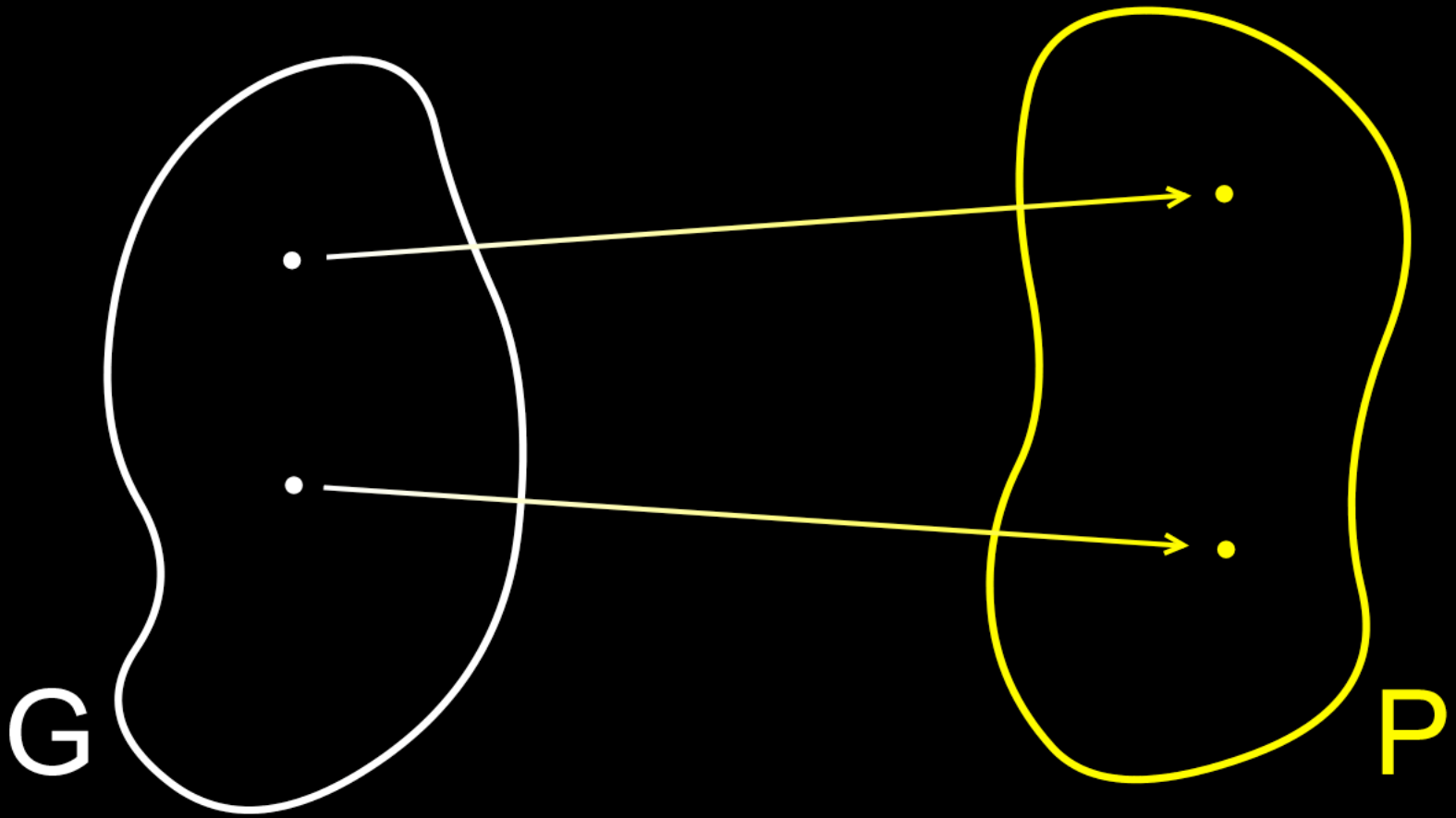


A fluorescence microscopy image of yeast cells. The cells are stained with three different dyes: green, blue, and red. The green signal highlights the cell walls, the blue signal highlights the nuclei, and the red signal highlights internal organelles, likely mitochondria. The cells are scattered across the field of view, with some appearing as single cells and others as budding pairs or small clusters. The background is black, making the colored cells stand out.

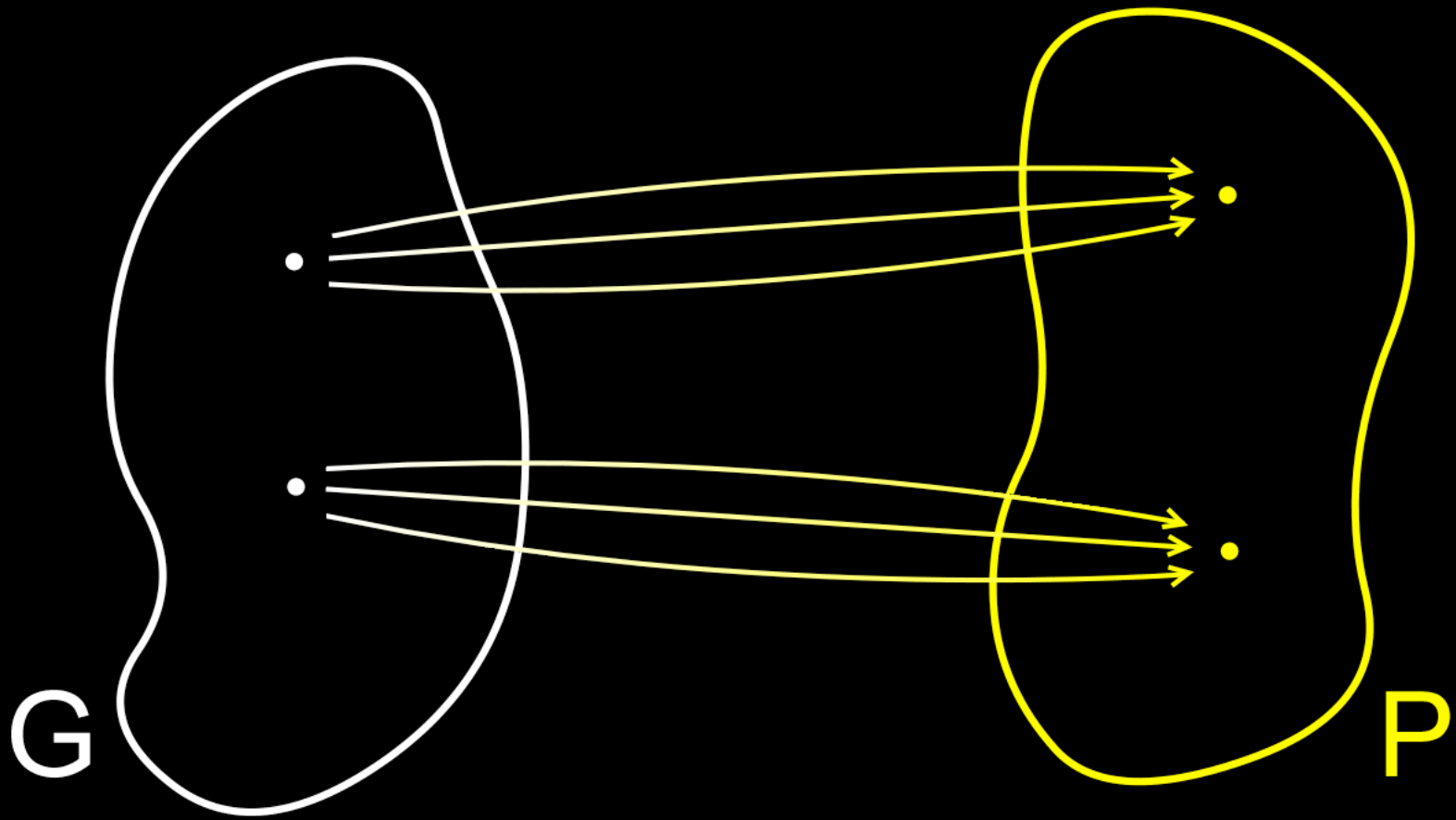
Phenotypic robustness and network centrality in yeast

Mark Siegal & Sasha Levy
Center for Genomics and Systems Biology
Department of Biology
New York University

