Transcriptomes as phenotypes

Bringing Genetics to Genomics

David Angeles-Albores, Ph.D.
Alm Laboratory
MIT

Online Slides Available at dangeles.github.io

The geneticist's arsenal

Null mutants (**Epistasis**)

Allelic series (**dominance**)

Crosses (**maternal effects**)

Genetics orders genes along pathways

Genes

a

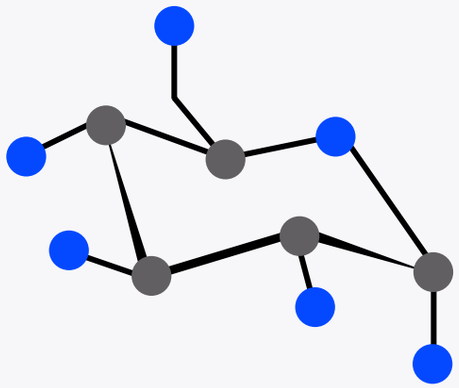
b

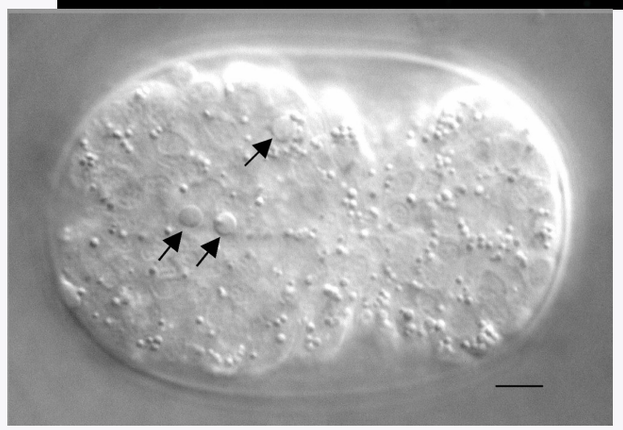
c

d

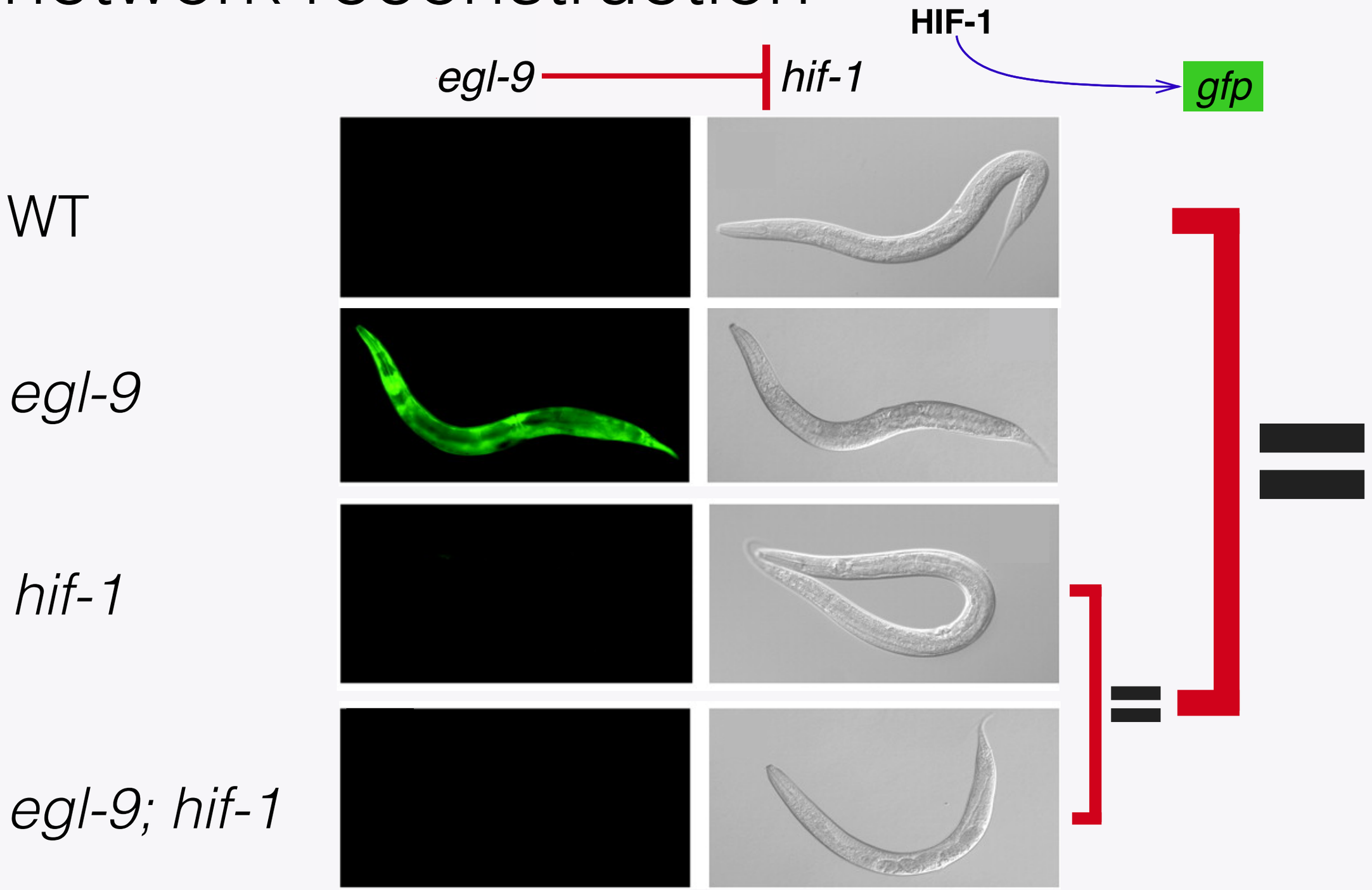
e

Phenotypes





Batesonian epistasis is a powerful method for network reconstruction



Shen *et al*, 2006

Epistasis analysis in a nutshell:

(A) Choose phenotype (based on expertise)
(B) Phenotype single, double NULL mutants
(C) Check if double mutant = a single mutant

Yes?

Infer pathway

No?

Genetic interaction is ‘complex’, need more information

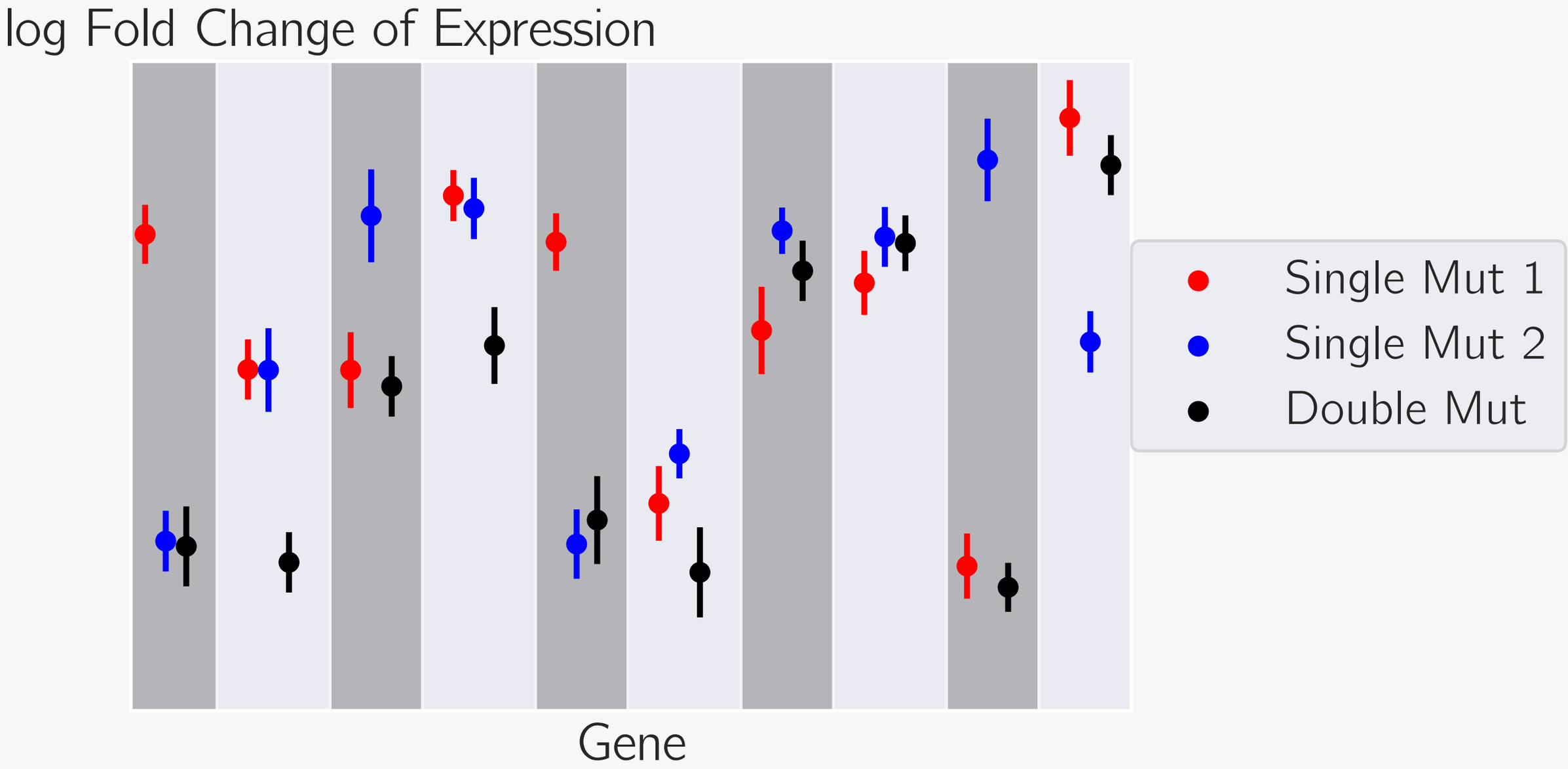
RNA-seq offers the possibility of a new kind of phenotypes

