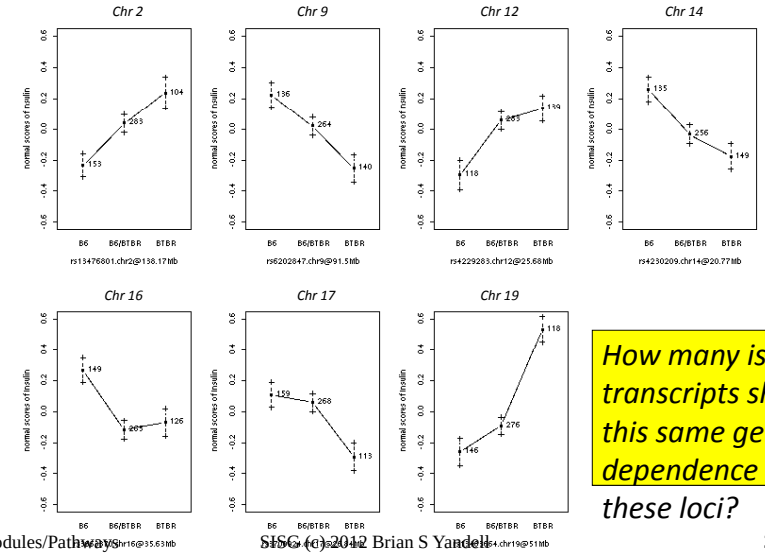


 # transcripts that match weighted insulin model in each of 4 tissues:

tissue	# transcripts
Islet	1984
Adipose	605
Liver	485
Gastroc	404

Ping Wang

insulin main effects



How many islet transcripts show this same genetic dependence at these loci?

Expression Networks
Zhang & Horvath (2005)

www.genetics.ucla/edu/labs/horvath/CoexpressionNetwork

- organize expression traits using correlation

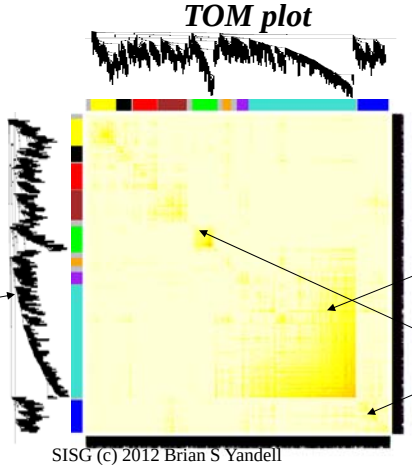
- adjacency $a_{ij} = |cor(x_i, x_j)|^{\beta}, \beta = 6$
- connectivity $k_i = \text{sum}_l(a_{il})$
- topological overlap $TOM_{ij} = \dfrac{a_{ij} + \text{sum}_l(a_{il}a_{jl})}{1 - a_{ij} + \min(k_i, k_j)}$

Using the topological overlap matrix (TOM) to cluster genes

– modules correspond to branches of the dendrogram

Genes correspond to rows and columns

TOM plot



Hierarchical clustering dendrogram

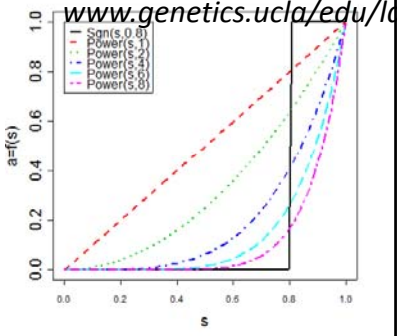
TOM matrix

Module: Correspond to branches

module traits highly correlated

- adjacency attenuates correlation
- can separate positive, nega
- summarize module
  - eigengene
  - weighted average of traits
- relate module
  - to clinical traits
  - map eigengene

www.genetics.ucla/edu/lc

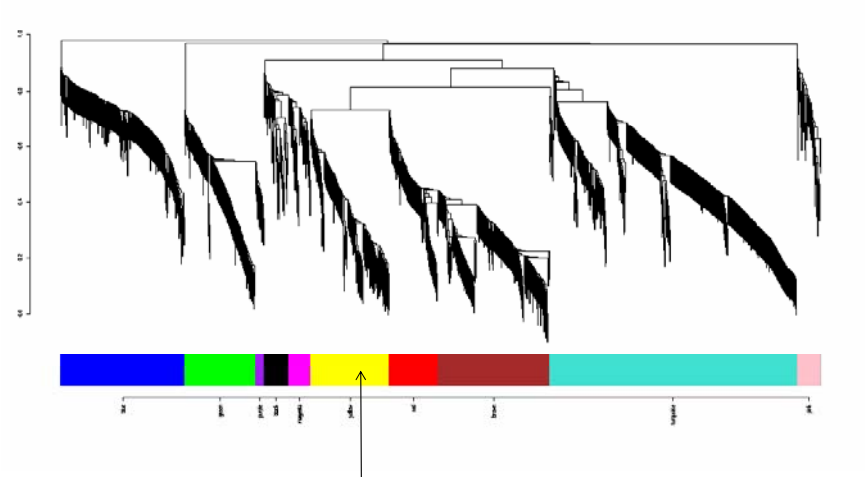


advantages of Horvath modules

- **emphasize modules (pathways) instead of individual genes**
  - Greatly alleviates the problem of multiple comparisons
  - ~20 module comparisons versus 1000s of gene comparisons
- intramodular connectivity k_i finds key drivers (hub genes)
  - quantifies module membership (centrality)
  - highly connected genes have an increased chance of validation
- module definition is based on gene expression data
  - no prior pathway information is used for module definition
  - two modules (eigengenes) can be highly correlated
- unified approach for relating variables
  - compare data sets on same mathematical footing
- scale-free: zoom in and see similar structure