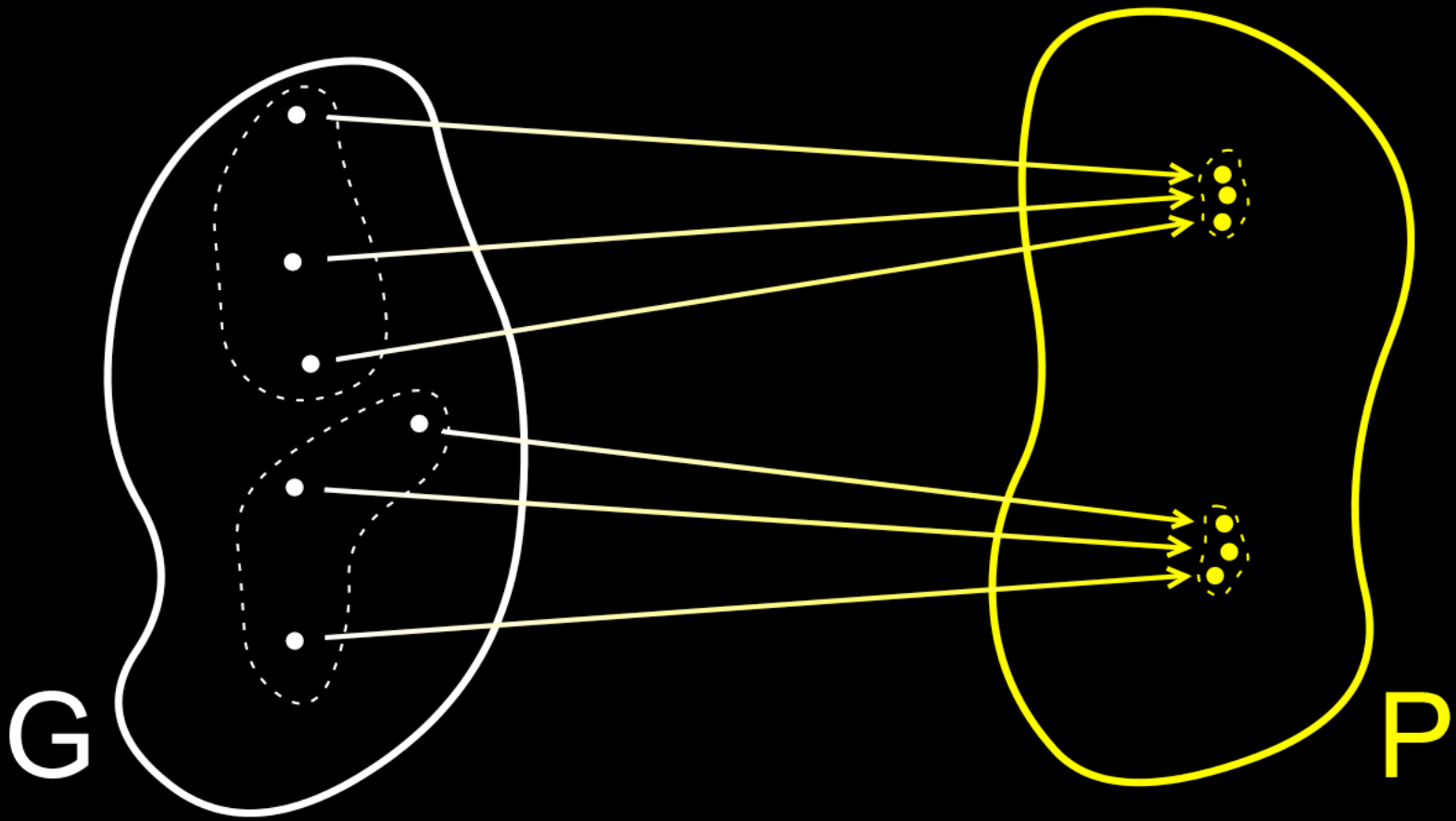
The genotype phenotype map



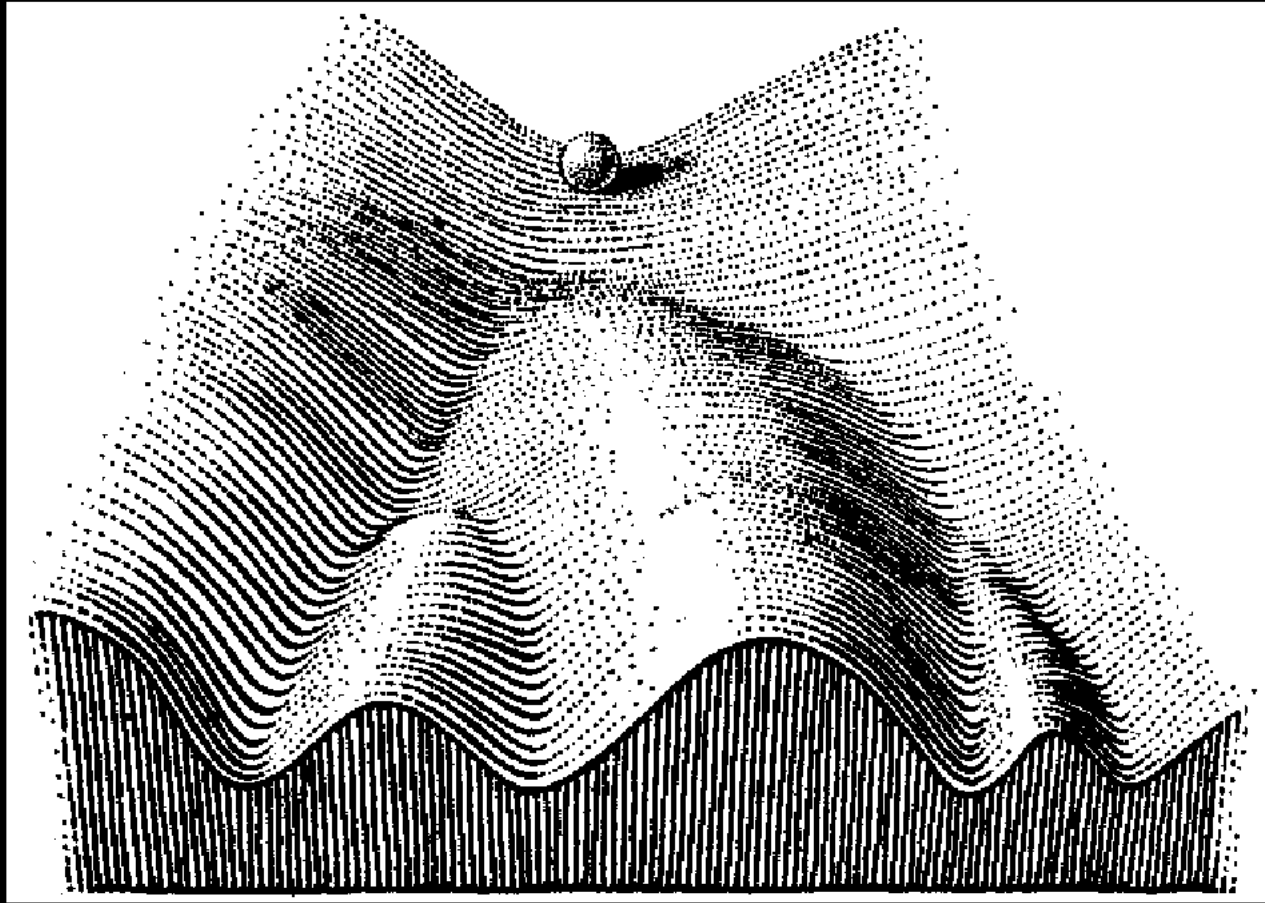
The genotype phenotype map: homeostasis



The genotype phenotype map: genetic robustness

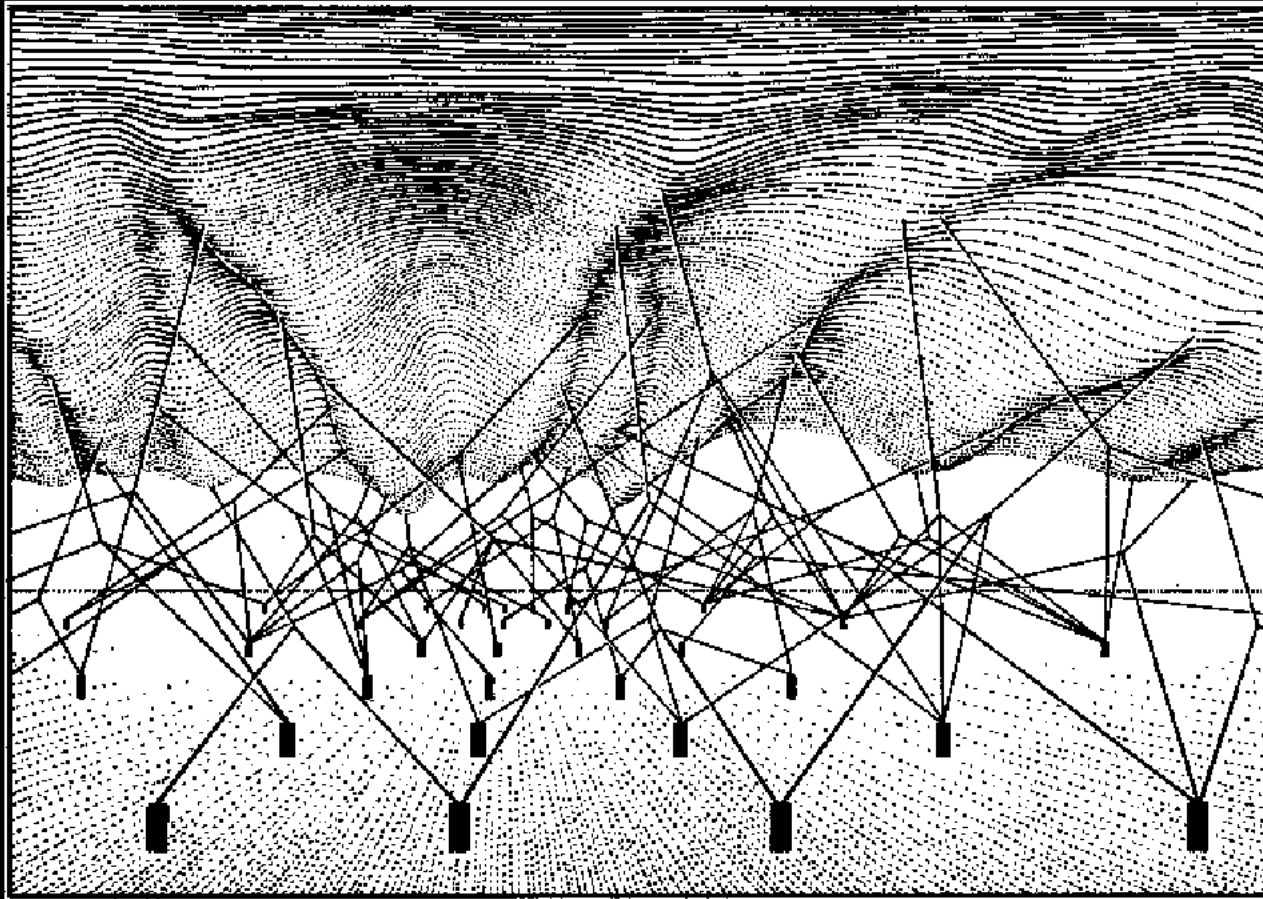


Canalization: the robustness of developmental pathways



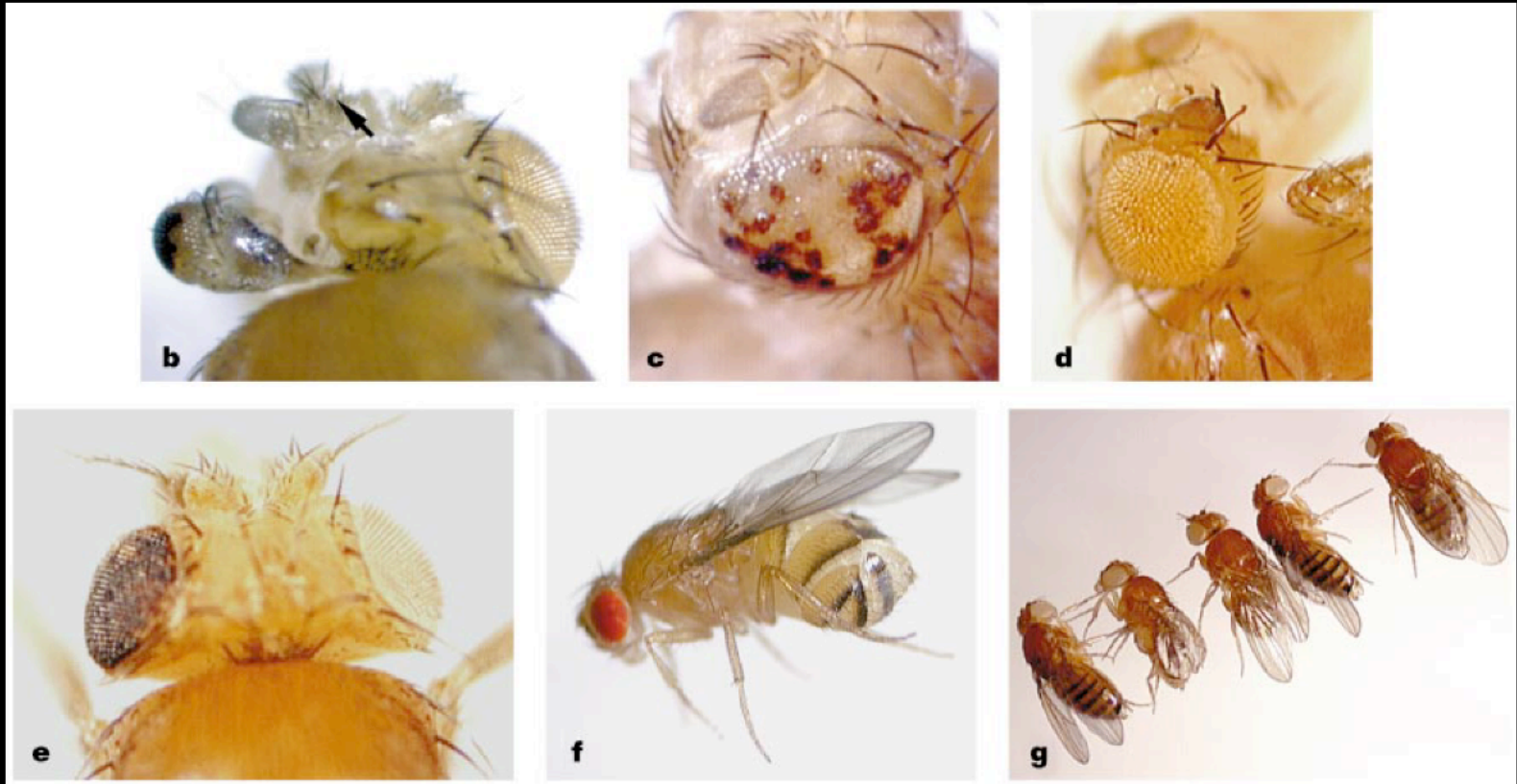
Waddington, *The Strategy of the Genes* (1957)