Genome-wide

Quantitative

Unbiased

Transcriptomes are powerful, but complicated



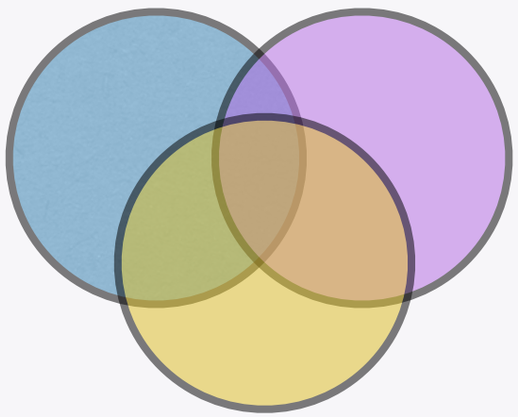
To use genetic methods in a genomic context, we need **specialized statistical machinery**

For details, see:

- **Epistasis**: Angeles-Albores *et al*, *PNAS*, 2018; Angeles-Albores *et al*, *G3*, 2017
- **Dominance**: Angeles-Albores, *Genetics*, 2018

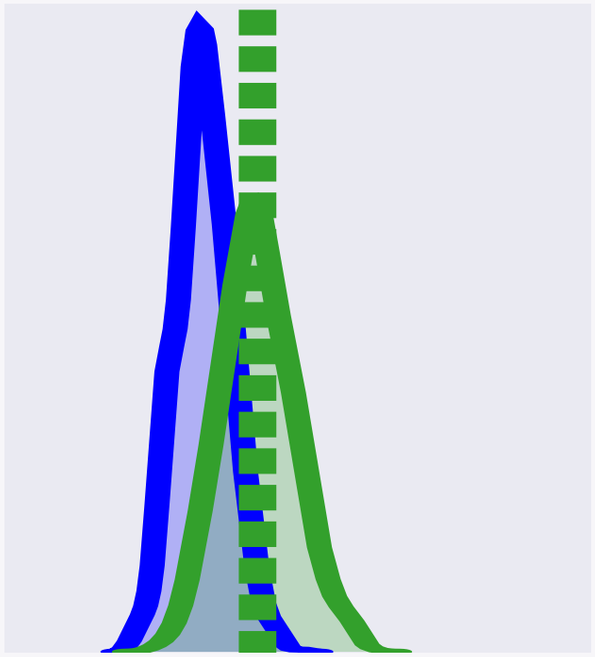
Transcriptome-wide epistasis analysis in a nutshell:

Choose phenotype

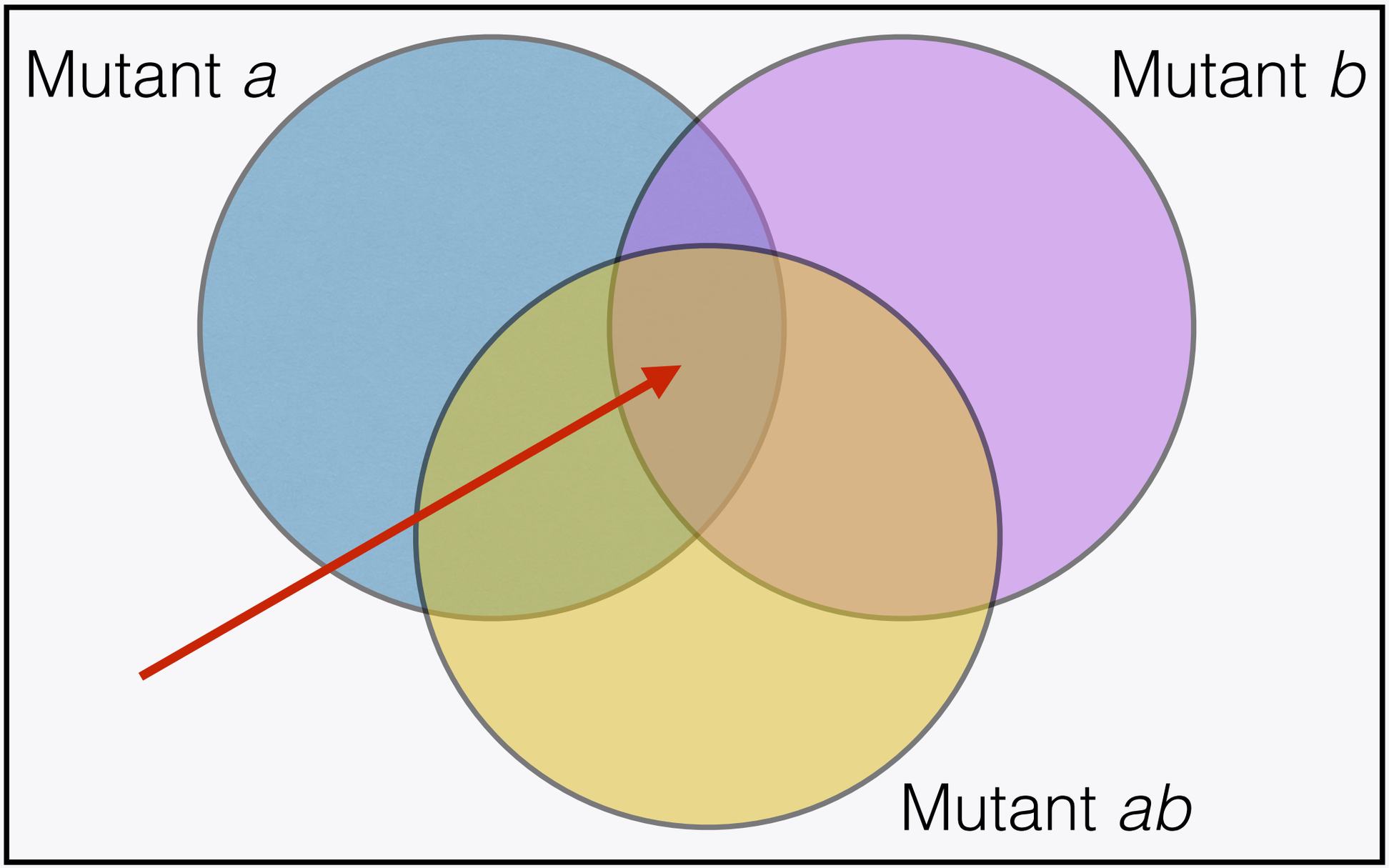


Compute a statistic for all genes in phenotype

Check if statistic is Batesonian



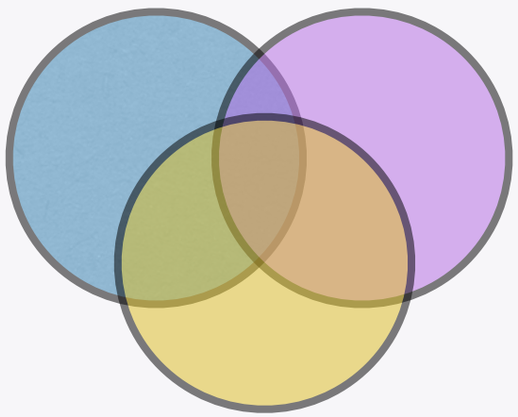
Transcriptome-wide epistasis: Defining a phenotype