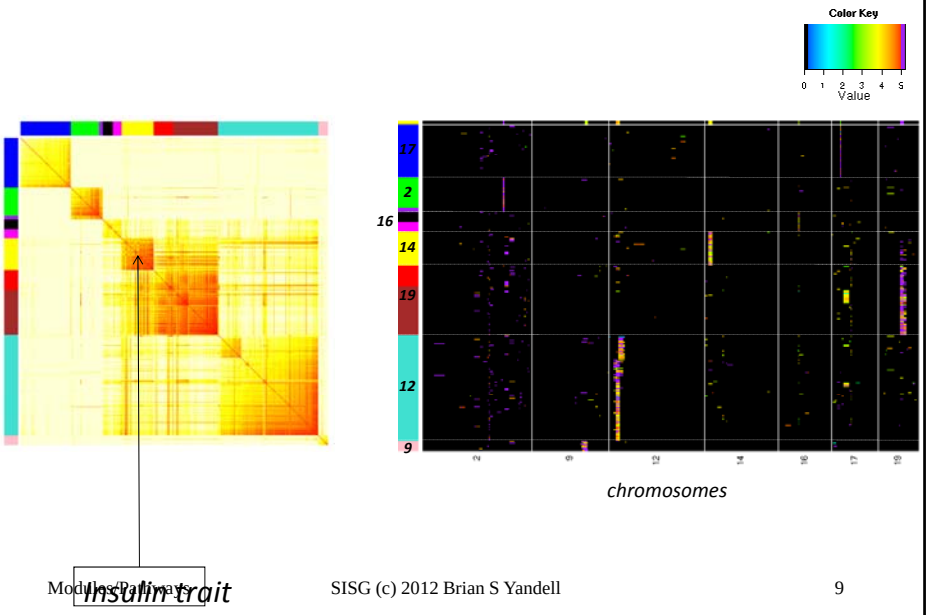
Ping Wang

modules for 1984 transcripts with similar genetic architecture as insulin



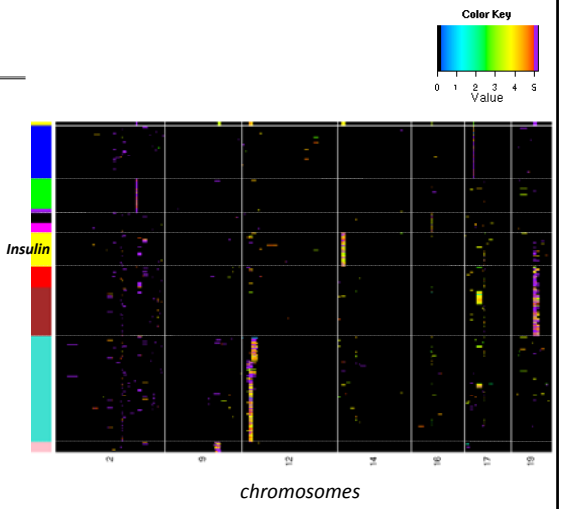
contains the insulin trait

Islet – modules



Islet – enrichment for modules

Module	Pvalue	Qvalue	Count	Size	Term
BLUE	0.0005	0.0463	30	1068	biosynthetic process
	0.0006	0.0470	18	511	cellular lipid metabolic process
	0.0009	0.0507	11	241	lipid biosynthetic process
	0.0012	0.0593	19	590	lipid metabolic process
GREEN	0.0008	0.0457	4	76	phosphate transport
	0.0055	0.0970	2	20	intermediate filament-based process
	0.0056	0.0970	10	707	ion transport
PURPLE	0.0011	0.0165	7	2769	nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
BLACK	0.0078	0.0138	2	68	sensory perception of sound
MAGENTA	**2.54E-05**	**0.0011**	**7**	**313**	**cell cycle process**
	0.0001	**0.0040**	**5**	**179**	**microtubule-based process**
	0.0004	**0.0040**	**5**	**225**	**mitotic cell cycle**
	0.0005	**0.0040**	**5**	**228**	**M phase**
	0.0006	**0.0040**	**5**	**239**	**cell division**
	0.0009	**0.0041**	**5**	**266**	**cell cycle phase**
	0.0011	**0.0041**	**4**	**162**	**mitosis**
	0.0012	**0.0041**	**4**	**163**	**M phase of mitotic cell cycle**
YELLOW	0.0026	0.0675	7	281	cell projection organization and biogenesis
	0.0026	0.0675	7	281	cell part morphogenesis
	0.0026	0.0675	7	281	cell projection morphogenesis
RED	0.0017	0.0619	2	13	steroid hormone receptor signaling pathway
	0.0026	0.0619	5	200	reproductive process
BROWN	0.0057	0.1442	4	96	response to pheromone
TURQUOISE	0.0002	0.0830	17	279	enzyme linked receptor protein signaling pathway
	0.0003	0.0830	10	115	morphogenesis of an epithelium
	0.0003	0.0830	7	57	morphogenesis of embryonic epithelium
	0.0004	0.0830	40	1021	anatomical structure morphogenesis
PINK	0.0004	0.0608	2	14	vesicle organization and biogenesis
	0.0092	0.0612	4	384	regulation of apoptosis

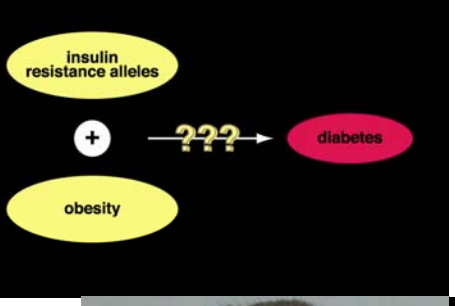


www.geneontology.org

- ontologies
  - Cellular component (GOCC)
  - Biological process (GOBP)
  - Molecular function (GOMF)
- hierarchy of classification
  - general to specific
  - based on extensive literature search, predictions
- prone to errors, historical inaccuracies

Bayesian causal phenotype network incorporating genetic variation and biological knowledge

Brian S Yandell, Jee Young Moon
University of Wisconsin-Madison
Elias Chaibub Neto, Sage Bionetworks
Xinwei Deng, VA Tech



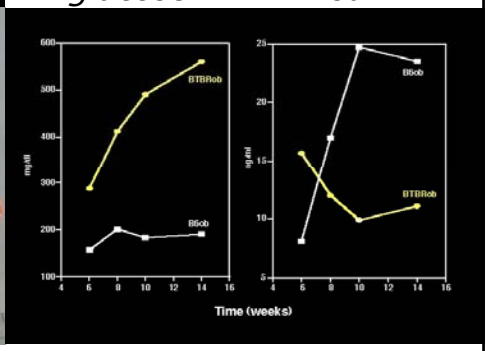
BTBR mouse is insulin resistant

B6 is not

make both obese…

Alan Attie
Biochemistr

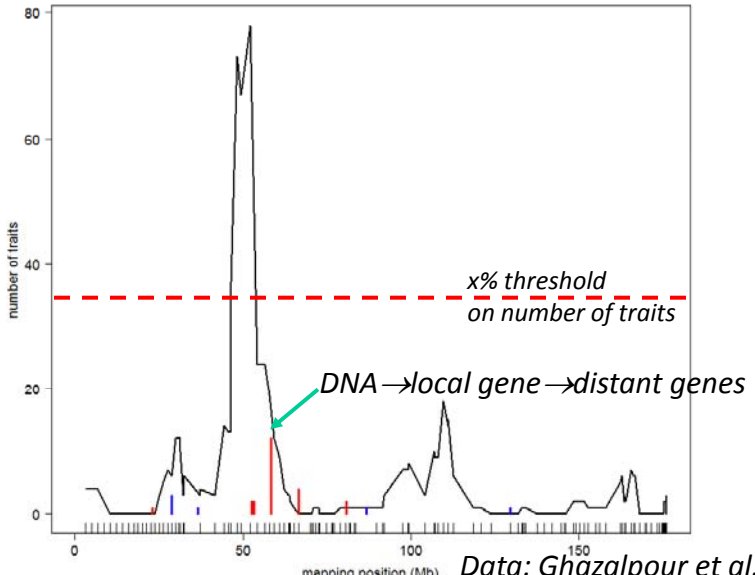
glucose *insulin*



bigger picture

- how do DNA, RNA, proteins, metabolites regulate each other?
- regulatory networks from microarray expression data
  - time series measurements or transcriptional perturbations
  - segregating population: **genotype as driving perturbations**
- goal: discover causal regulatory relationships among phenotypes
- use knowledge of regulatory relationships from databases