Canalization as an emergent property of a complex network



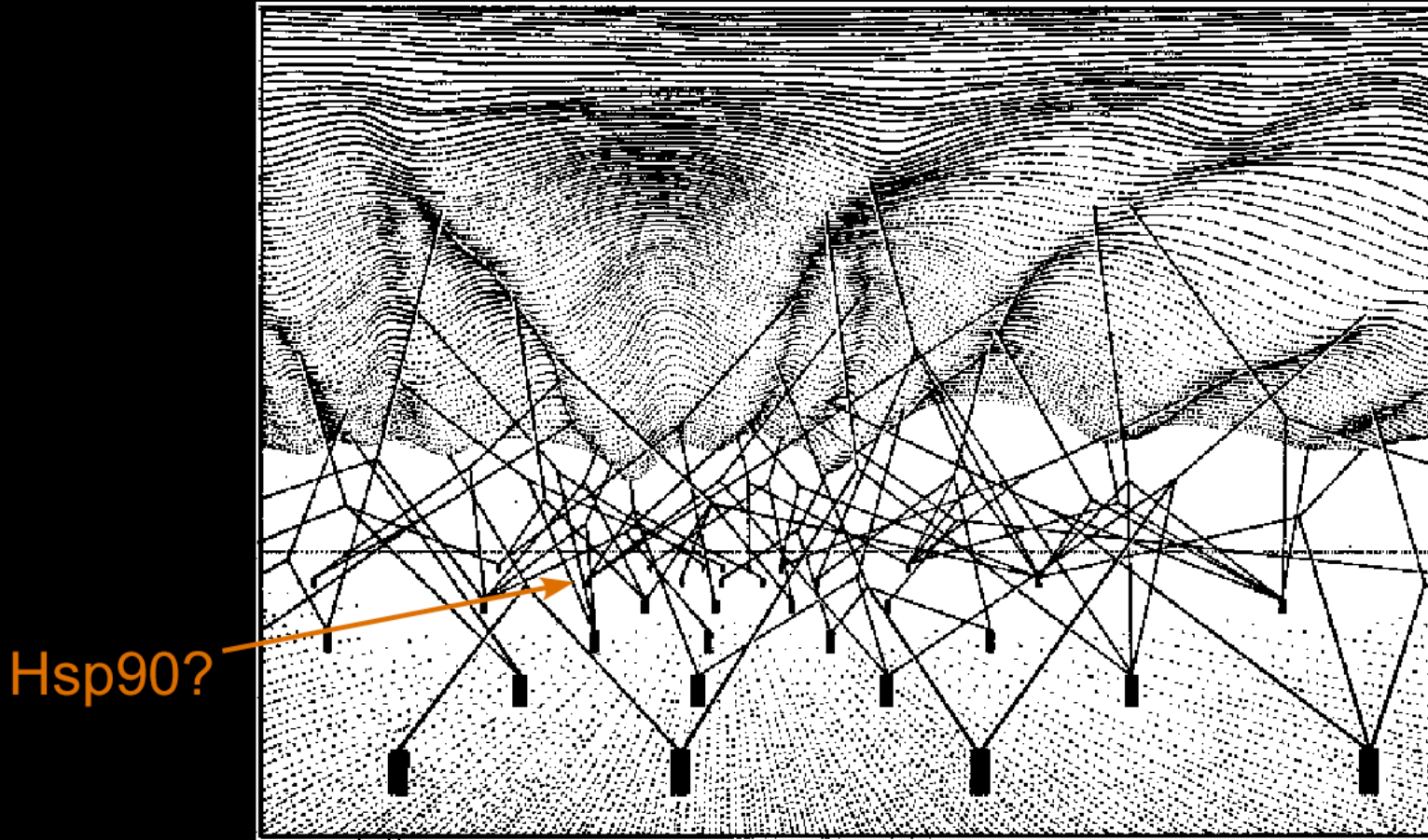
Waddington, *The Strategy of the Genes* (1957)

Hsp90: a “phenotypic capacitor”



Rutherford and Lindquist, *Nature* 396:336 (1998)

How many capacitors exist? Do they have particular properties?
Does their impairment always create monsters?



Hsp90?

Waddington, *The Strategy of the Genes* (1957)

Experimental goals

Systematically screen the genome of *Saccharomyces cerevisiae* for genes encoding phenotypic capacitors

Characterize the identified capacitors:

evolutionary parameters

network parameters

Yeast as a model system for phenotypic capacitance

Advantages

comprehensive collection of isogenic knockout strains

many high-throughput, genome-wide data sets

growth rates

gene and protein interactions

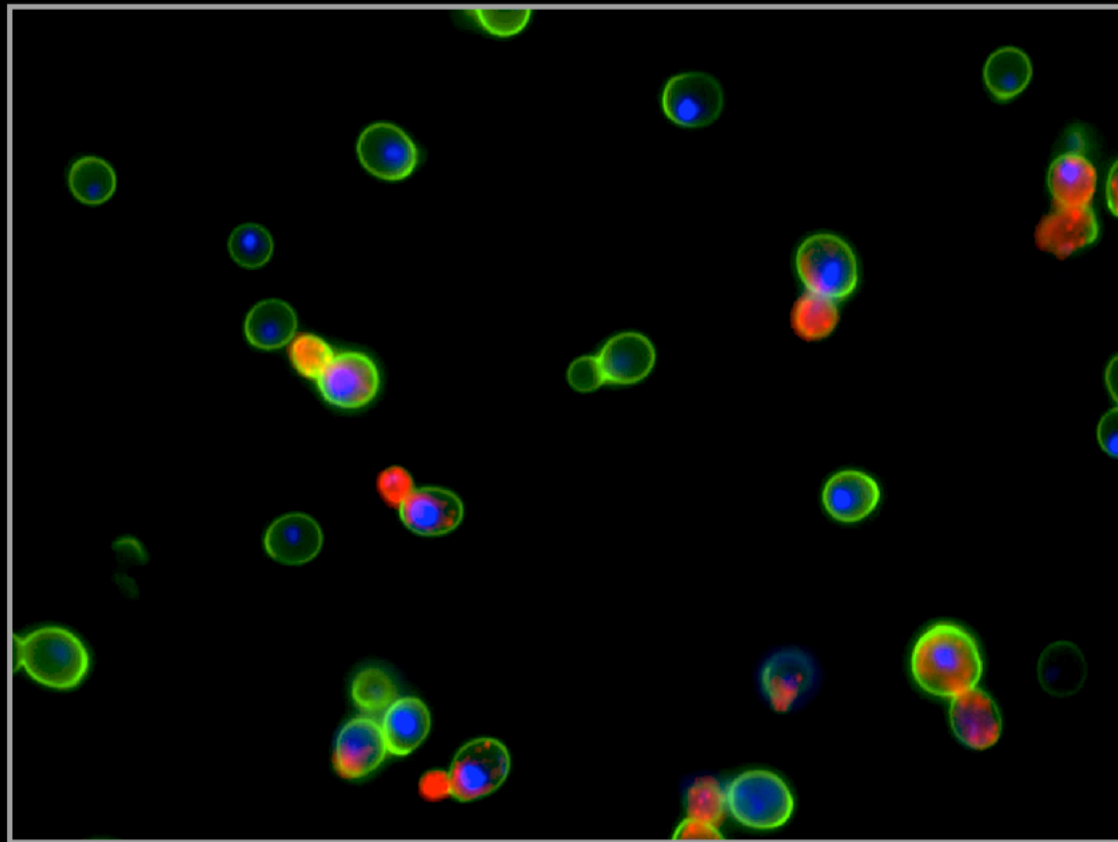
gene expression

ease of culturing and genetic manipulation

Disadvantages

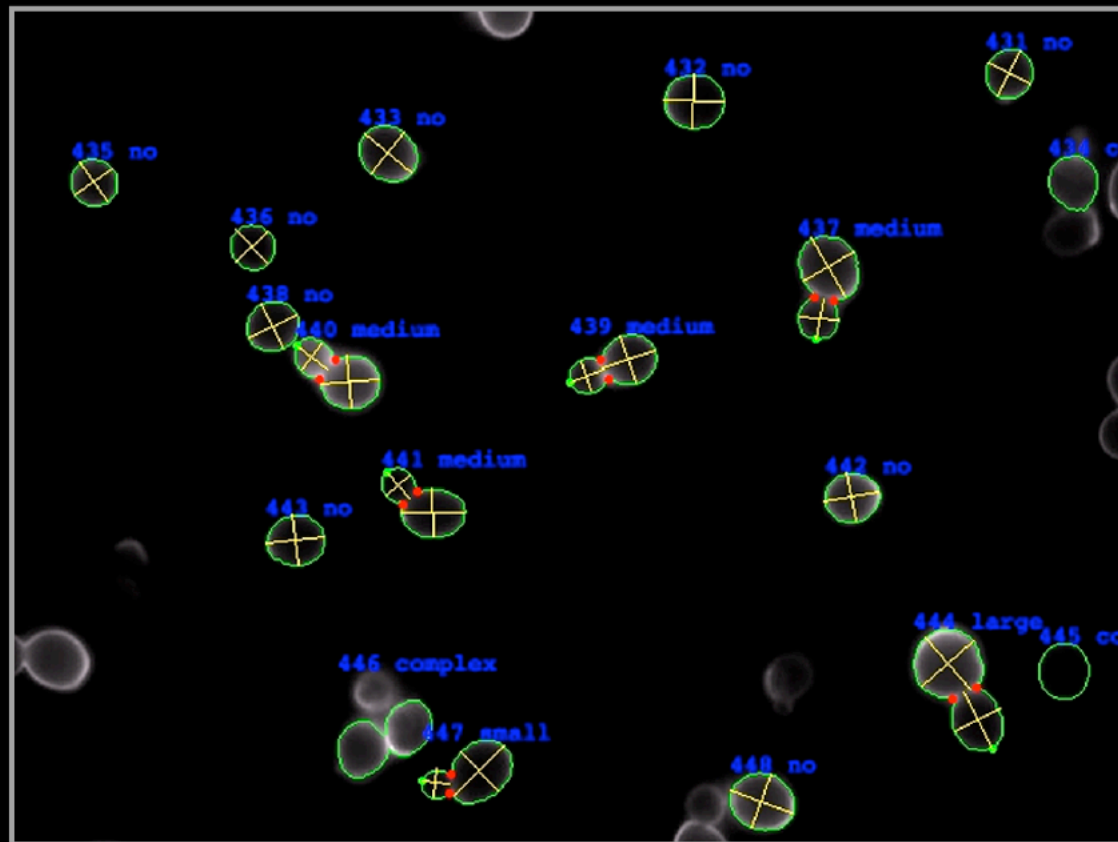
most measured yeast phenotypes are population properties, not individual ones (*e.g.*, growth rate)

High-throughput morphological phenotyping of yeast cells (Ohya *et al.*, 2005, *PNAS* 102:19015)



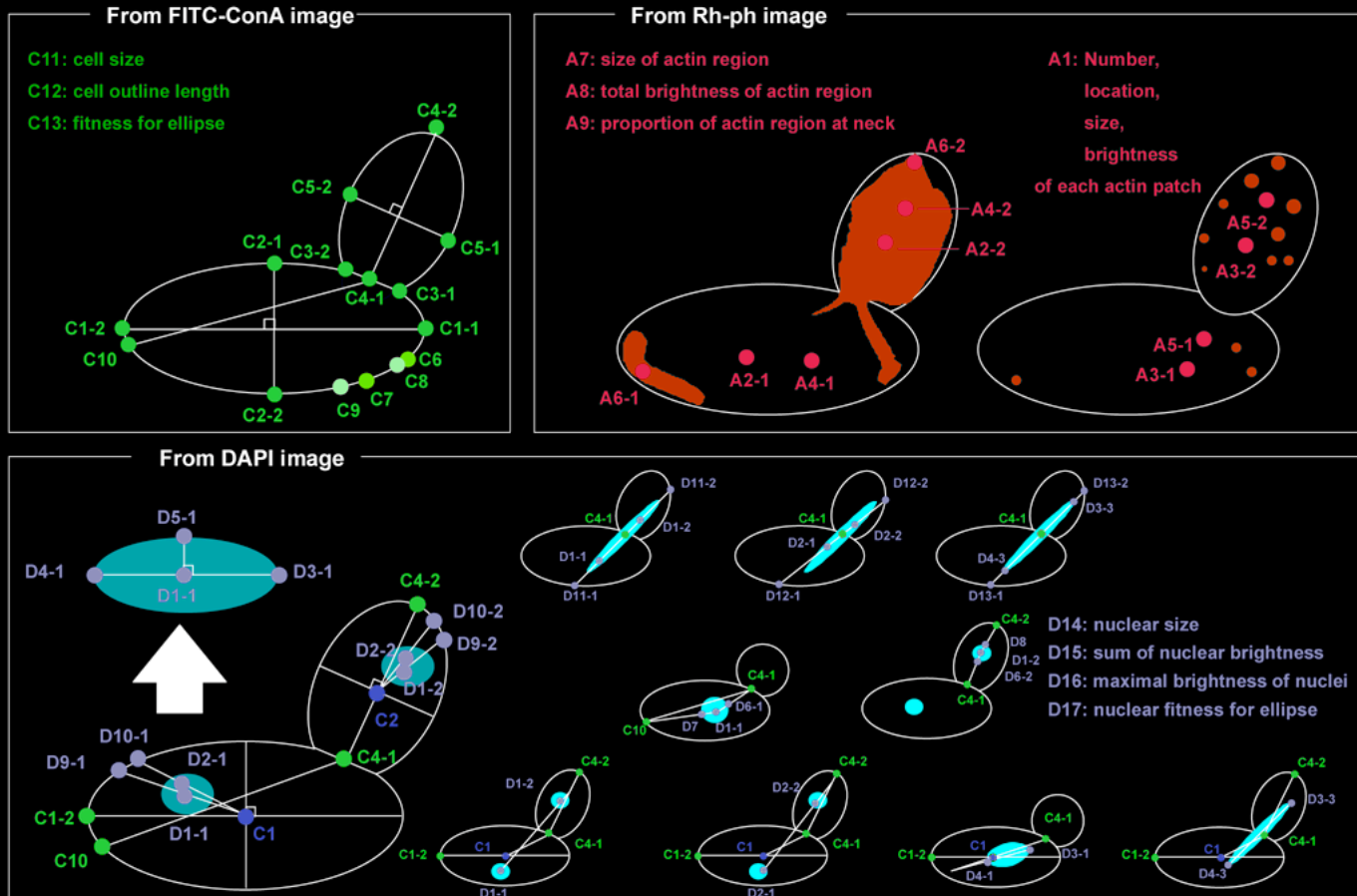
ConA
DAPI
phalloidin

High-throughput morphological phenotyping of yeast cells (Ohya *et al.*, 2005, *PNAS* 102:19015)