Diff. Exp. Genes relative to WT

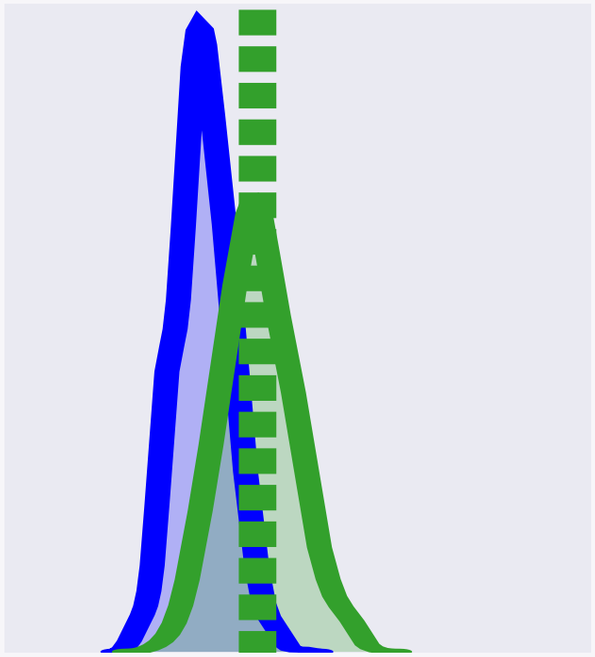
Transcriptome-wide epistasis analysis in a nutshell:

Choose phenotype



Compute a statistic for all genes in phenotype

Check if statistic is Batesonian

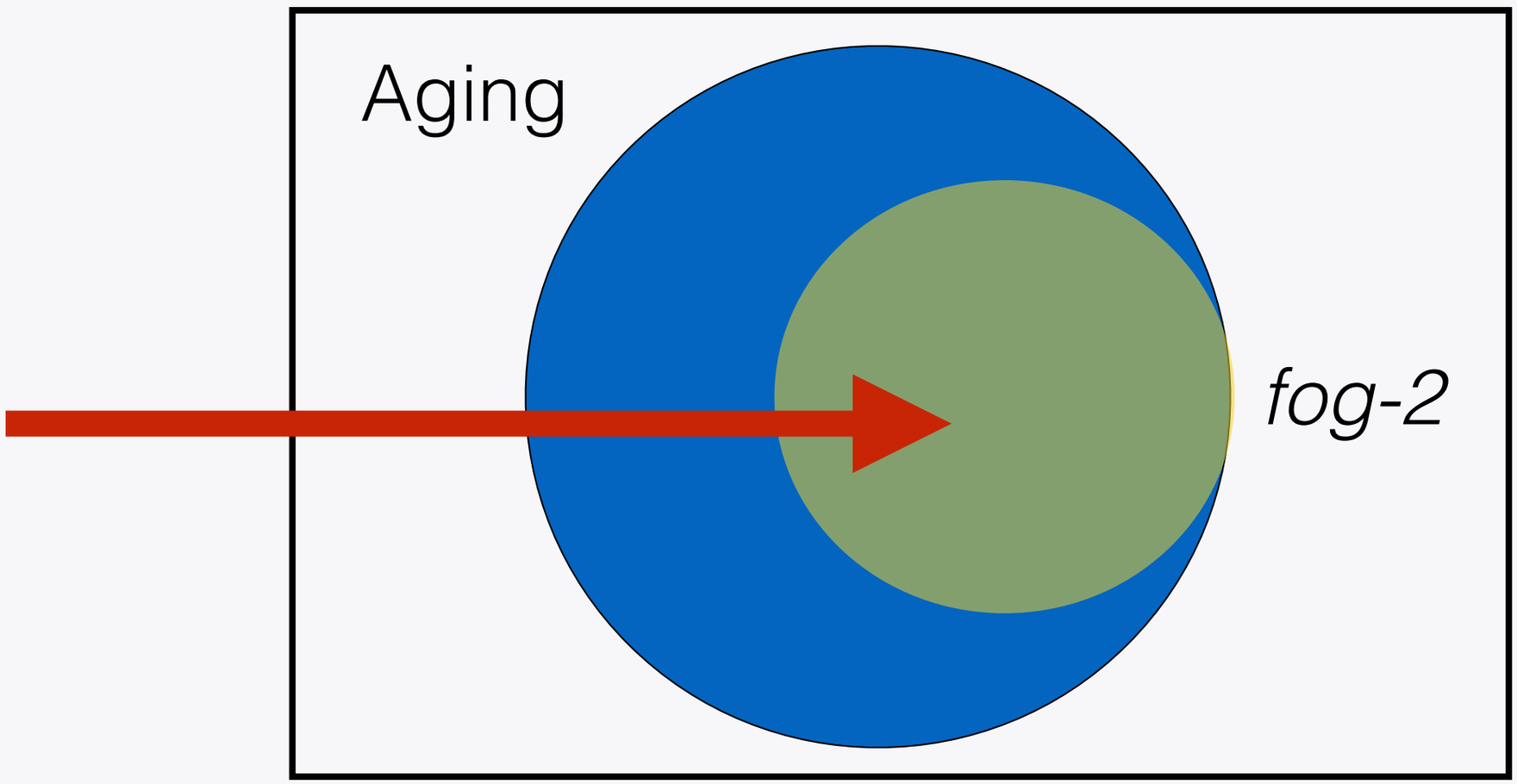


An example: Does sperm status have effects independent of aging?

	WT	***fog-2***
Young adult	Young, Sperm	Young, NO Sperm,
‘Middle-aged’ adult	Aged, NO Sperm,	Aged, NO Sperm,

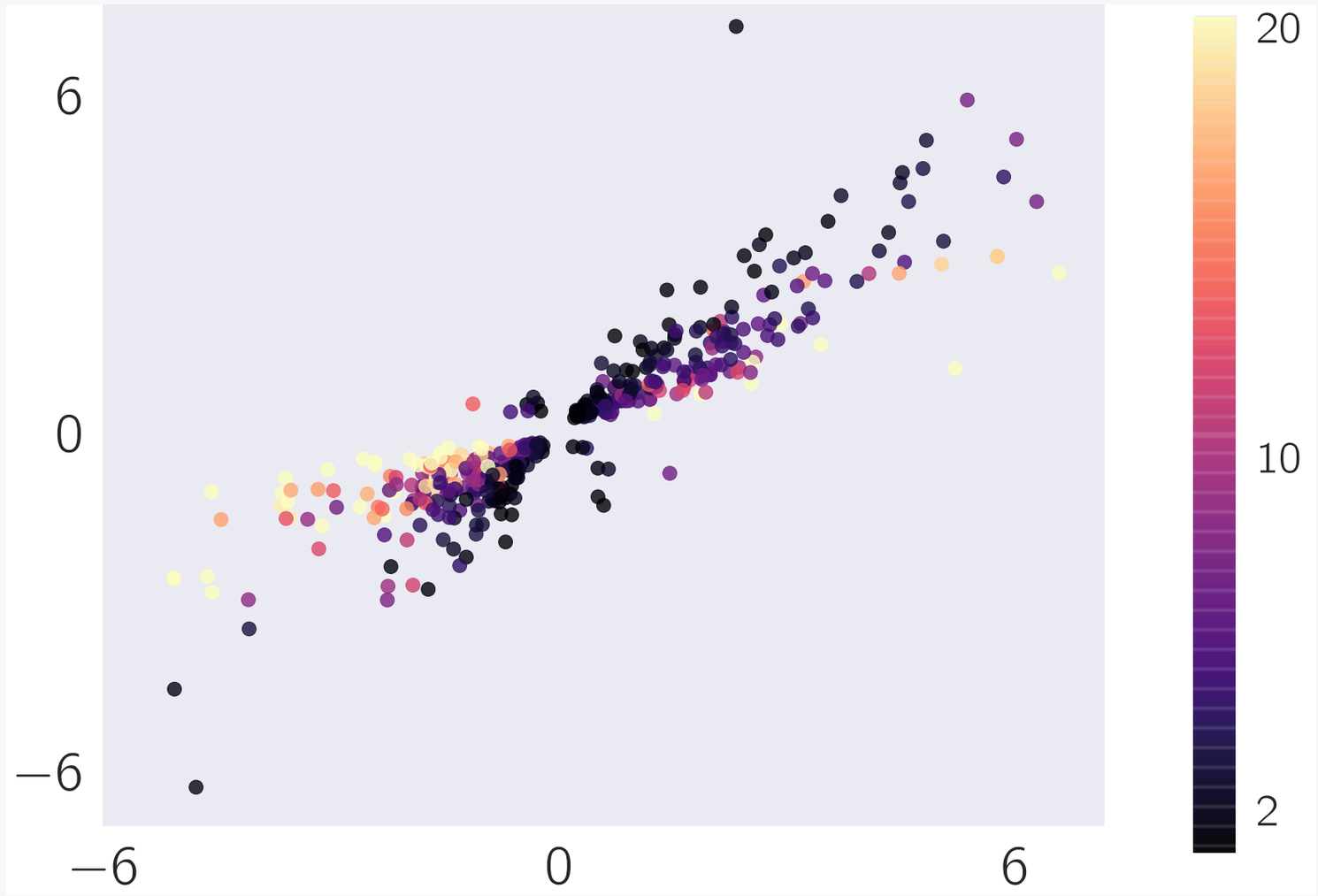
Angeles-Albores, Leighton and Sternberg, *G3,* 2017

Age affects more genes than *fog-2*, so we find the commonly affected subset



Diff. Exp. Genes relative to WT

First, show that both perturbations have equivalent effects



What does our black box do?