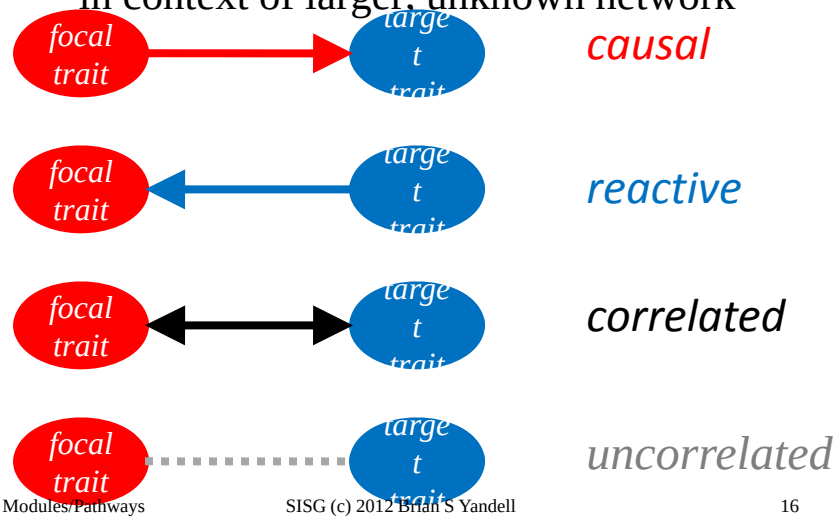
BxH ApoE-/- chr 2: hotspot



Data: Ghazalpour et al.(2006) PLo

causal model selection choices

in context of larger, unknown network



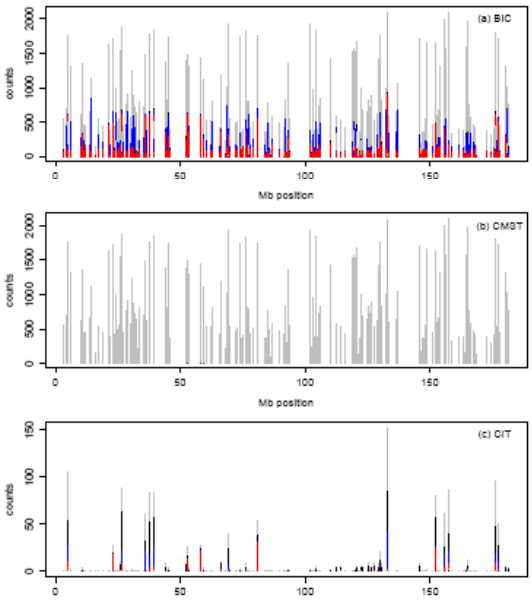
causal architecture references

- BIC: Schadt et al. (2005) *Nature Genet*
- CIT: Millstein et al. (2009) *BMC Genet*
- Aten et al. Horvath (2008) *BMC Sys Bio*
- CMST: Chaibub Neto et al. (2010) PhD thesis
  - Chaibub Neto et al. (2012) *Genetics* (in review)

Extends Vuong's model selection tests to the comparison of 3, possibly **misspecified**, models.

(M_1) (M_2) (M_3)

$Q_1 \rightarrow Y_1 \rightarrow Y_2 \leftarrow Q_{2|1}$ $Q_{1|2} \rightarrow Y_1 \leftarrow Y_2 \leftarrow Q_2$ $Q_1 \rightarrow Y_1 \quad Y_2 \leftarrow Q_2$

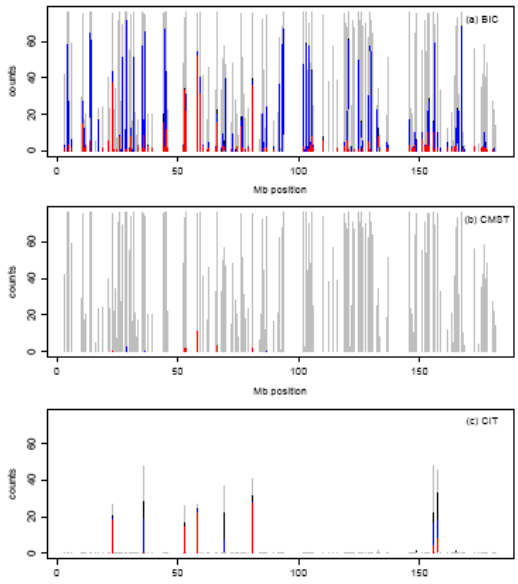


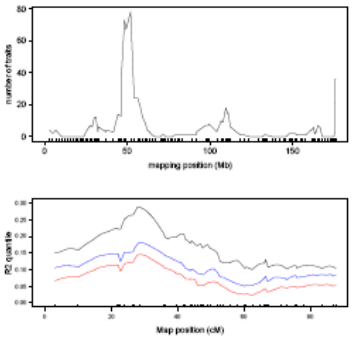
BxH ApoE-/- study
Ghazalpour et al. (2008)
PLoS Genetics

Liver expression data in a mice intercross.

3,421 transcripts and 1,065 markers.

261 transcripts physically located on chr 2.





Analysis restricted to 78 traits composing a hotspot around 54.2Mb.