4718 haploid-viable yeast knockout (YKO) strains phenotyped
220 phenotypes with mean and variance

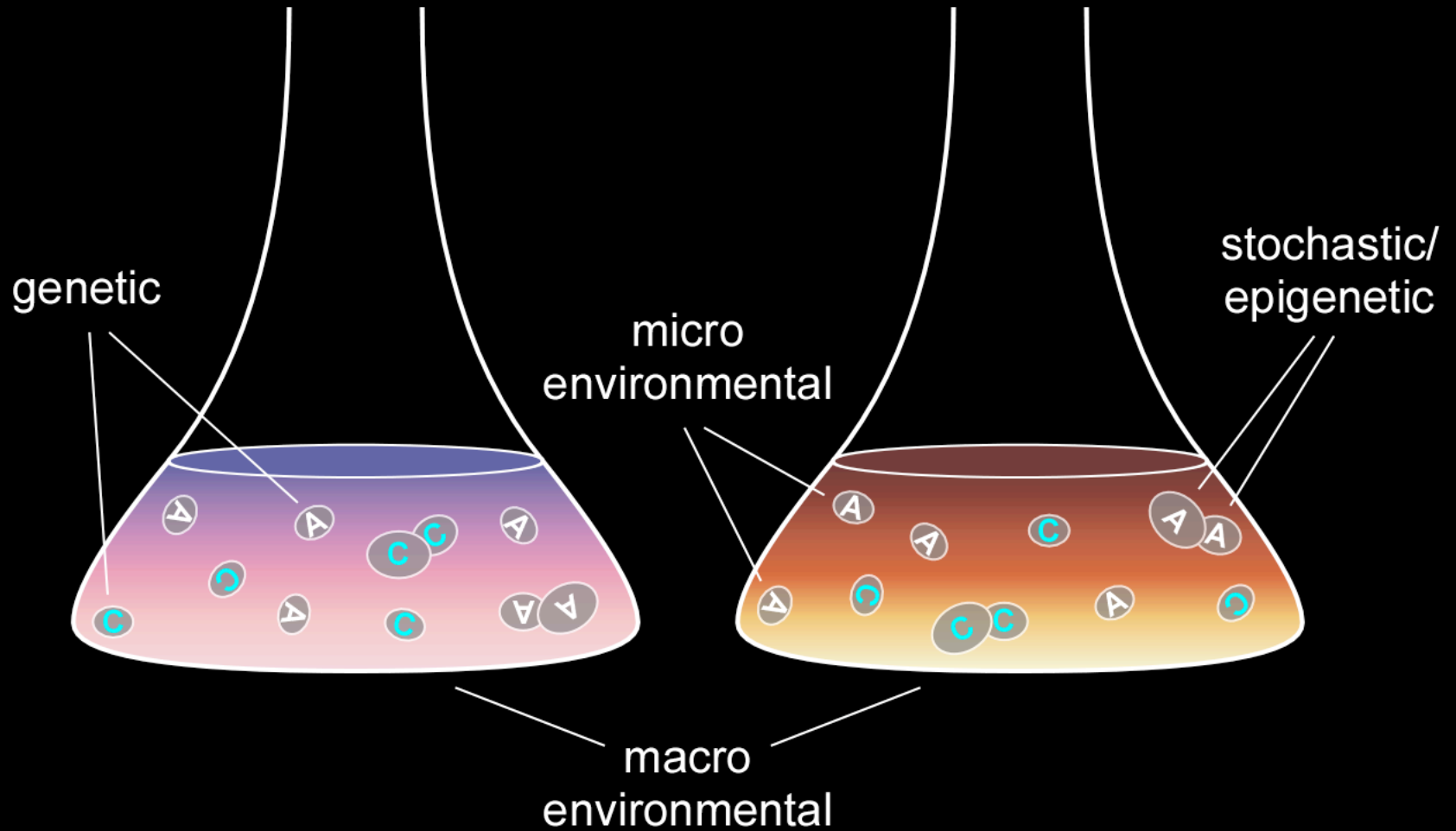
High-throughput morphological phenotyping of yeast cells (Ohya *et al.*, 2005, *PNAS* 102:19015)



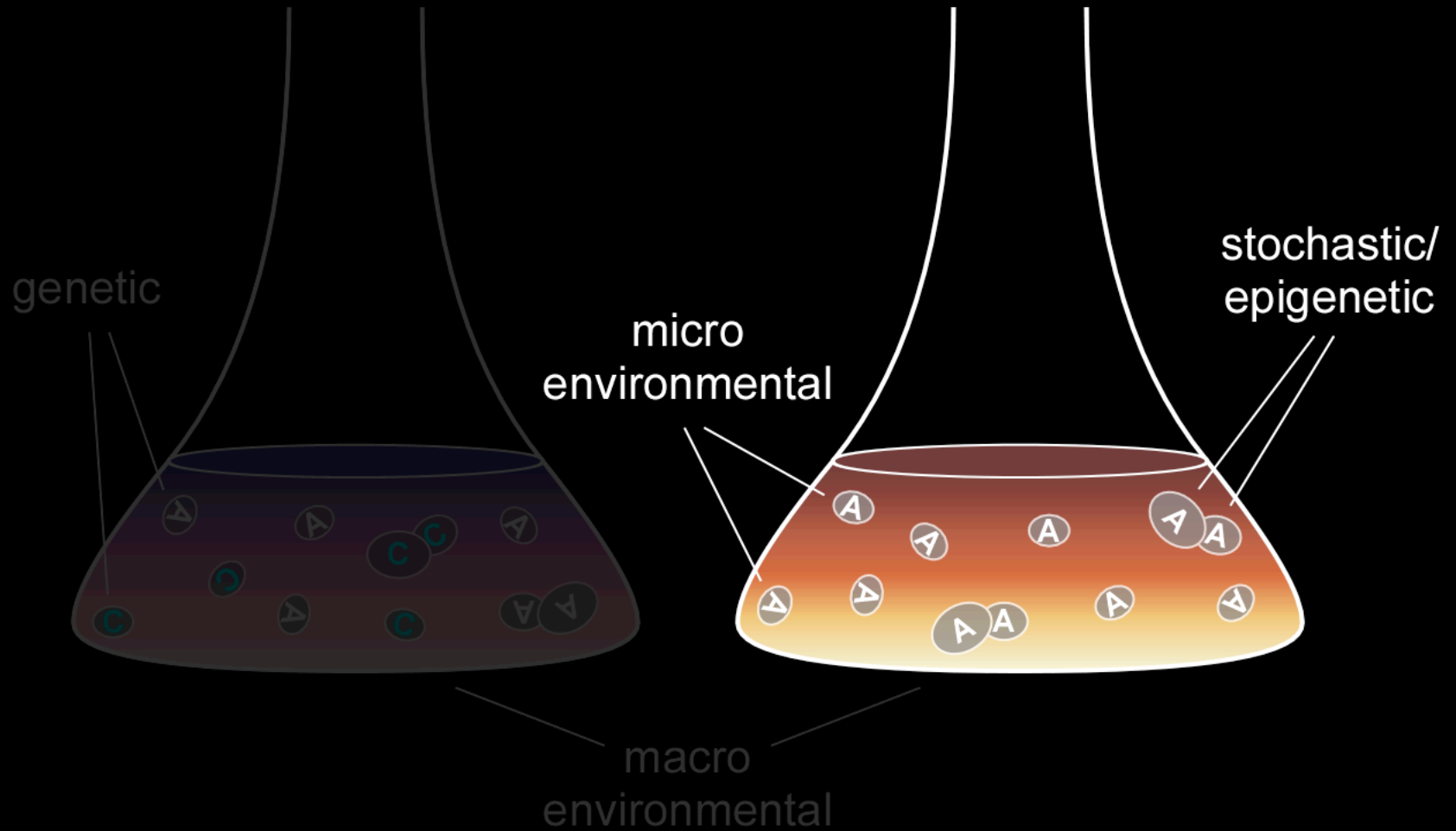
Ohya *et al.*, 2005, *PNAS* 102:19015

4718 haploid-viable yeast knockout (YKO) strains phenotyped
220 phenotypes with mean and variance

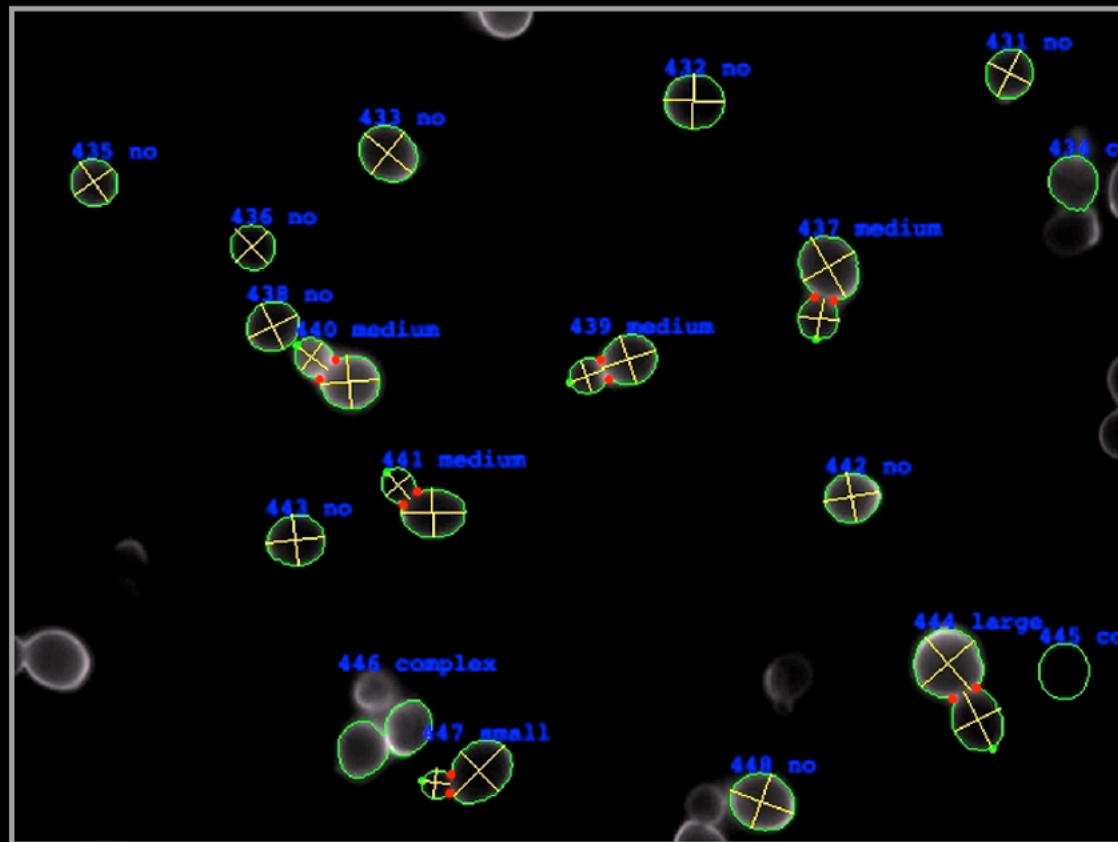
Sources of variation in yeast cultures



Sources of variation in YKO morphological phenotypes



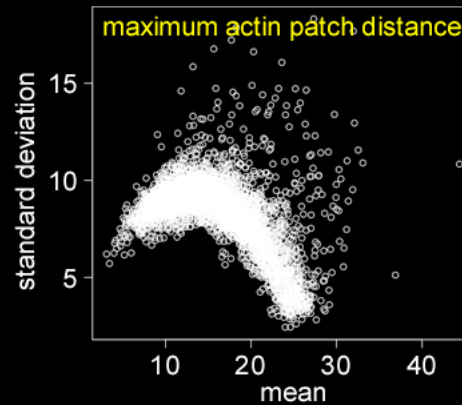
High-throughput morphological phenotyping of yeast cells (Ohya *et al.*, 2005, *PNAS* 102:19015)