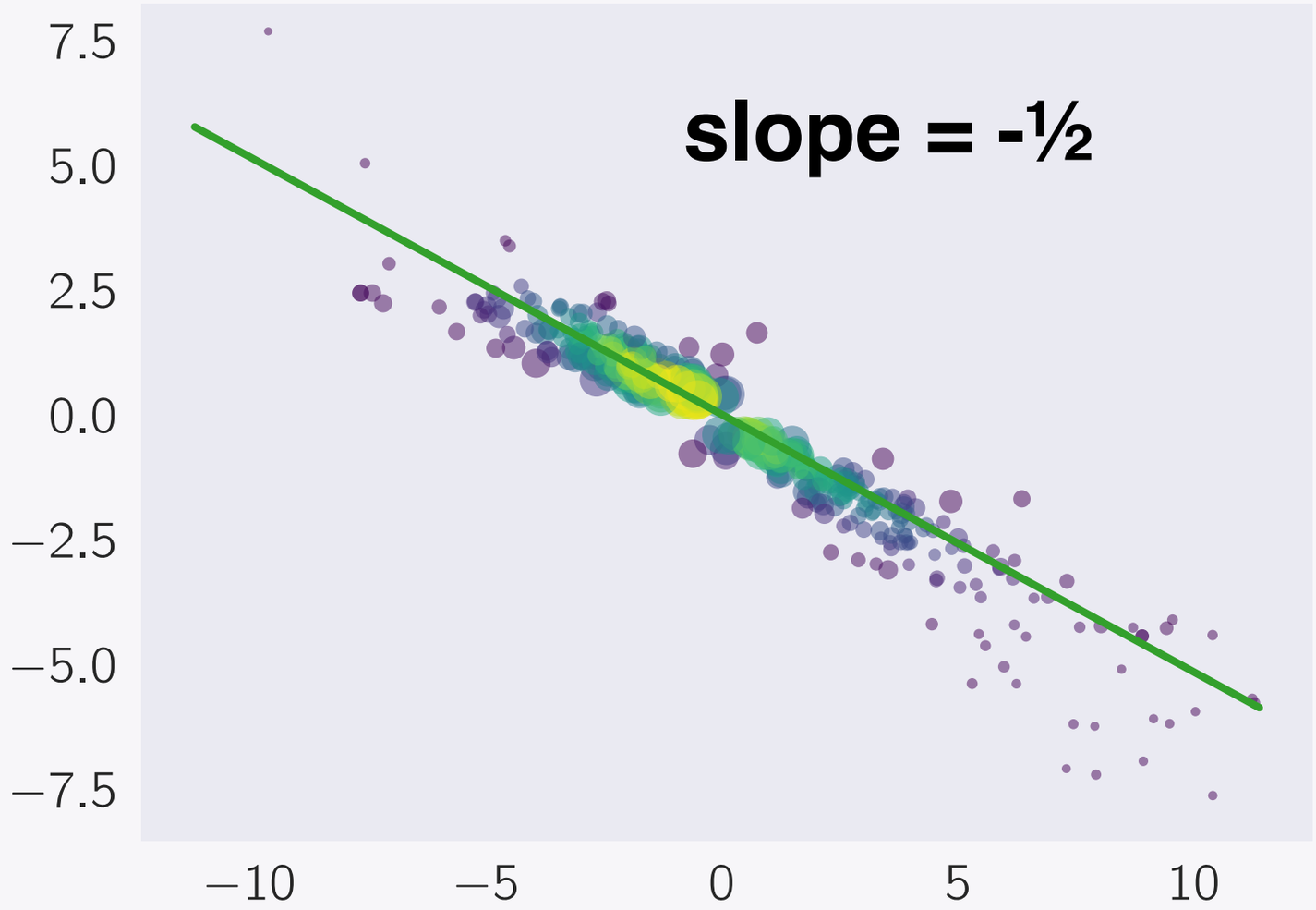
(1) Calculate **expected** double mutant value
(Add the single mutant log Fold Changes)

(2) Compute **observed - expected**

(3) Plot expected vs. (observed - expected)
for all transcripts and **determine line of best fit**

A slope of -½ indicates that sperm loss through aging is the same as never having sperm

Observed - Expected



Behind the math:

Observed

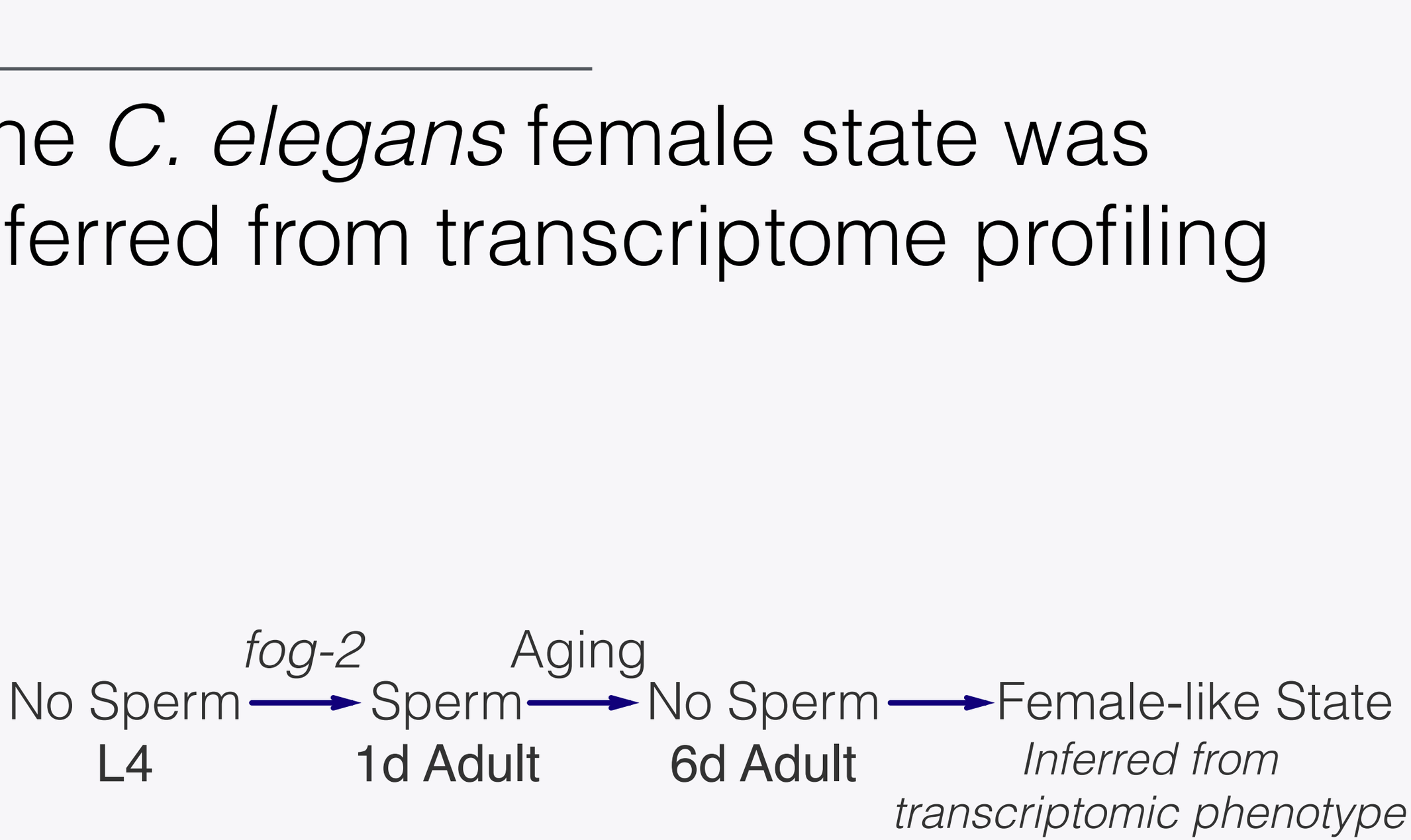
=

ln(FC) time

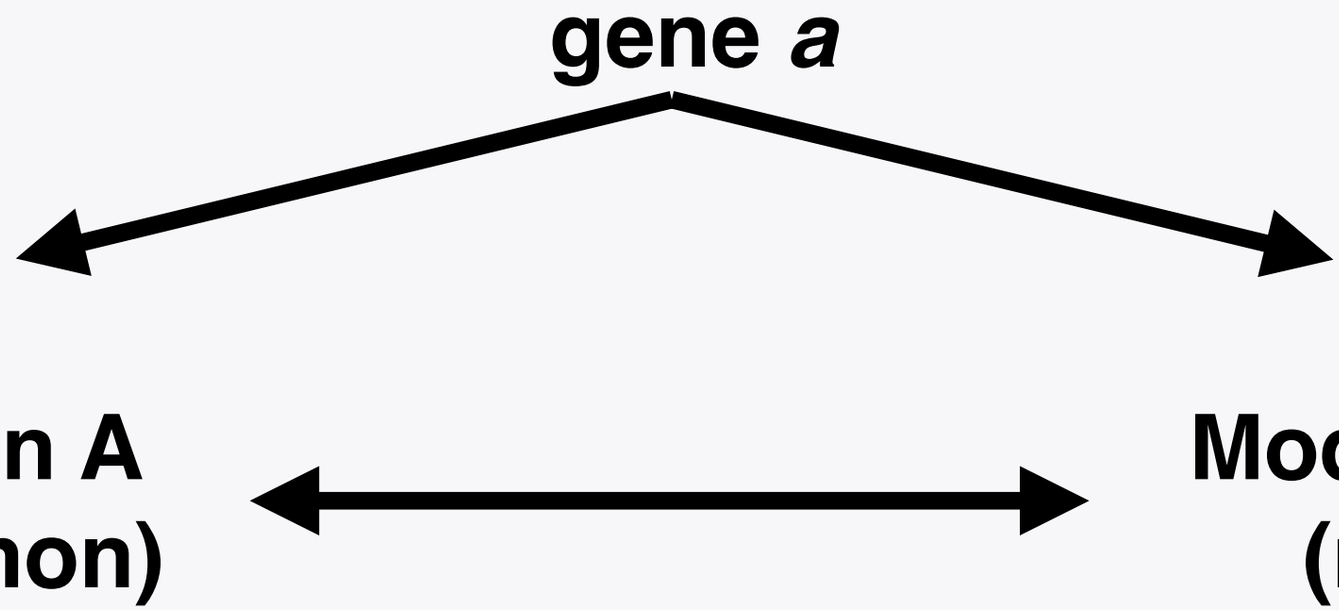
=

ln(FC) fog-2

The *C. elegans* female state was inferred from transcriptome profiling



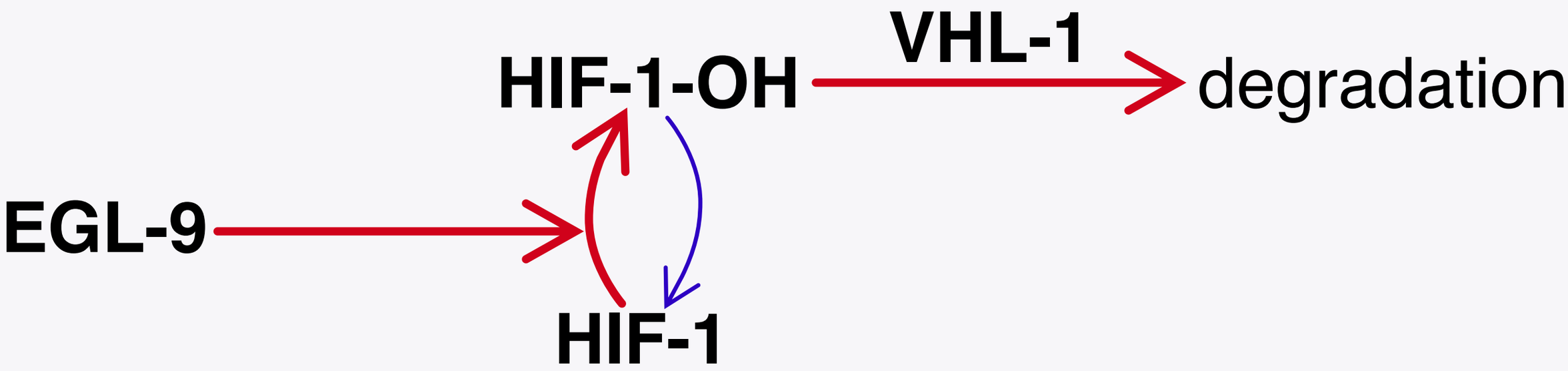
Transcriptomes can be used to think about biochemistry



Accounts for most effects of knocking out ***a***

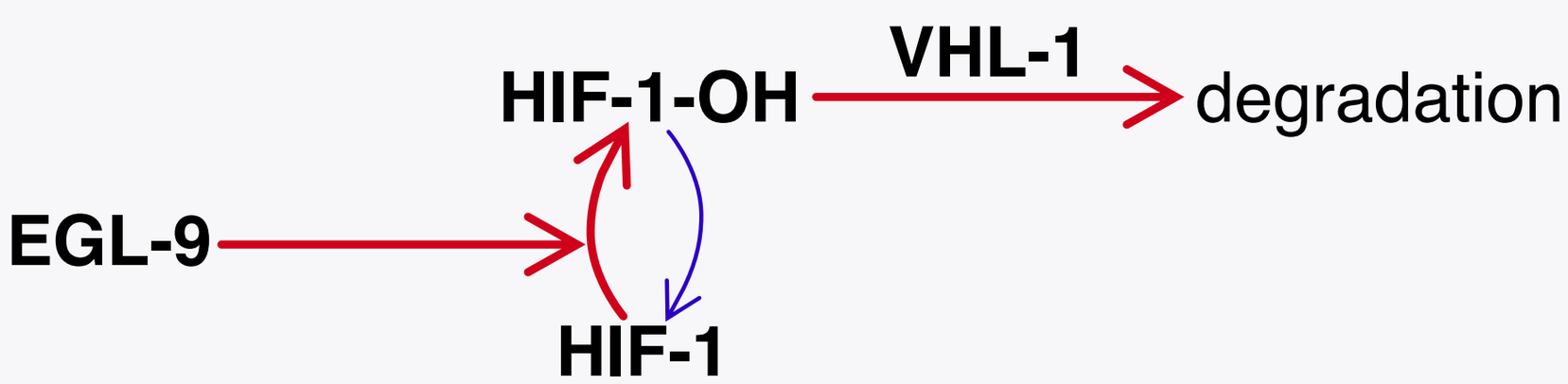
Accounts for a few effects of knocking out ***a***

Hypoxia factor 1 (*hif-1*) is degraded by VHL-1 in an EGL-9 dependent manner

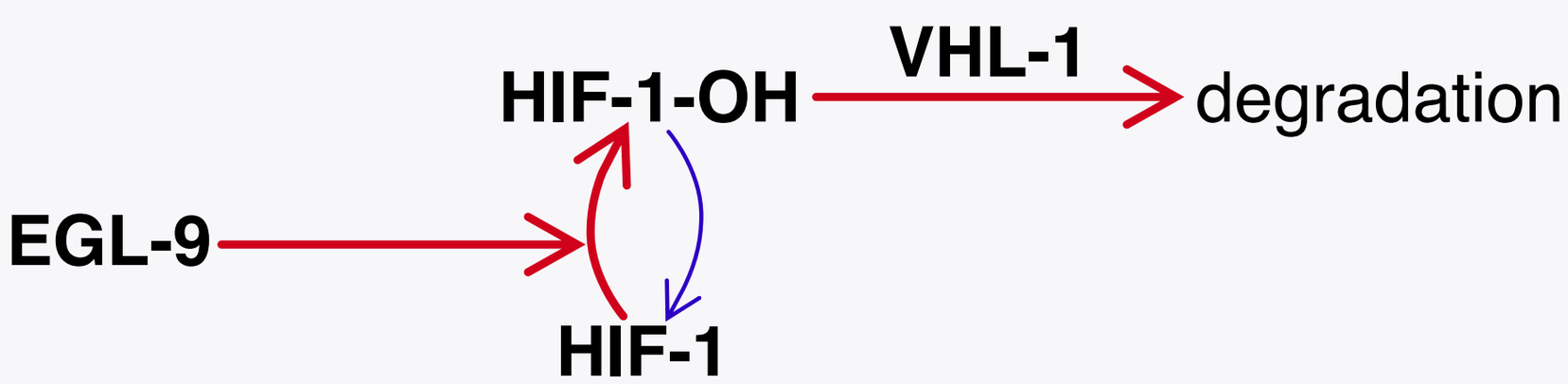


Angeles Albores, Puckett Robins, *et al.*, *PNAS*, 2018

Using HIF-1 abundance as phenotype leads to the canonical genetic diagram:



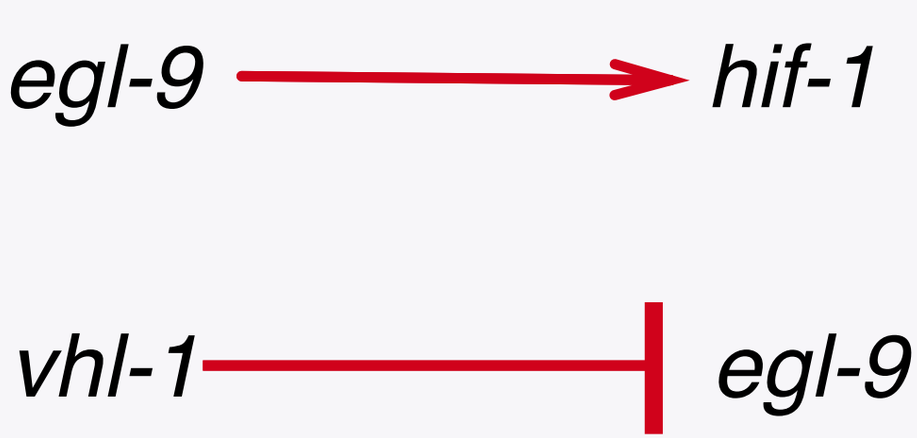
If we could measure HIF-1-OH abundance, we would write the genetic pathway as:



Choosing a phenotype affects the outcome of the genetic reconstruction:

HIF-1 abundance as phenotype

HIF-1-OH abundance as phenotype

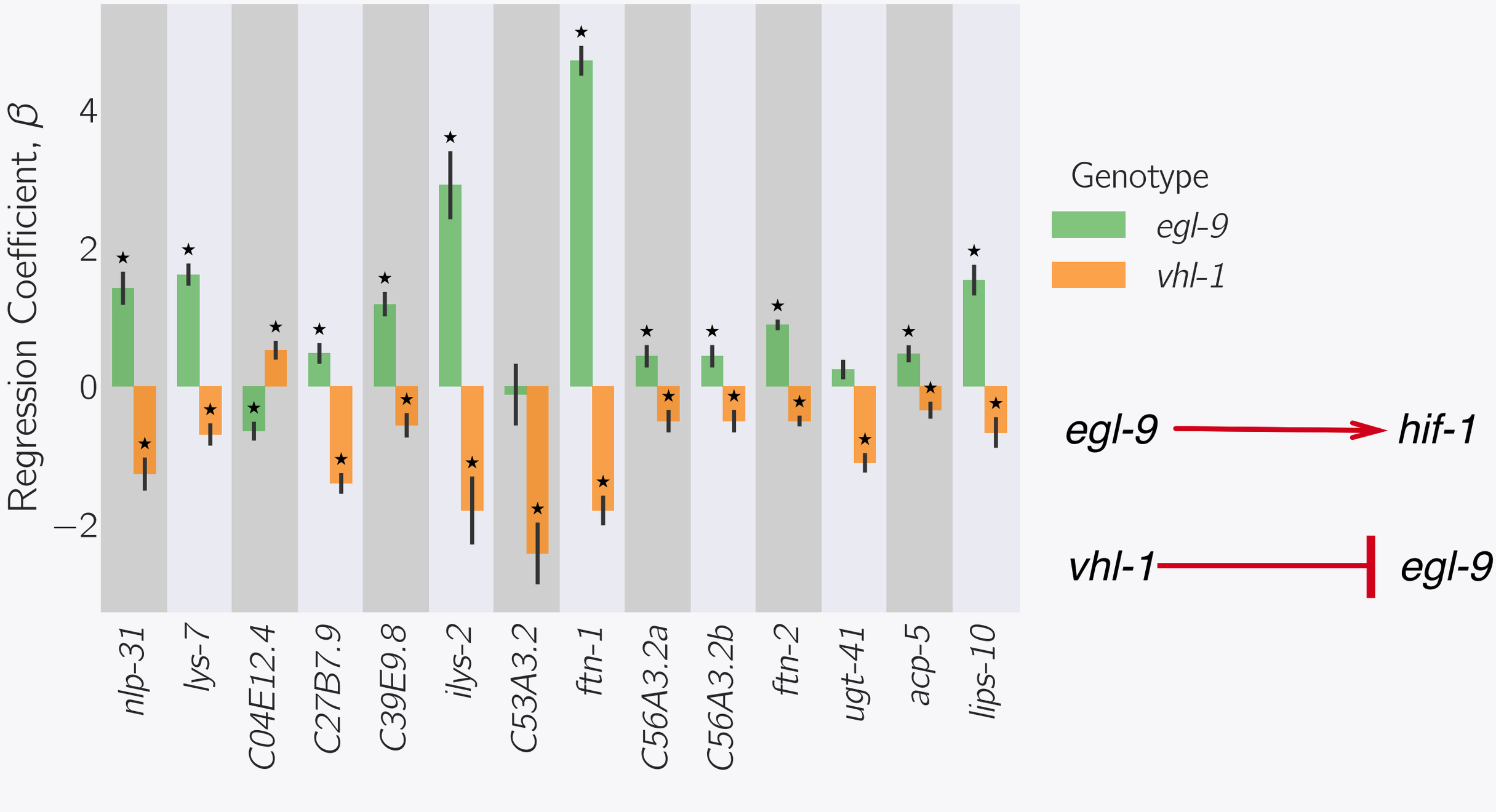


However, both pathways obey the same set of epistatic rules!

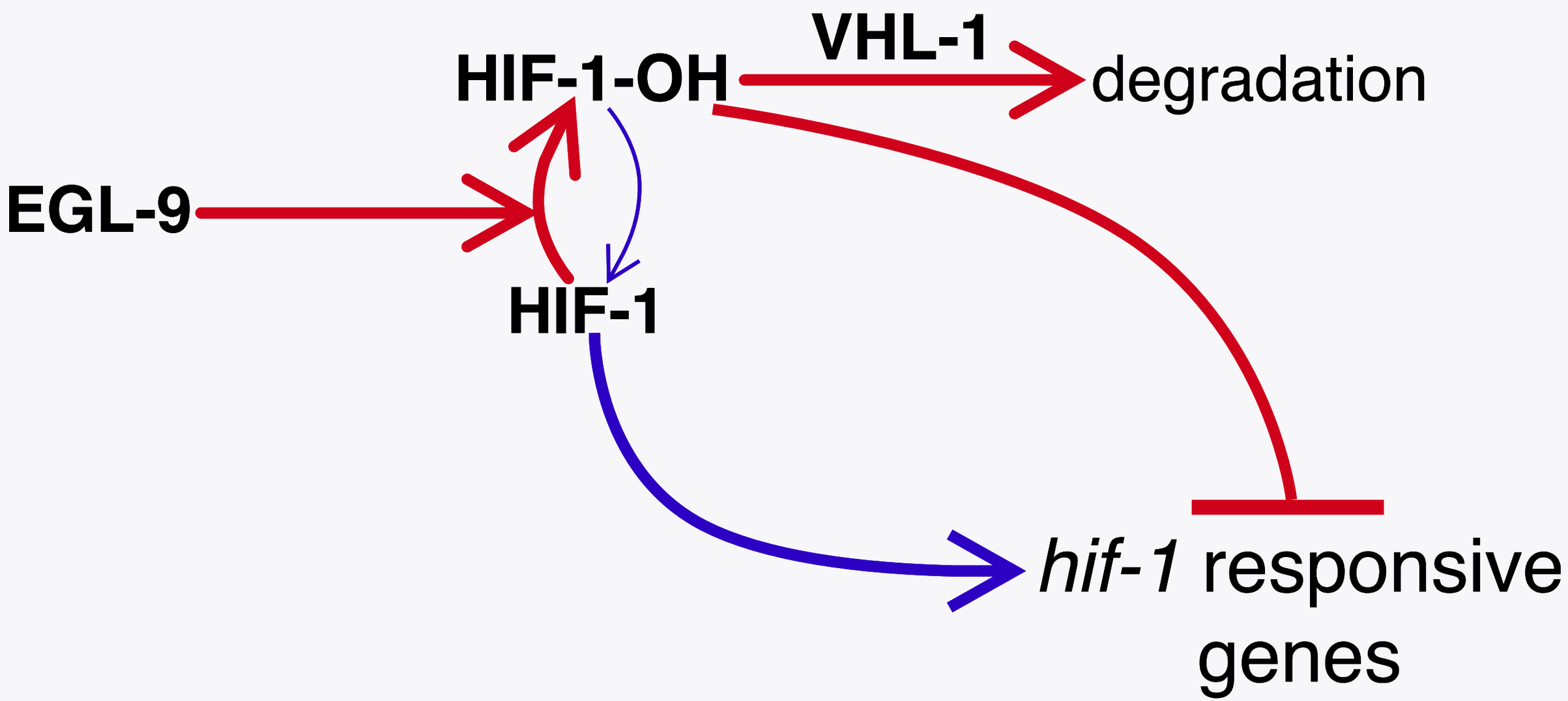
egl-9 = egl-9;vhl-1

hif-1 = egl-9;hif-1

Sequencing hypoxia pathway mutants reveal ~50 genes that behave as if controlled by HIF-1-OH



Hypothesis: A subset of genes is strongly responsive to HIF-1-OH levels



Transcriptomes + Biochemical Models can lead to testable hypotheses about molecular functions.