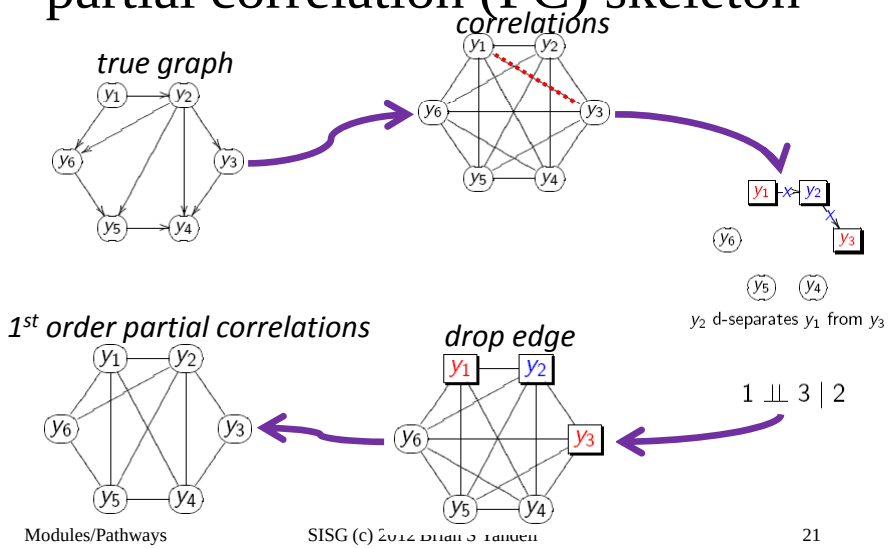
This collection of traits enriches for "immune system process".

Pscdbp, the local trait at 58.4Mb, is a transcription factor.

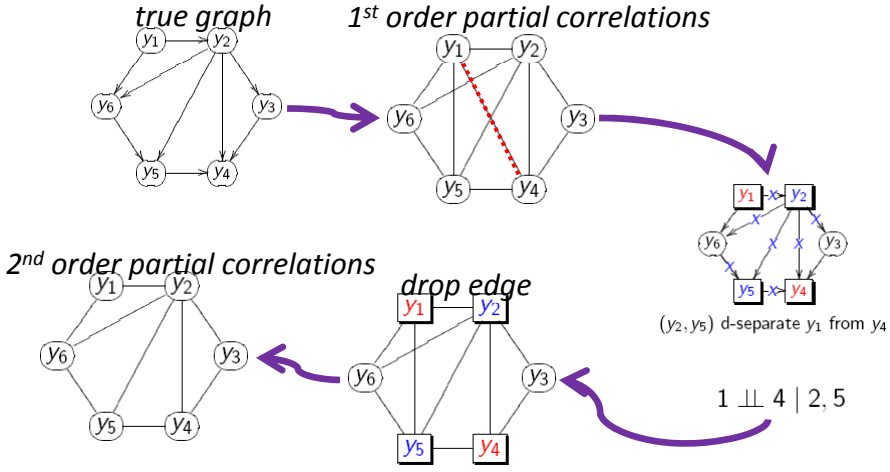
QTL-driven directed graphs

- given genetic architecture (QTLs), what causal network structure is supported by data?
- R/qdg available at www.github.org/byandell
- references
  - Chaibub Neto, Ferrara, Attie, Yandell (2008) Inferring causal phenotype networks from segregating populations. *Genetics 179*: 1089-1100. [doi:genetics.107.085167]
  - Ferrara et al. Attie (2008) Genetic networks of liver metabolism revealed by integration of metabolic and transcriptomic profiling. *PLoS Genet 4*: e1000034. [doi:10.1371/journal.pgen.1000034]

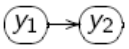
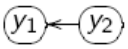
partial correlation (PC) skeleton



partial correlation (PC) skeleton

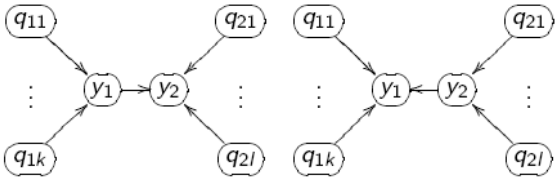


edge direction: which is causal?

M_1 :  $M2$: 

the above models are likelihood equivalent,

$$f(y_1)f(y_2 \mid y_1) = f(y_1, y_2) = f(y_2)f(y_1 \mid y_2)$$

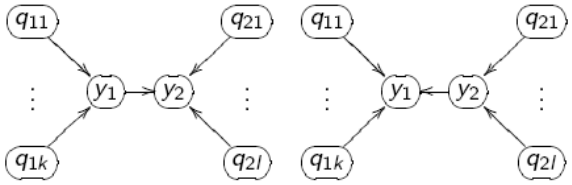


not likelihood equivalent *due to QTL*

$$f(\mathbf{q}_1)f(y_1 \mid \mathbf{q}_1)f(y_2 \mid y_1, \mathbf{q}_2)f(\mathbf{q}_2)$$
$$\neq$$
$$f(\mathbf{q}_2)f(y_2 \mid \mathbf{q}_2)f(y_1 \mid y_2, \mathbf{q}_1)f(\mathbf{q}_1)$$

test edge direction using LOD score

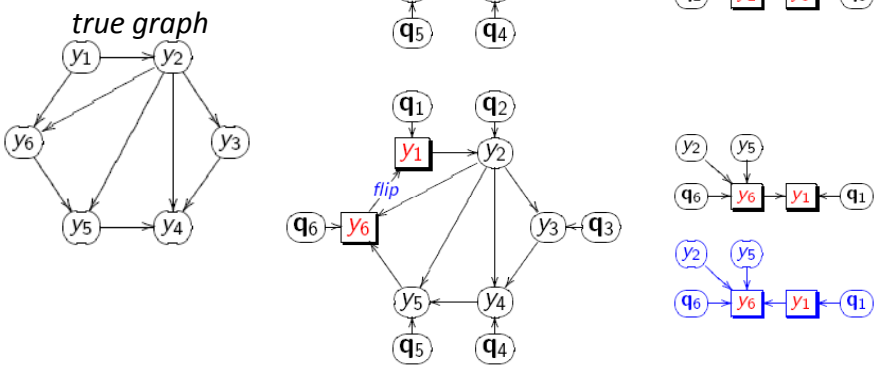
$$LOD = \log_{10}\left\{\frac{\prod_{i=1}^{n} f(y_{1i} \mid \mathbf{q}_{1i})f(y_{2i} \mid y_{1i}, \mathbf{q}_{2i})}{\prod_{i=1}^{n} f(y_{2i} \mid \mathbf{q}_{2i})f(y_{1i} \mid y_{2i}, \mathbf{q}_{1i})}\right\}$$



not likelihood equivalent because

$$f(\mathbf{q}_1)f(y_1 \mid \mathbf{q}_1)f(y_2 \mid y_1, \mathbf{q}_2)f(\mathbf{q}_2)$$
$$\neq$$
$$f(\mathbf{q}_2)f(y_2 \mid \mathbf{q}_2)f(y_1 \mid y_2, \mathbf{q}_1)f(\mathbf{q}_1)$$

reverse edges using QTLs



causal graphical models in systems genetics

- What if genetic architecture and causal network are unknown? jointly infer both using iteration
- Chaibub Neto, Keller, Attie, Yandell (2010) Causal Graphical Models in Systems Genetics: a unified framework for joint inference of causal network and genetic architecture for correlated phenotypes. *Ann Appl Statist 4*: 320-339. [doi:10.1214/09-AOAS288]
- R/qtlnet available from www.github.org/byandell
- Related references
  - Schadt et al. Lusis (2005 *Nat Genet*); Li et al. Churchill (2006 *Genetics*); Chen Emmert-Streib Storey(2007 *Genome Bio*); Liu de la Fuente Hoeschele (2008 *Genetics*); Winrow et al. Turek (2009 *PLoS ONE*); Hageman et al. Churchill (2011 *Genetics*)