4718 haploid-viable yeast knockout (YKO) strains phenotyped
220 phenotypes with mean and variance

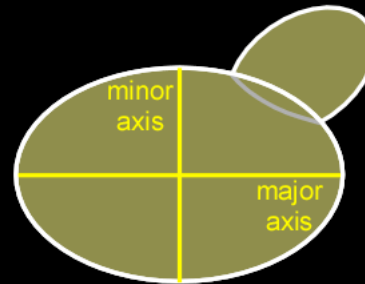
Challenges in identifying capacitors using yeast morphological data

variance depends on mean



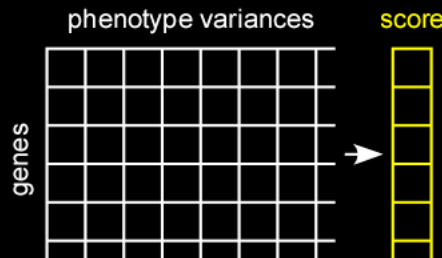
lowess regression

phenotypes might be redundant



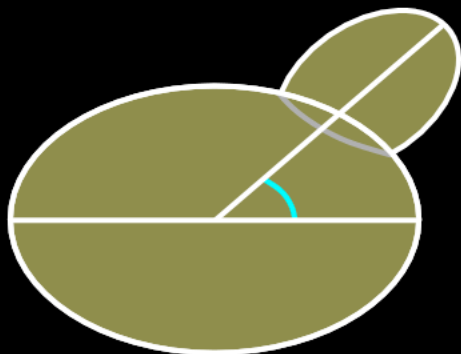
dimensional reduction by PAM

need single score and significance for each KO

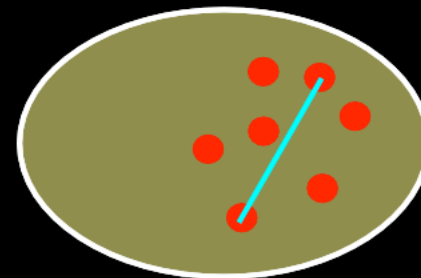


asymmetric trimmed mean and permutations

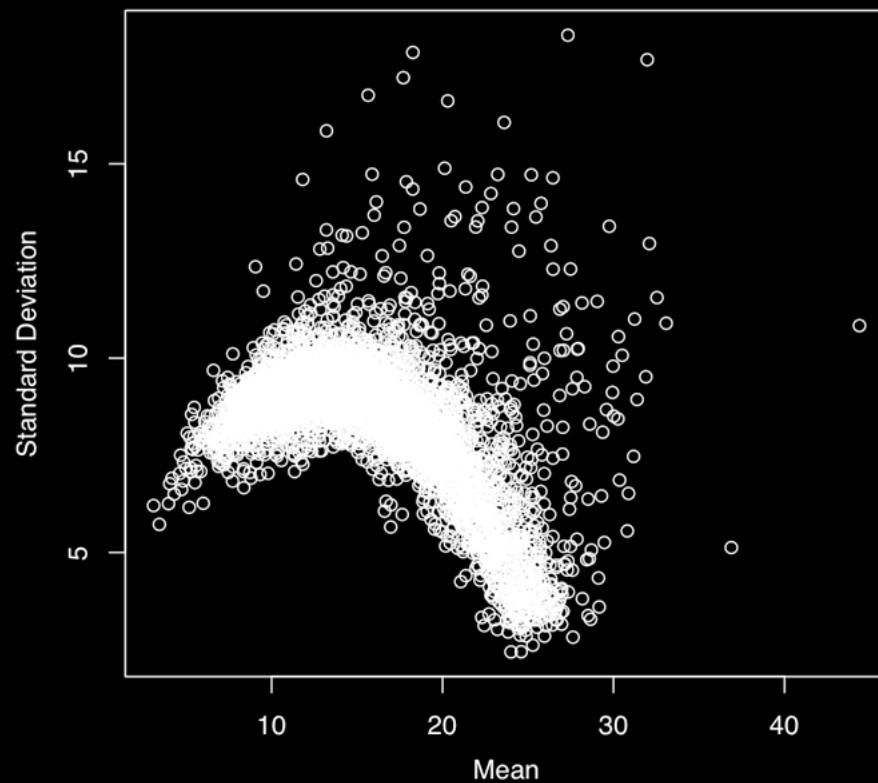
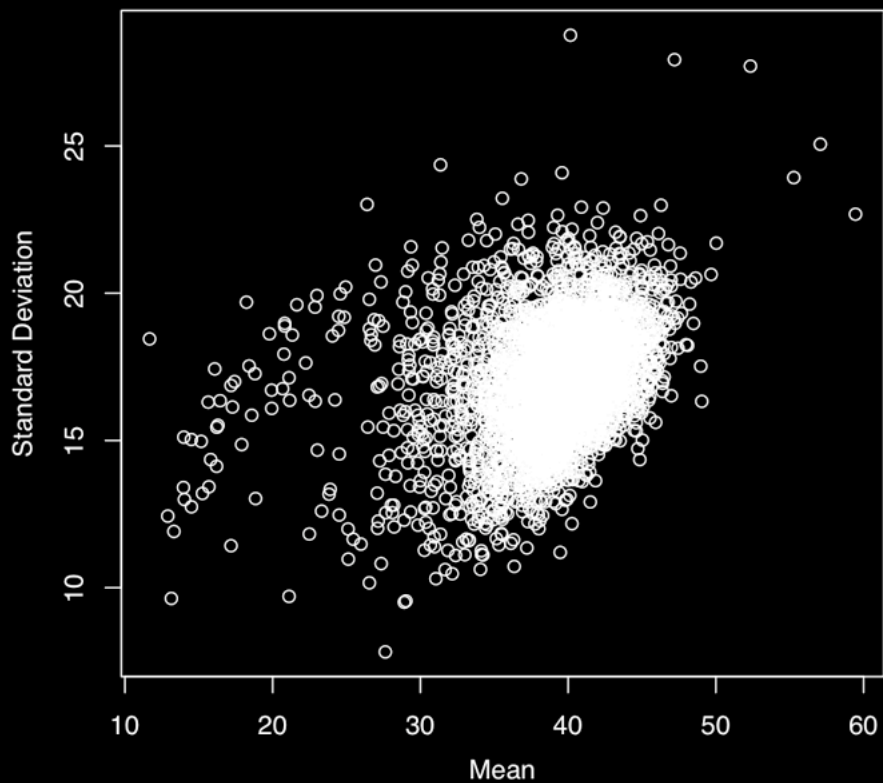
Phenotypic variance depends on the mean



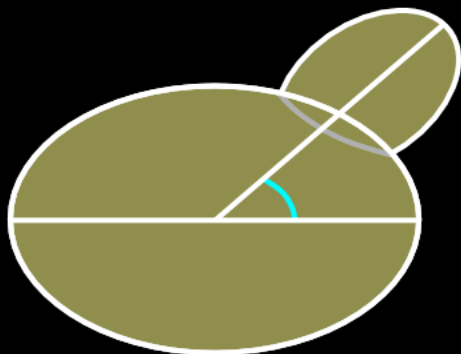
bud direction



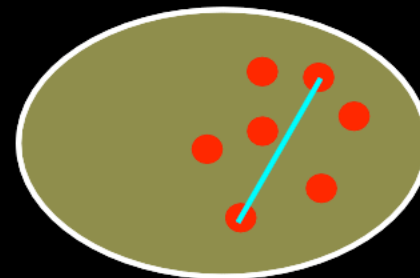
maximum actin patch distance



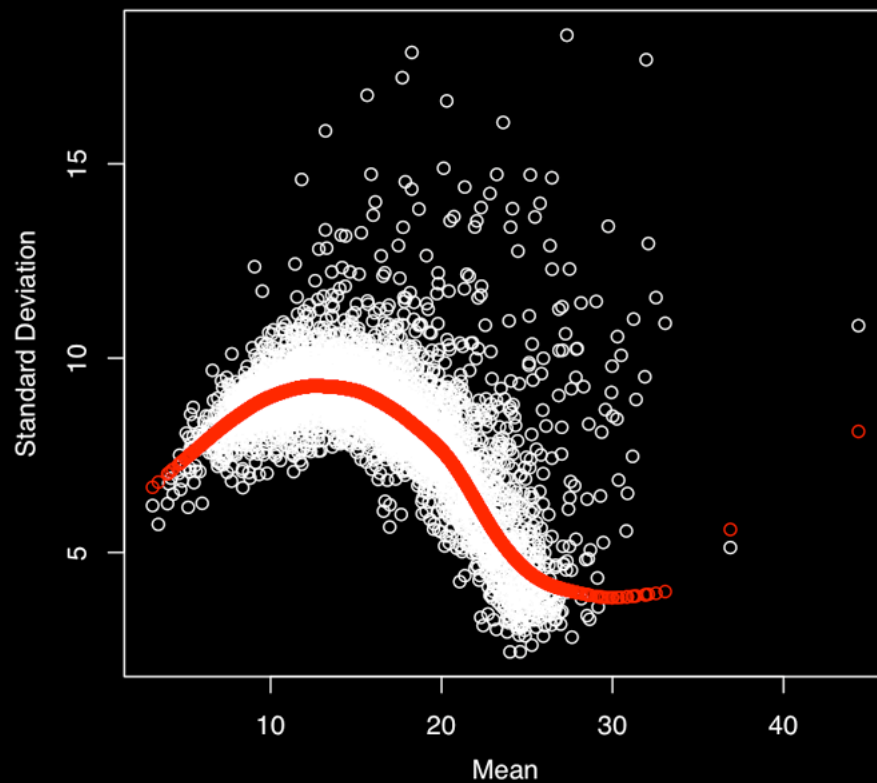
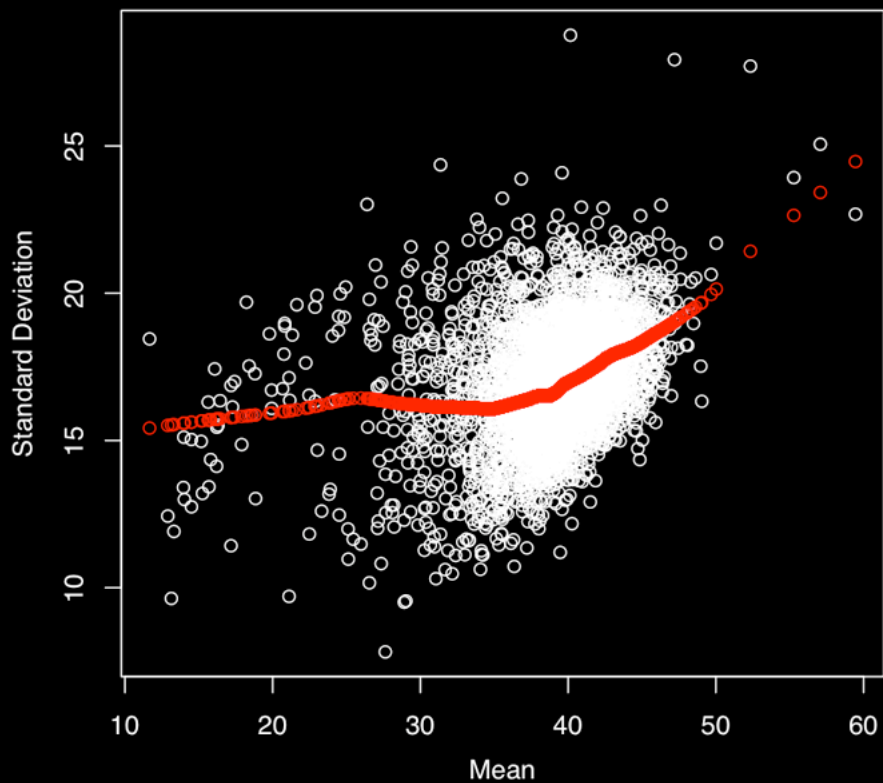
Lowess regression to identify high variance given the mean