Transcriptomes are phenotypes in other organisms, such as bacteria!

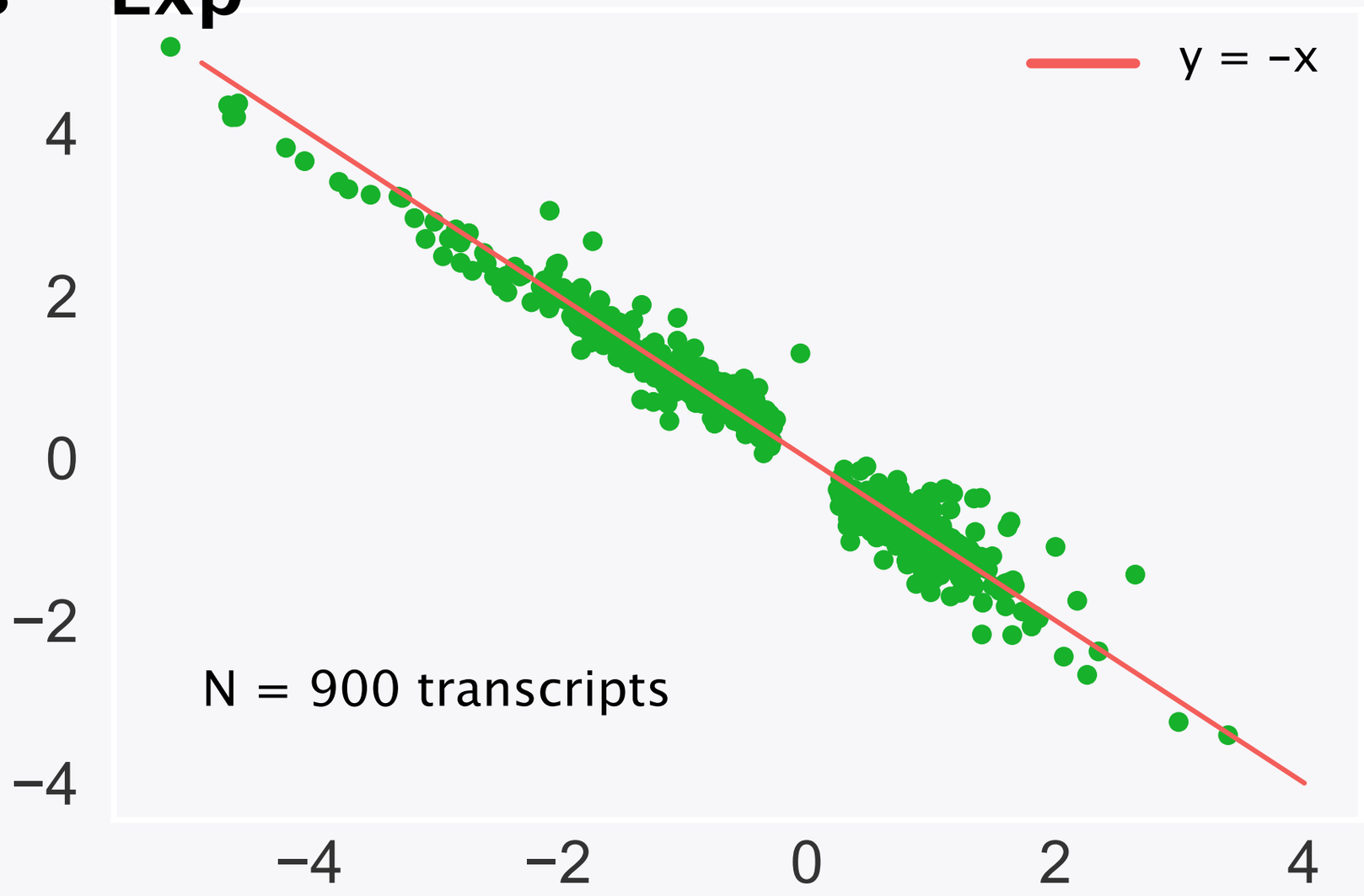
Fuqing's Question:

Do prebiotics affect antibiotic response in a gut bacterium?

+/- Prebiotic
+/- Antibiotic

Work by Fuqing Wu

A slope of -1 indicates complete inhibition of the effect of antibiotics by probiotics for a subset of genes



Work by Fuqing Wu

Transcriptomes are phenotypes in other organisms, such as bacteria!

Prebiotic — **Antibiotic**

Work by Fuqing Wu

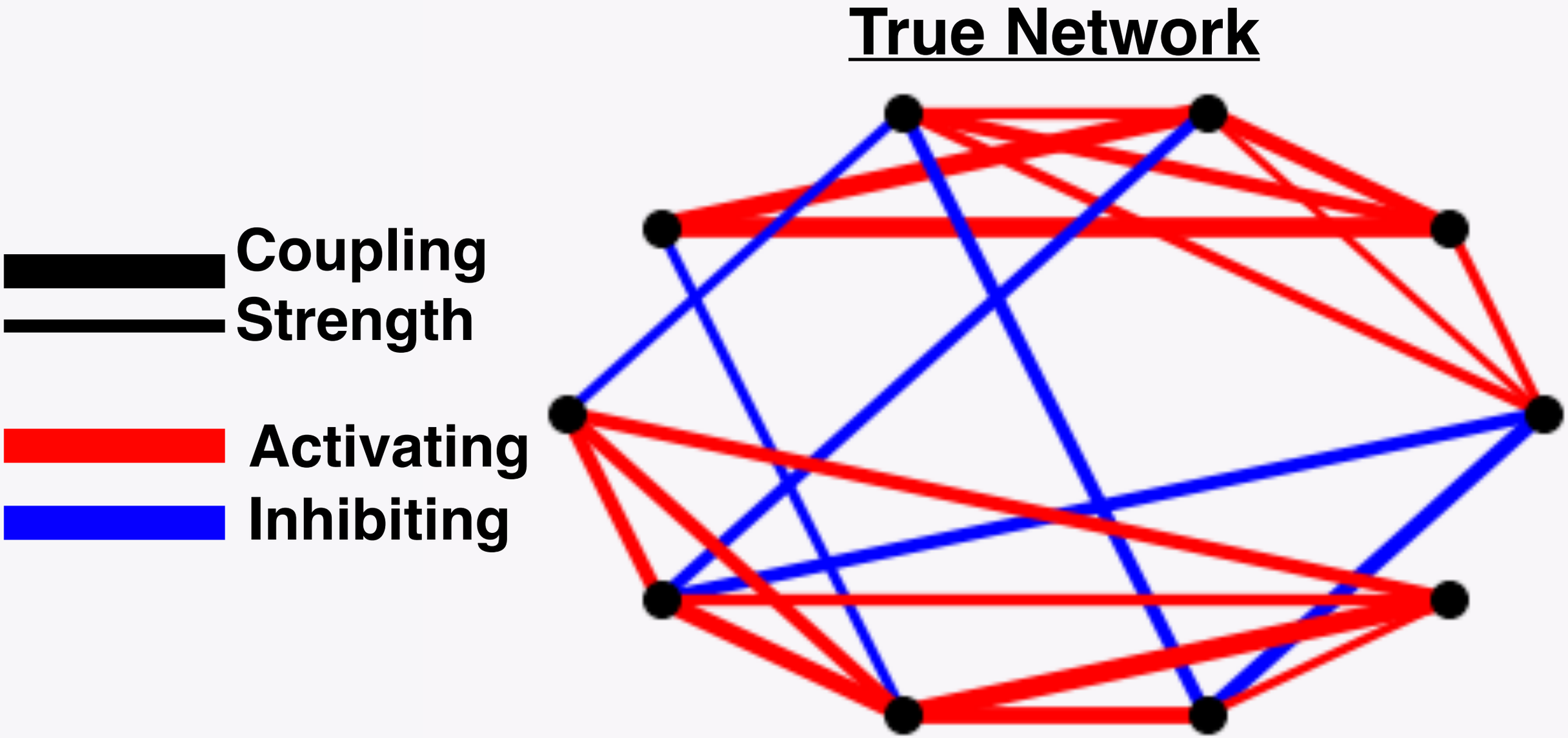
Transcriptomes as phenotypes: The geneticist's new arsenal

Null mutants (**Transcriptome-wide Epistasis**)