Basic idea of QTLnet

- iterate between finding QTL and network
- genetic architecture given causal network
  - trait y depends on parents pa(y) in network
  - QTL for y found conditional on pa(y)
    - Parents pa(y) are interacting covariates for QTL scan
- causal network given genetic architecture
  - build (adjust) causal network given QTL
  - each direction change may alter neighbor edges

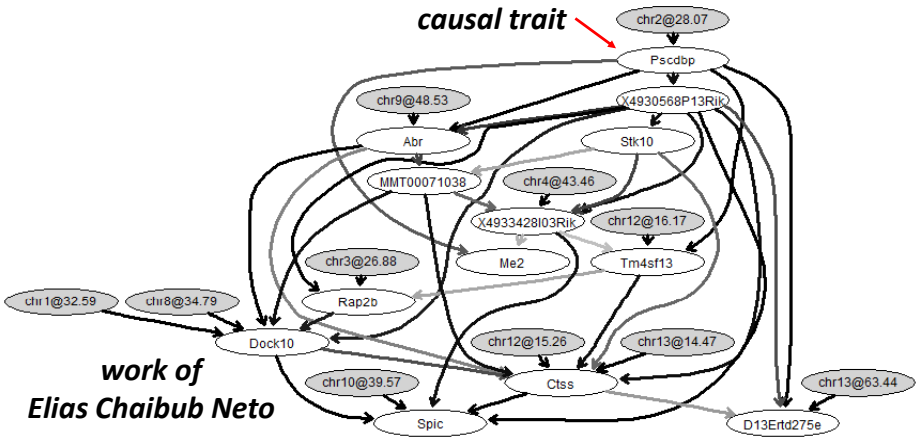
missing data method: MCMC

- known phenotypes Y, genotypes Q
- unknown graph G
- want to study $\Pr(Y \mid G, Q)$
- break down in terms of individual edges
  - $\Pr(Y|G,Q)$ = sum of $\Pr(Y_i \mid \text{pa}(Y_i), Q)$
- sample new values for individual edges
  - given current value of all other edges
- repeat many times and average results

MCMC steps for QTLnet

- propose new causal network G
  - with simple changes to current network:
  - change edge direction
  - add or drop edge
- find any new genetic architectures Q
  - update phenotypes when parents pa(y) change in new G
- compute likelihood for new network and QTL
  - $\Pr(Y \mid G, Q)$
- accept or reject new network and QTL
  - usual Metropolis-Hastings idea

BxH ApoE-/- causal network for transcription factor Pscdbp

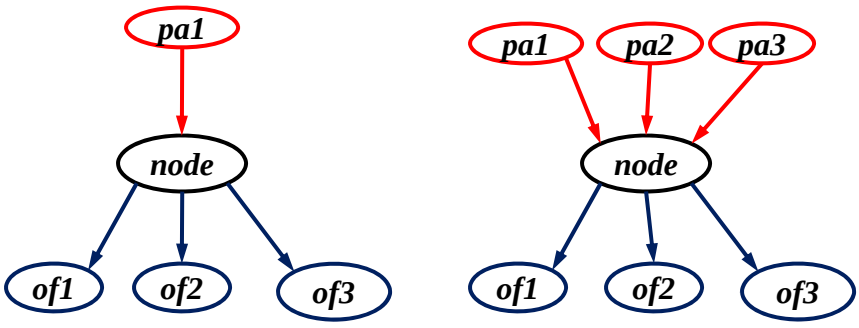


Data: Ghazalpour et al.(2006) PLoS Gen

scaling up to larger networks

- reduce complexity of graphs
  - use prior knowledge to constrain valid edges
  - restrict number of causal edges into each node
- make task parallel: run on many machines
  - pre-compute conditional probabilities
  - run multiple parallel Markov chains
- rethink approach
  - LASSO, sparse PLS, other optimization methods