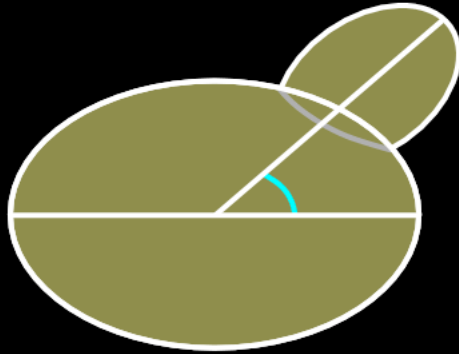
bud direction



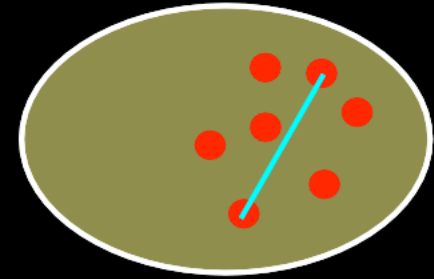
maximum actin patch distance



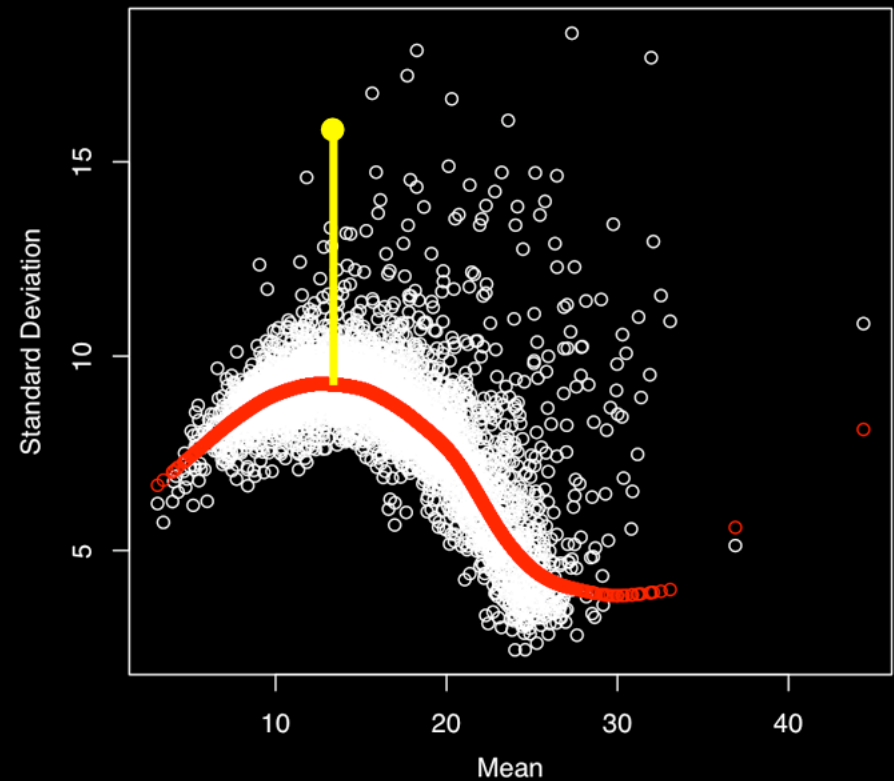
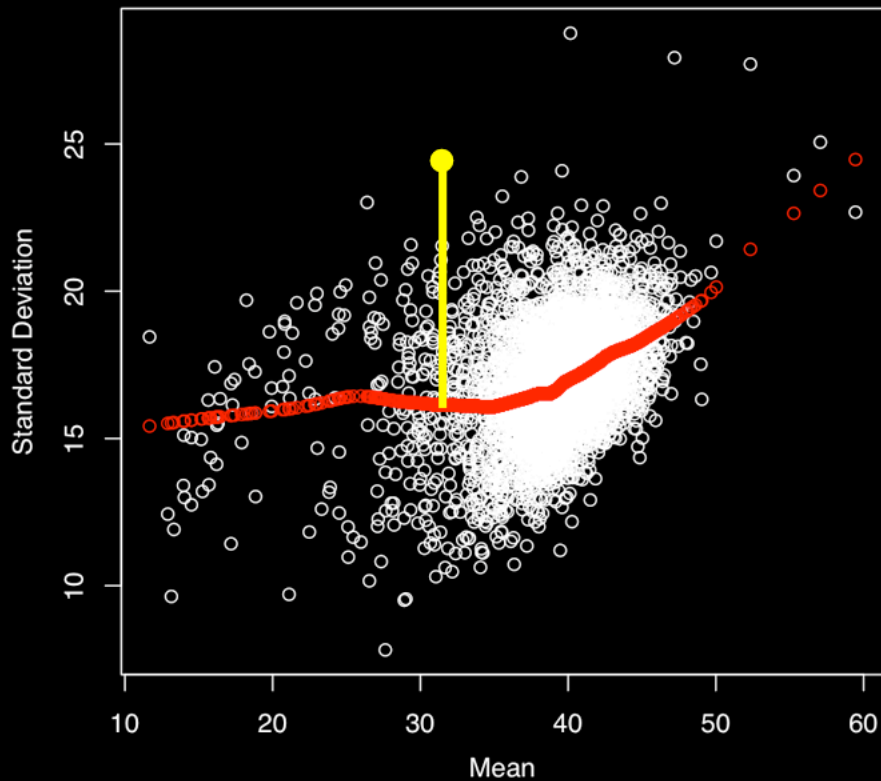
Residuals of SD are a measure of variance relative to mean



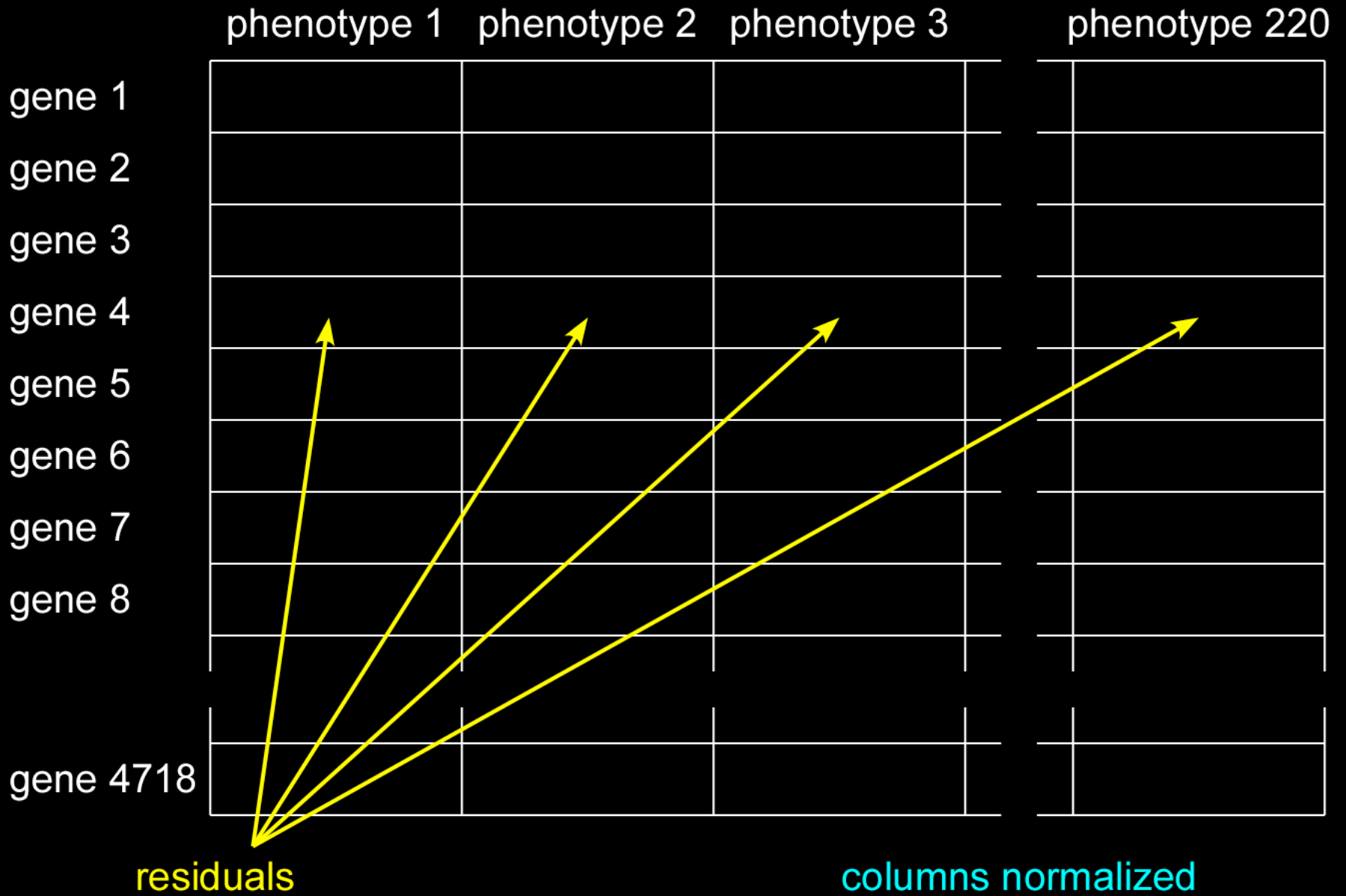
bud direction



maximum actin patch distance

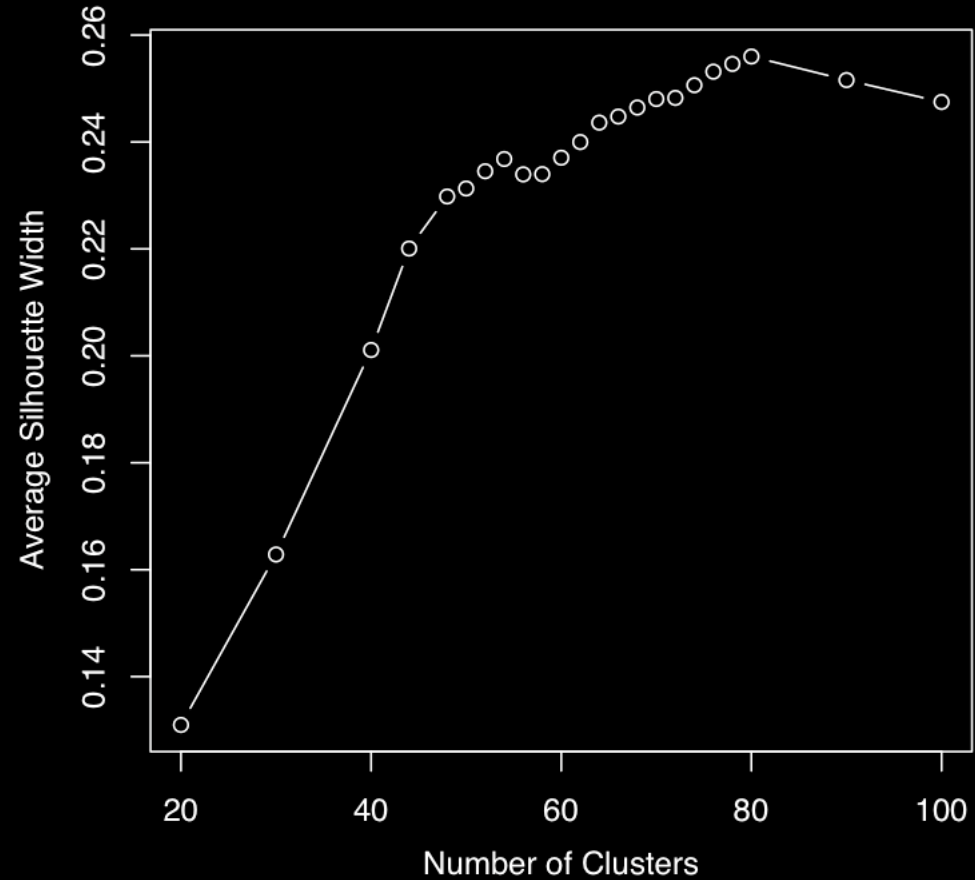
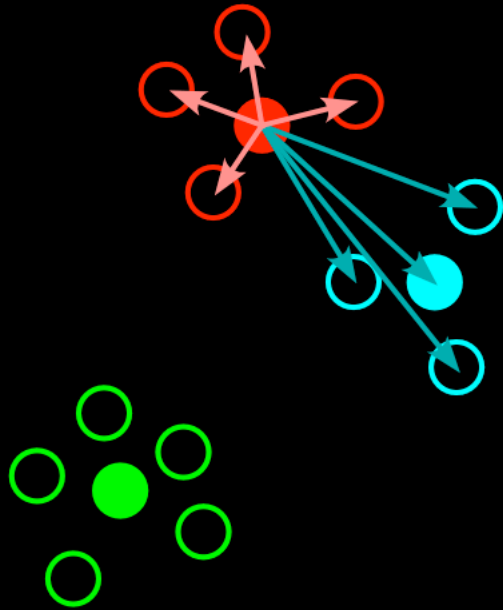