Allelic series (**Transcriptome-wide dominance**)

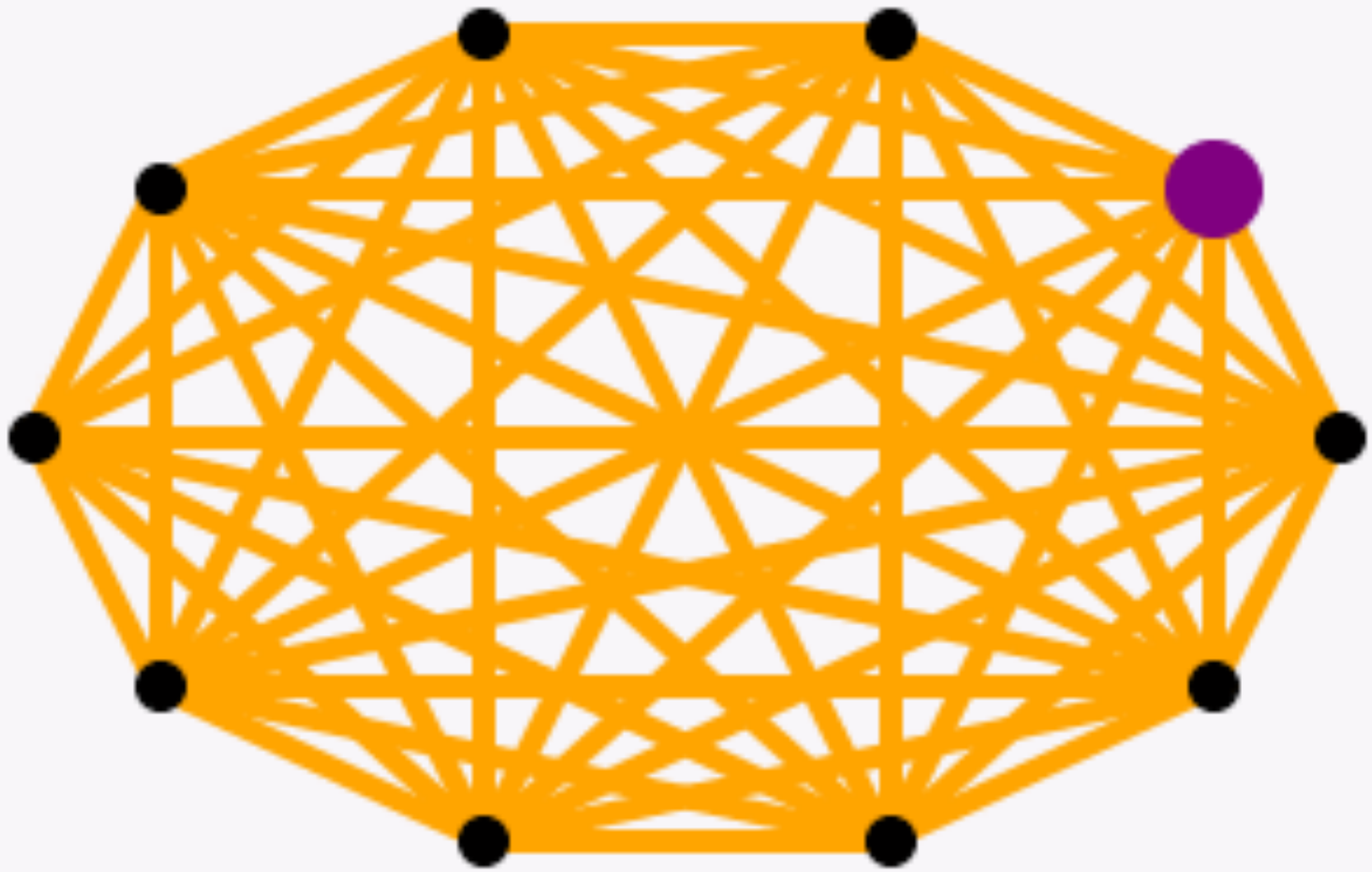
Crosses (**Transcriptome-wide maternal effects**)

Epistasis analyses can be automated



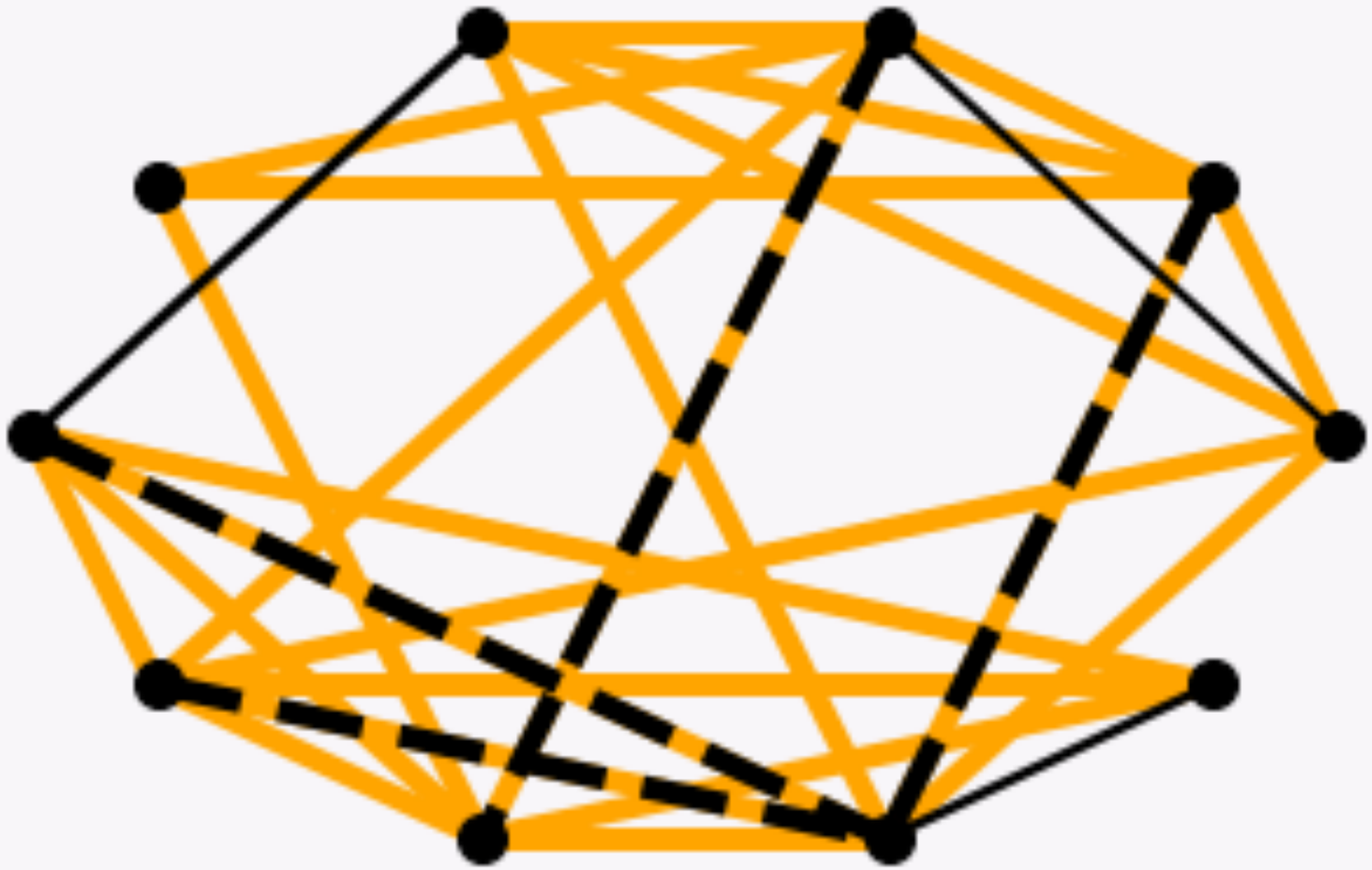
Collaboration with Matt Thomson

An example of automated reconstruction



Mutated Gene (Null)

Reconstructed network structure (no valences!)



Real edges

Missing edges (smaller = weaker)

Extra edges (should not be there)

Transcriptomes are phenotypes

Deploying transcriptomes in a **rich experimental context** makes them powerful

We now have both **statistical and conceptual machinery** to use transcriptomes productively

Transcriptomes are Phenotypes!

Paul Sternberg

hhmi | Howard Hughes Medical Institute

Sternberg Lab
Carmie Puckett Robinson
Daniel Leighton
Tiffany Khaw
Tiffany Tsou
Hillel Schwartz

Barbara Wold
Brian Williams

Matt Thomson

Millard and Muriel Jacobs Genetics and Genetics Lab
Igor Antoshechkin
Vijaya Kumar

Erich Schwarz