graph complexity with node parents



parallel phases for larger projects

Phase 1: identify parents

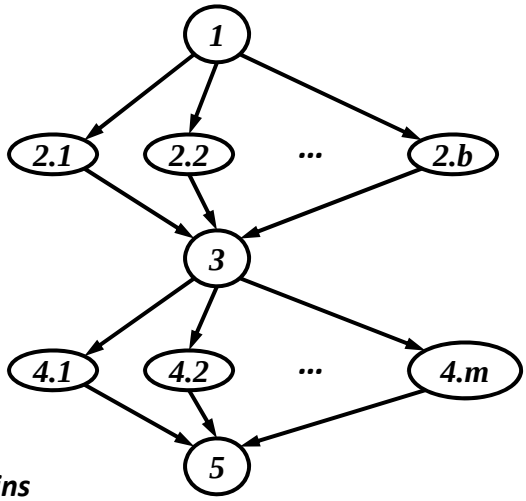
Phase 2: compute BICs

BIC = LOD – penalty

all possible parents to all nodes

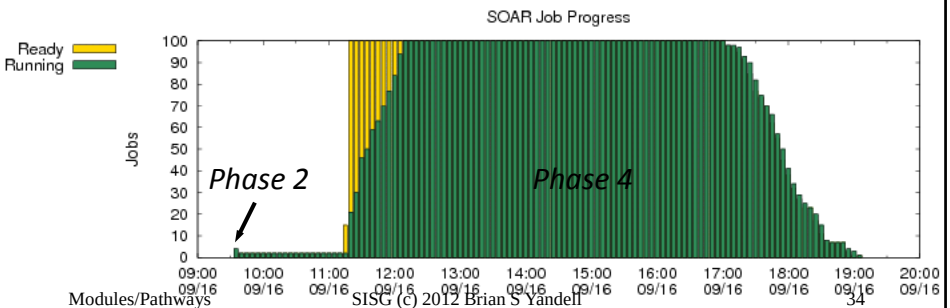
Phase 3: store BICs

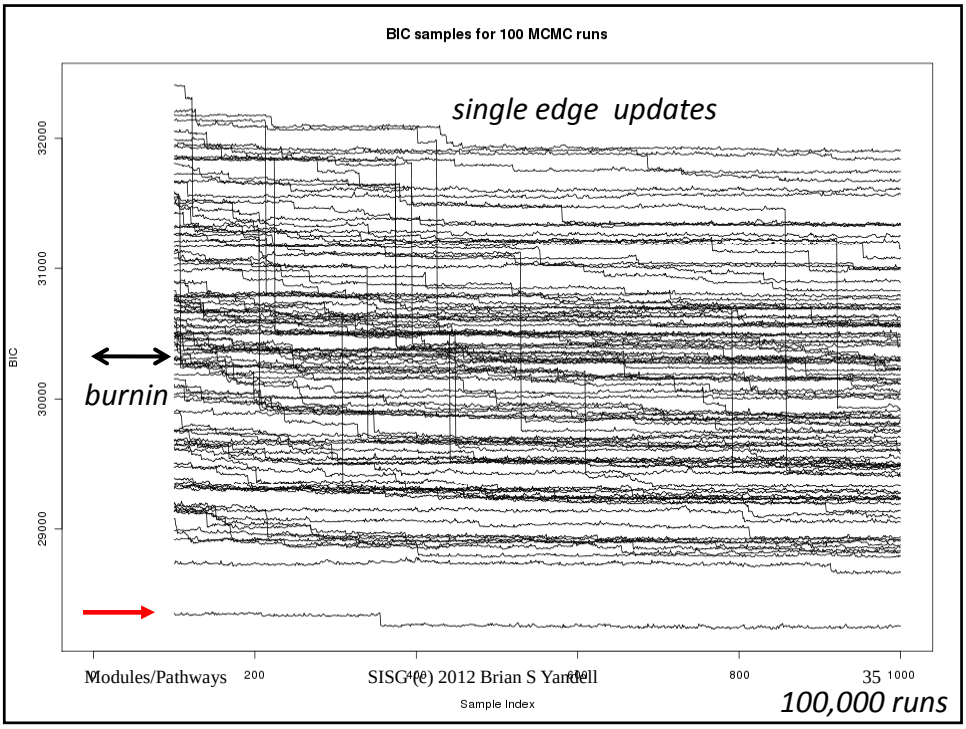
Phase 4: run Markov chains



parallel implementation

- R/qtlnet available at www.github.org/byandell
- Condor cluster: chtc.cs.wisc.edu
  - System Of Automated Runs (SOAR)
    - ~2000 cores in pool shared by many scientists
    - automated run of new jobs placed in project

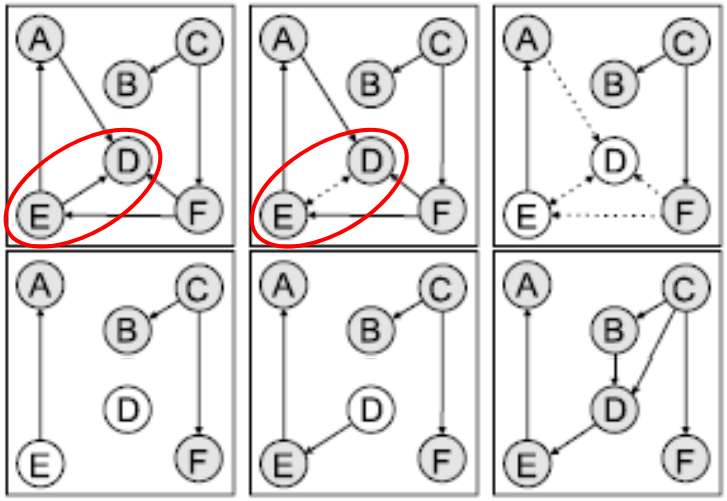




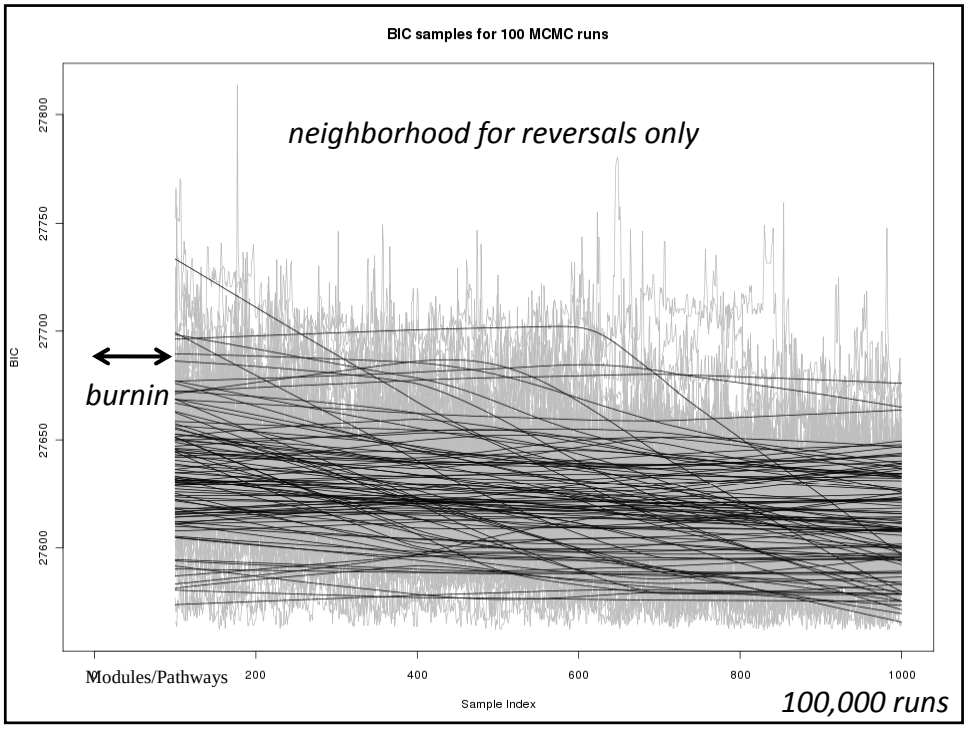
neighborhood edge reversal

select edge
drop edge
identify parents

orphan nodes
reverse edge
find new parents



Grzegorczyk M. and Husmeier D. (2008) Machine Learning 71 (2-3),



how to use functional information?