The data matrix




Clustering to remove physically or biologically redundant phenotypes

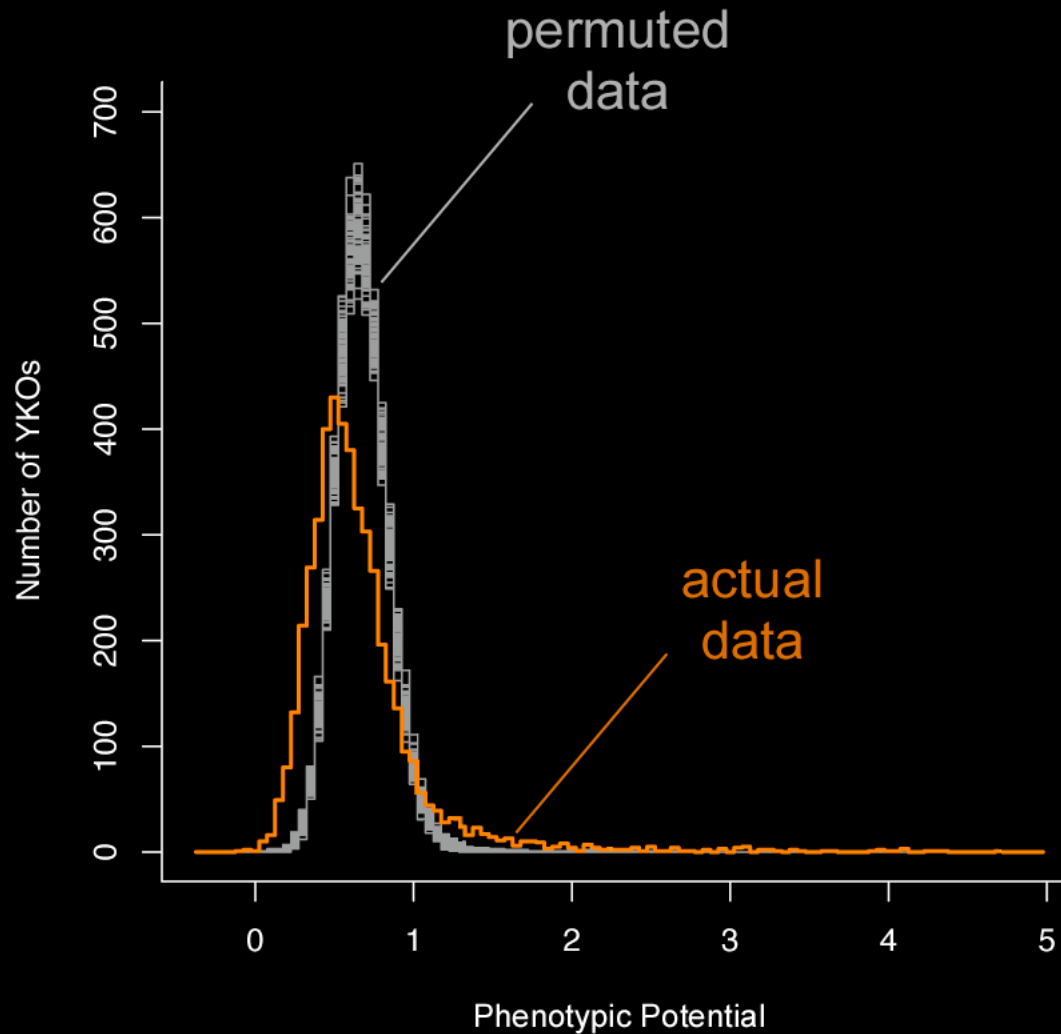
partitioning around medoids (PAM)



Calculation of phenotypic potential

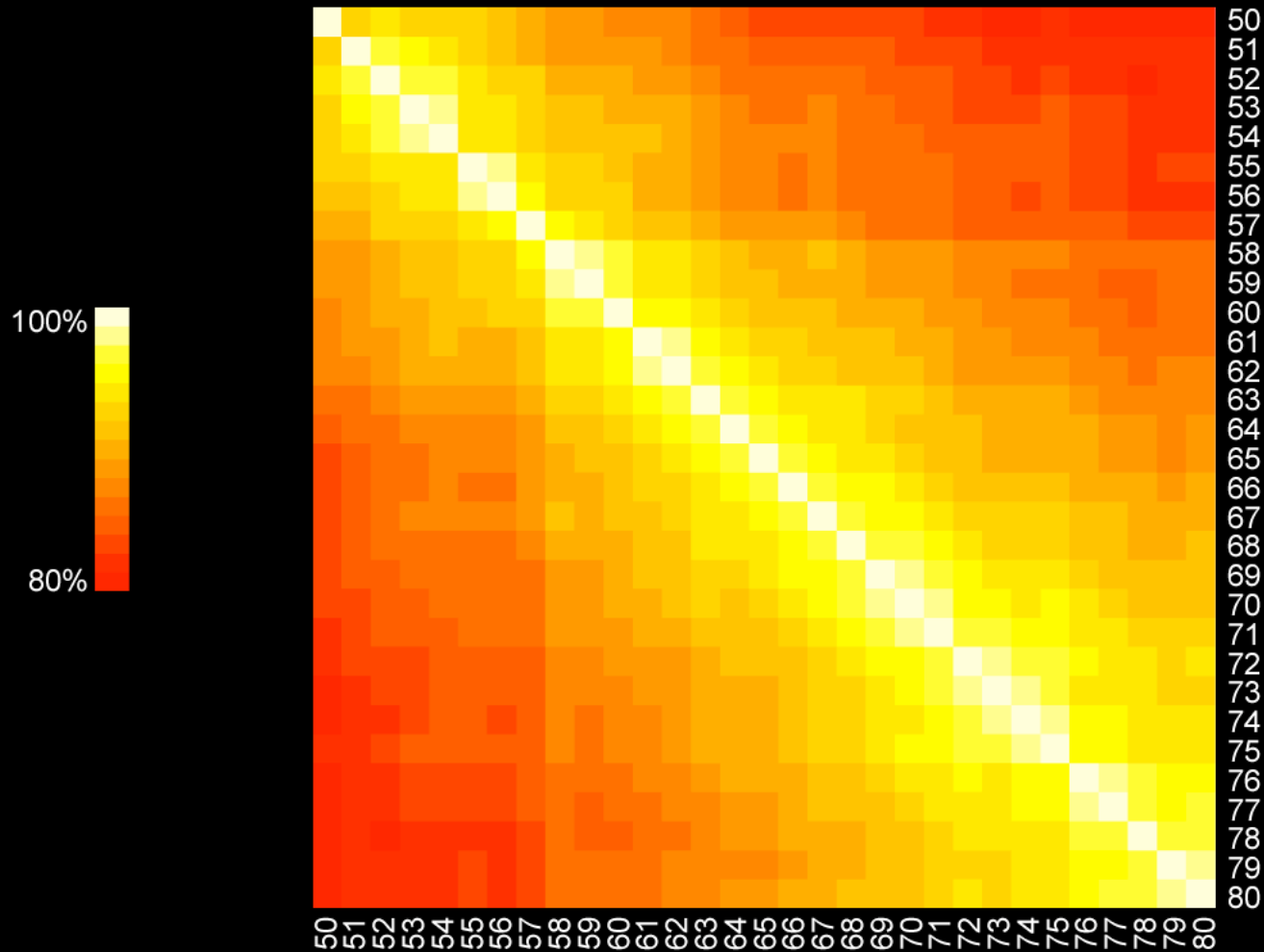
	medoid 1	medoid 2	medoid 70	phenotypic potential
gene 1				average top 35
gene 2				
gene 3				
gene 4				
gene 5				
gene 6				
gene 7				
gene 8				
gene 4718				

Significant excess of genes with high “phenotypic potential”



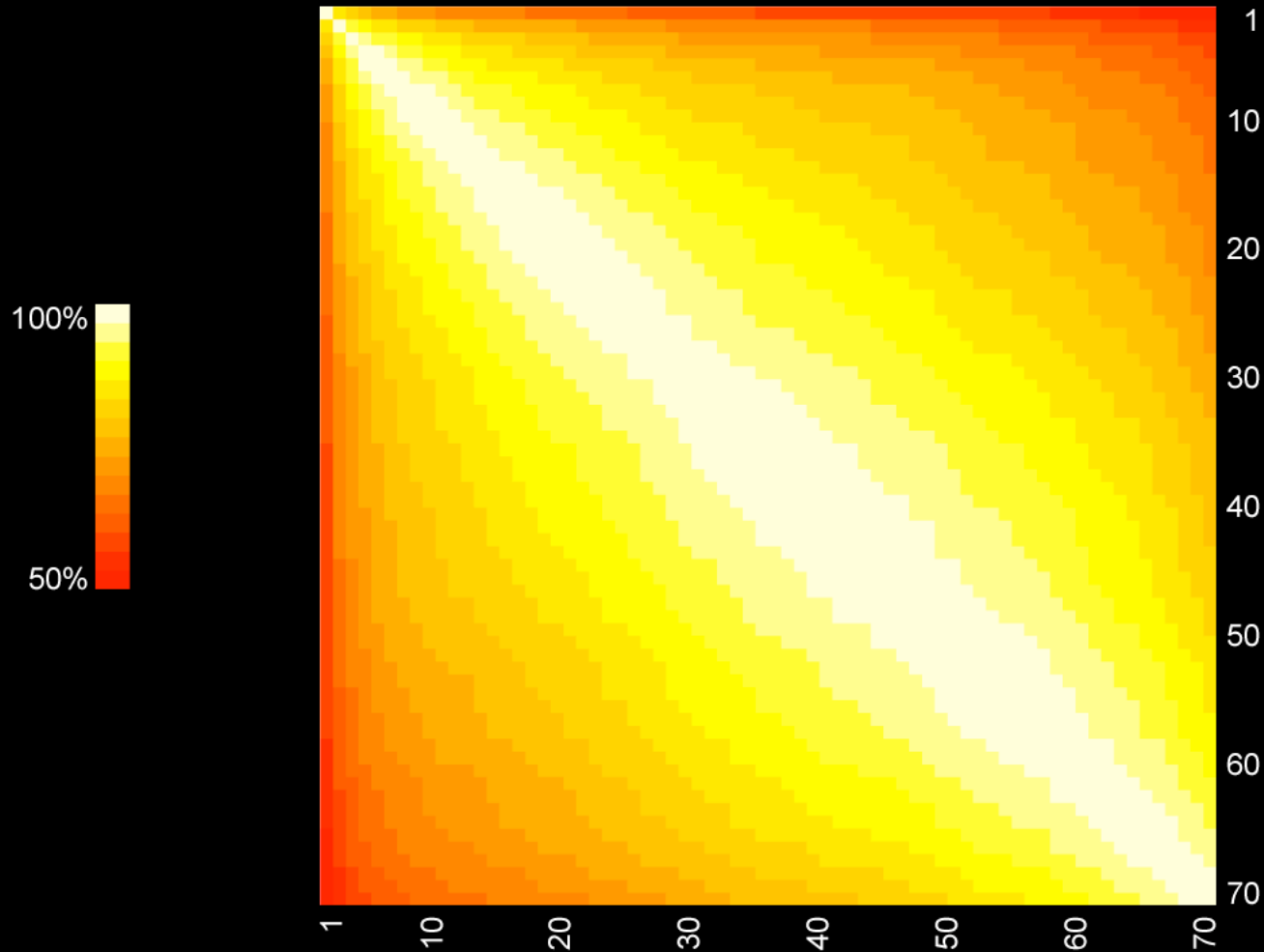
based on FDR, estimated 333 phenotypic capacitors identified