- functional grouping from prior studies
  - may or may not indicate direction
  - gene ontology (GO), KEGG
  - knockout (KO) panels
  - protein-protein interaction (PPI) database
  - transcription factor (TF) database
- methods using only this information
- priors for QTL-driven causal networks
  - more weight to local (*cis*) QTLs?

modeling biological knowledge

- infer graph G_Y from biological knowledge B
  - $\Pr(G_Y \mid B, W) = \exp(- W * |B - G_Y|) /$ constant
  - B = prob of edge given TF, PPI, KO database
    - derived using previous experiments, papers, etc.
  - G_Y = 0-1 matrix for graph with directed edges
- W = inferred weight of biological knowledge
  - W=0: no influence; W large: assumed correct
  - $P(W|B) = \phi \exp(- \phi W)$ exponential
- Werhli and Husmeier (2007) *J Bioinfo Comput Biol*

combining eQTL and bio knowledge

- probability for graph G and bio-weights W
  - given phenotypes Y, genotypes Q, bio info B
- $\Pr(G, W \mid Y, Q, B) = c \Pr(Y|G,Q)\Pr(G|B,W,Q)\Pr(W|B)$
  - $\Pr(Y|G,Q)$ is genetic architecture (QTLs)
    - using parent nodes of each trait as covariates
  - $\Pr(G|B,W,Q) = \Pr(G_Y|B,W) \Pr(G_{Q \to Y}|Q)$
    - $\Pr(G_Y|B,W)$ relates graph to biological info
    - $\Pr(G_{Q \to Y}|Q)$ relates genotype to phenotype

Moon JY, Chaibub Neto E, Deng X, Yandell BS (2011) Growing graphical models to infer causal phenotype networks. In *Probabilistic Graphical Models Dedicated to Applications in Genetics.* Sinoquet C, Mourad R, eds. (in review)

encoding biological knowledge *B*

transcription factors, DNA binding (causation)

$$B_{ij} = \frac{\lambda e^{-\lambda p}}{\lambda e^{-\lambda p} + (1 - e^{-\lambda})}$$

- p = p-value for TF binding of $i \rightarrow j$
- truncated exponential (λ) when TF $i \rightarrow j$
- uniform if no detection relationship
- Bernard, Hartemink (2005) *Pac Symp Biocomp*

encoding biological knowledge *B*

protein-protein interaction (association)

$$B_{ij} = B_{ji} = \frac{\text{posterior odds}}{1 + \text{posterior odds}}$$

- post odds = prior odds * LR
- use positive and negative gold standards
- Jansen et al. (2003) *Science*

encoding biological knowledge *B*

gene ontology(association)

$$B_{ij} = B_{ji} = c \bullet mean(sim(GO_i, GO_j))$$

- GO = molecular function, processes of gene
- sim = maximum information content across common parents of pair of genes
- Lord et al. (2003) *Bioinformatics*

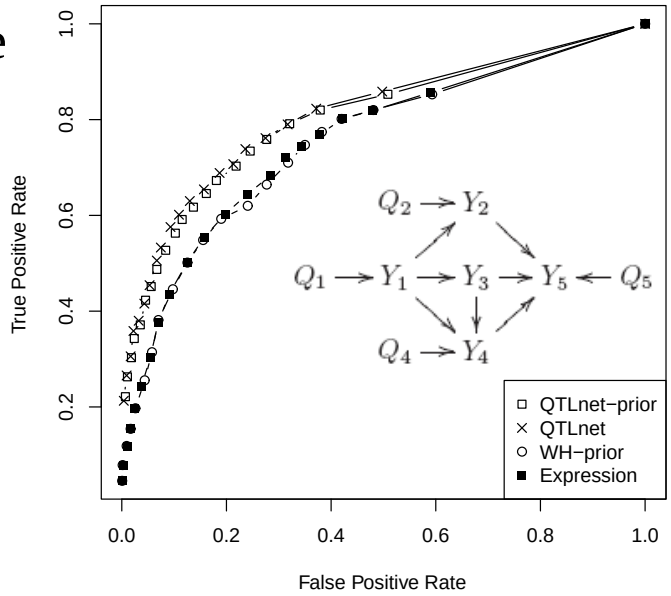
MCMC with pathway information

- sample new network G from proposal $R(G^*|G)$
  - add or drop edges; switch causal direction
- sample QTLs Q from proposal $R(Q^*|Q,Y)$
  - e.g. Bayesian QTL mapping given pa(Y)
- accept new network (G*,Q*) with probability
- A = min(1, $f(G,Q|G^*,Q^*)/ f(G^*,Q^*|G,Q)$)
  - $f(G,Q|G^*,Q^*)$ = Pr(Y|G*,Q*)Pr(G*|B,W,Q*)/$R(G^*|G)R(Q^*|Q,Y)$
- sample W from proposal R(W*|W)
- accept new weight W* with probability …

ROC curve simulation

open = QTLnet

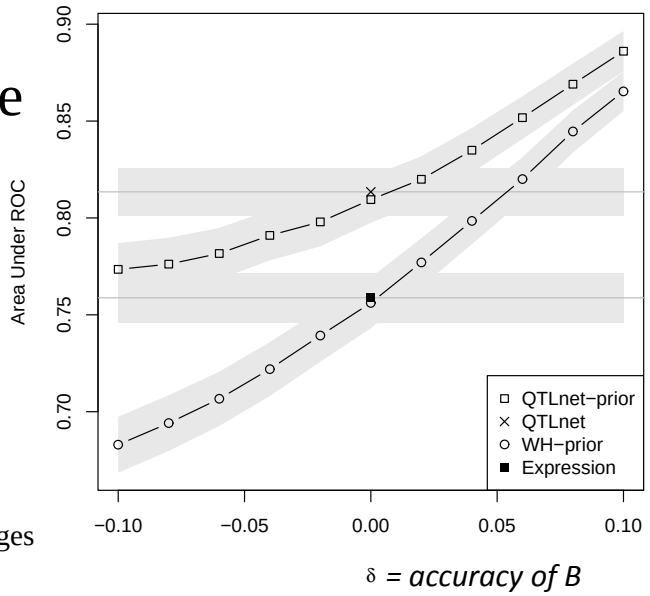
closed = phenotypes only



integrated ROC curve

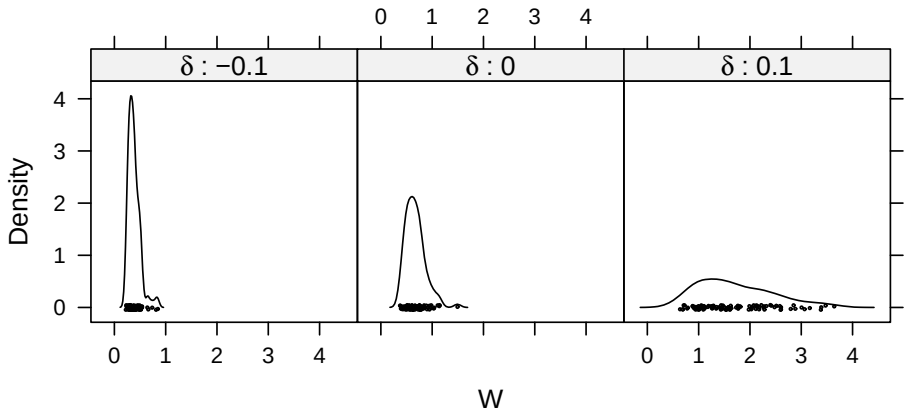
2x2: genetics pathways

probability classifier ranks true > false edges



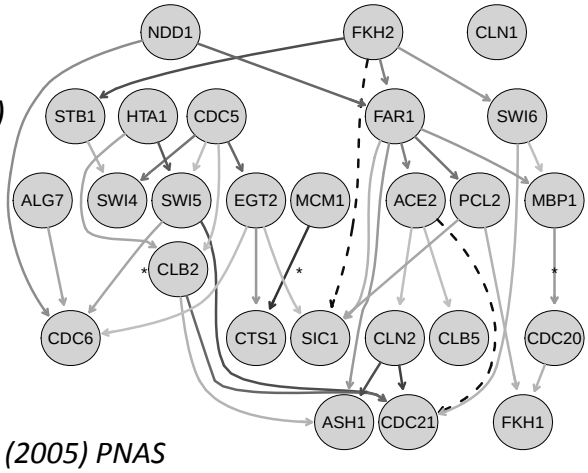
weight on biological knowledge

incorrect *non-informative* *correct*



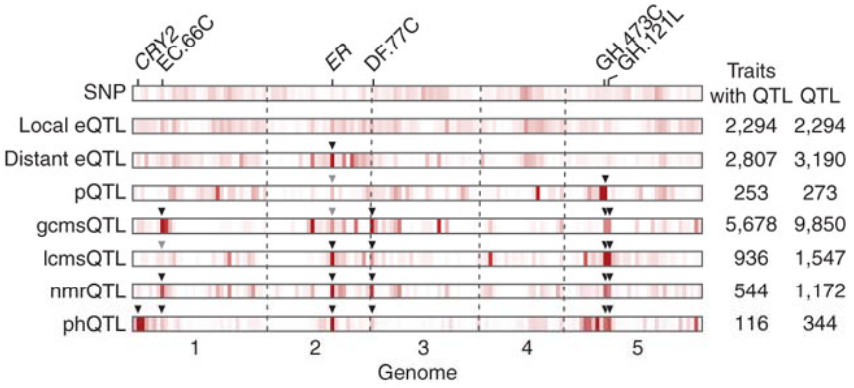
yeast data—partial success

26 genes
36 inferred edges
dashed: indirect (2)
starred: direct (3)
missed (39)
reversed (0)



Data: Brem, Kruglyak (2005) PNAS

phenotypic buffering of molecular QTL

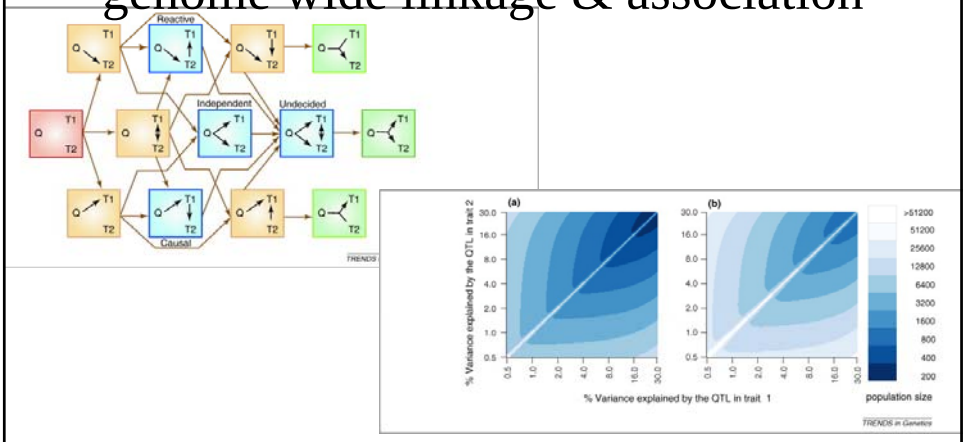


Fu et al. Jansen (2009 Nature Genetics)

limits of causal inference

- Computing costs already discussed
- Noisy data leads to false positive causal calls
  - Unfaithfulness assumption violated
  - Depends on sample size and omic technology
  - And on graph complexity (d = maximal path length i→j)
  - Profound limits
- Uhler C, Raskutti G, Buhlmann P, Yu B (2012 in prep) Geometry of faithfulness assumption in causal inference.
- Yang Li, Bruno M. Tesson, Gary A. Churchill, Ritsert C. Jansen (2010) Critical reasoning on causal inference in genome-wide linkage and association studies. *Trends in Genetics 26*: 493-498.

sizes for reliable causal inference genome wide linkage & association



Li, Tesson, Churchill, Jansen (2010) Trends in Genetics

limits of causal inference

unfaithful: false positive edges

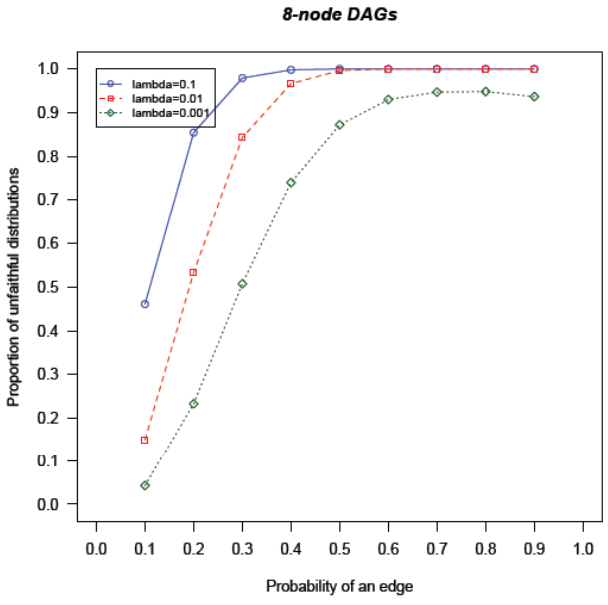
$\lambda = \min|\text{cor}(Y_i, Y_j)|$

$\lambda = c \bullet \text{sqrt}(dp/n)$

d=max degree

p=# nodes

n=sample size



Uhler, Raskutti, Buhlmann, Yu (2012 in p

Thanks!

- Grant support
  - NIH/NIDDK 58037, 66369
  - NIH/NIGMS 74244, 69430
  - NCI/ICBP U54-CA149237
  - NIH/R01MH090948
- Collaborators on papers and ideas
  - Alan Attie & Mark Keller, Biochemistry
  - Karl Broman, Aimee Broman, Christina Kendziorski