Identification of PCs is robust to changes in the procedure



percent match of top 502 PCs with different numbers of medoids
(top 35 averaged)

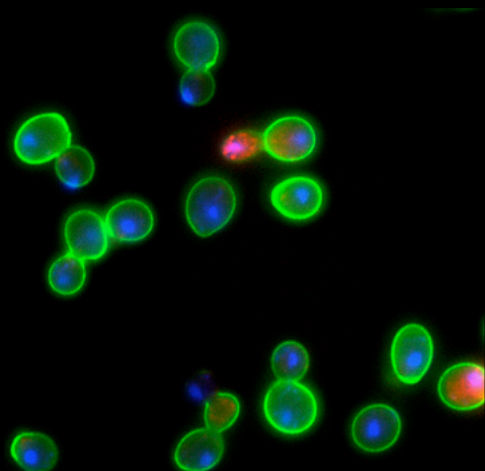
Identification of PCs is robust to changes in the procedure



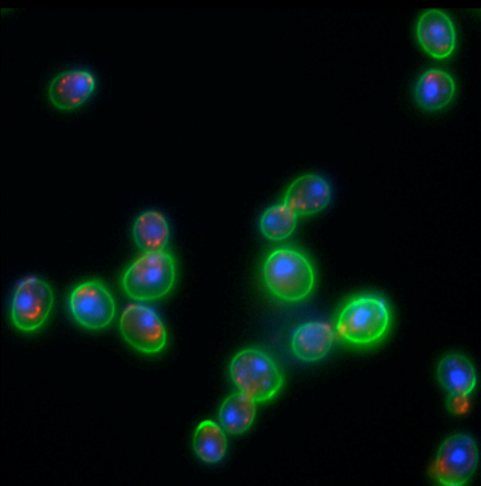
percent match of top 502 PCs with different numbers of medoids averaged
(out of 70 total)

Control (non-PC) yeast knockout strains

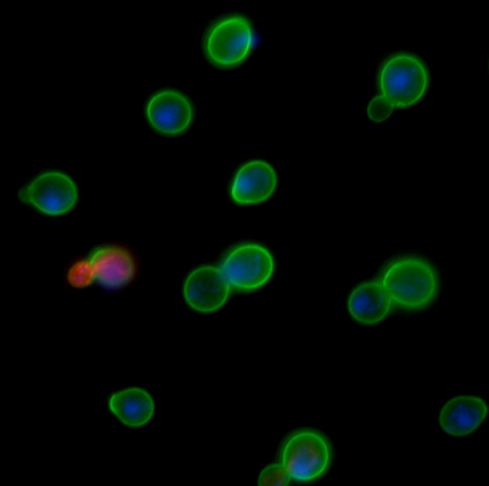
HXT8



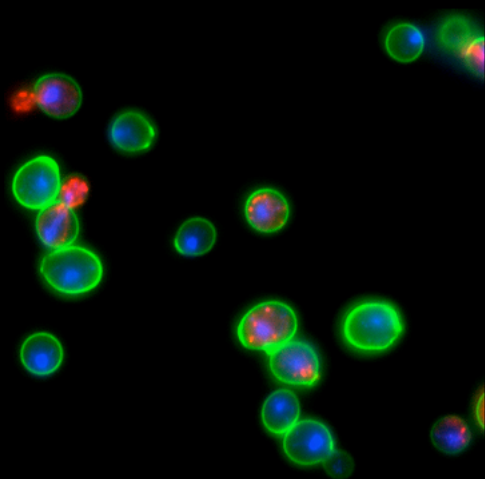
MRP49



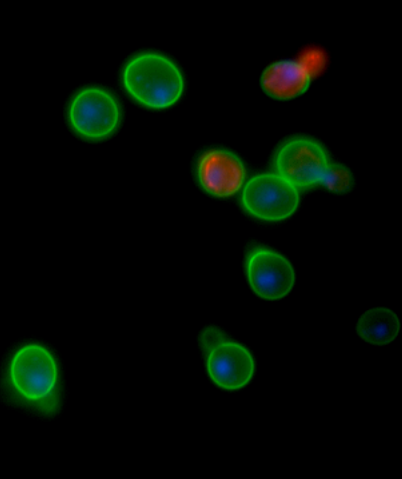
RNH202



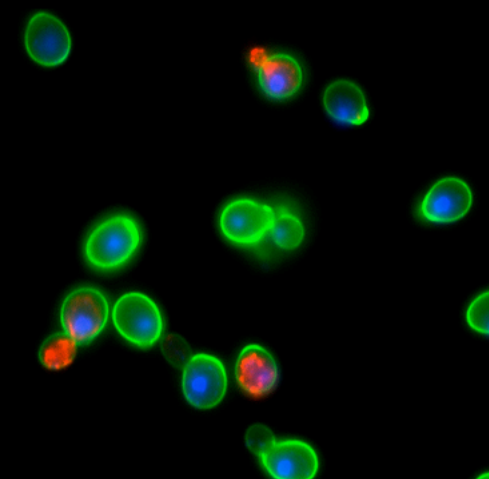
ROM1



YCR100C

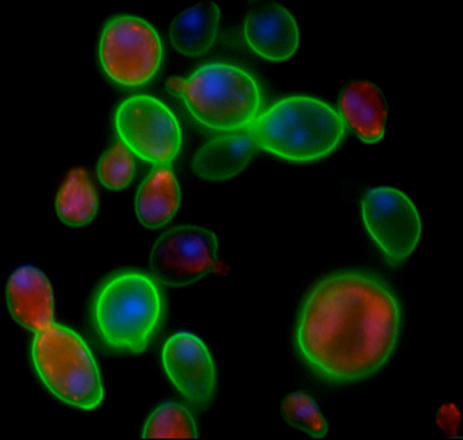


YDR101W

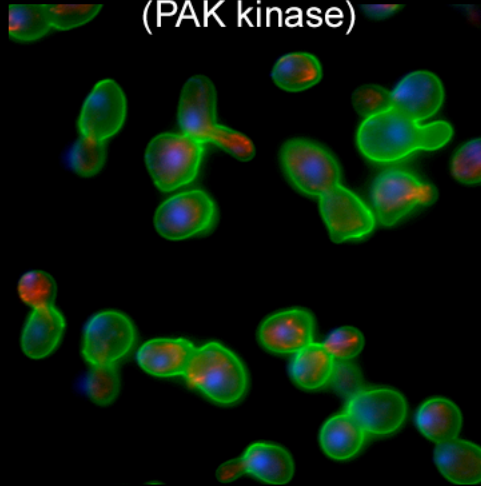


Phenotypic capacitor yeast knockout strains

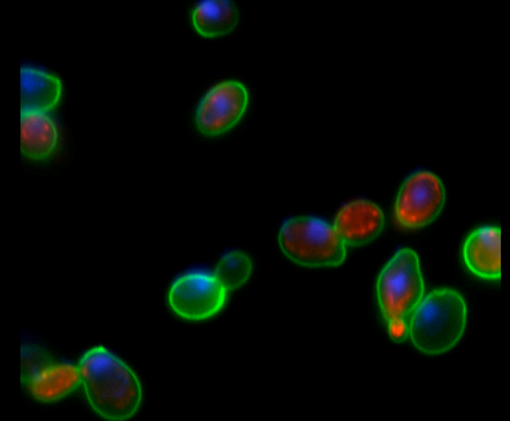
BEM2
(RhoGAP)



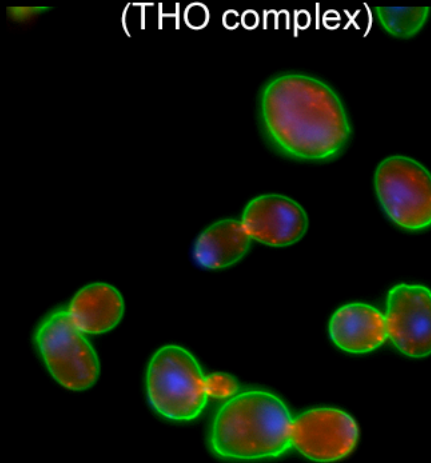
CLA4
(PAK kinase)



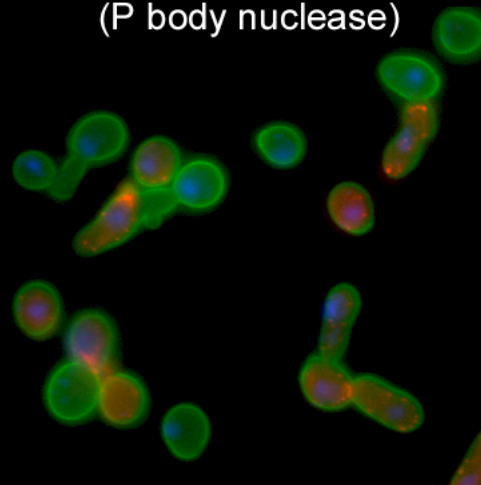
DIA2
(F-box protein)



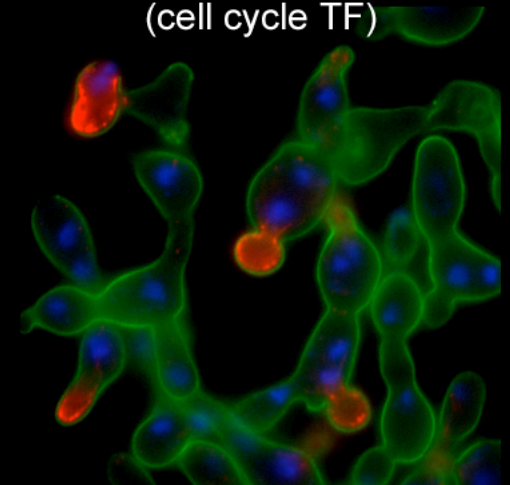
HPR1
(THO complex)



KEM1
(P body nuclease)



SWI6
(cell cycle TF)



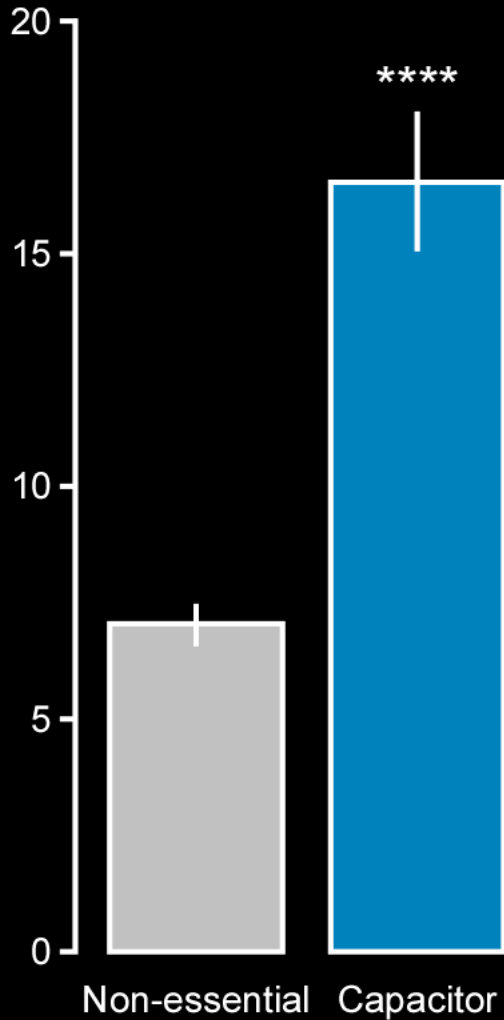
Gene ontology (GO) category enrichment of PCs

GO Process	<i>P</i> value*
chromosome organization and biogenesis	7.18×10^{-27}
└ telomere organization and biogenesis	2.11×10^{-25}
response to stimulus	1.29×10^{-4}
└ response to stress	4.91×10^{-3}
cell cycle	2.65×10^{-4}
RNA elongation	5.74×10^{-3}
post-translational protein modification	1.96×10^{-2}

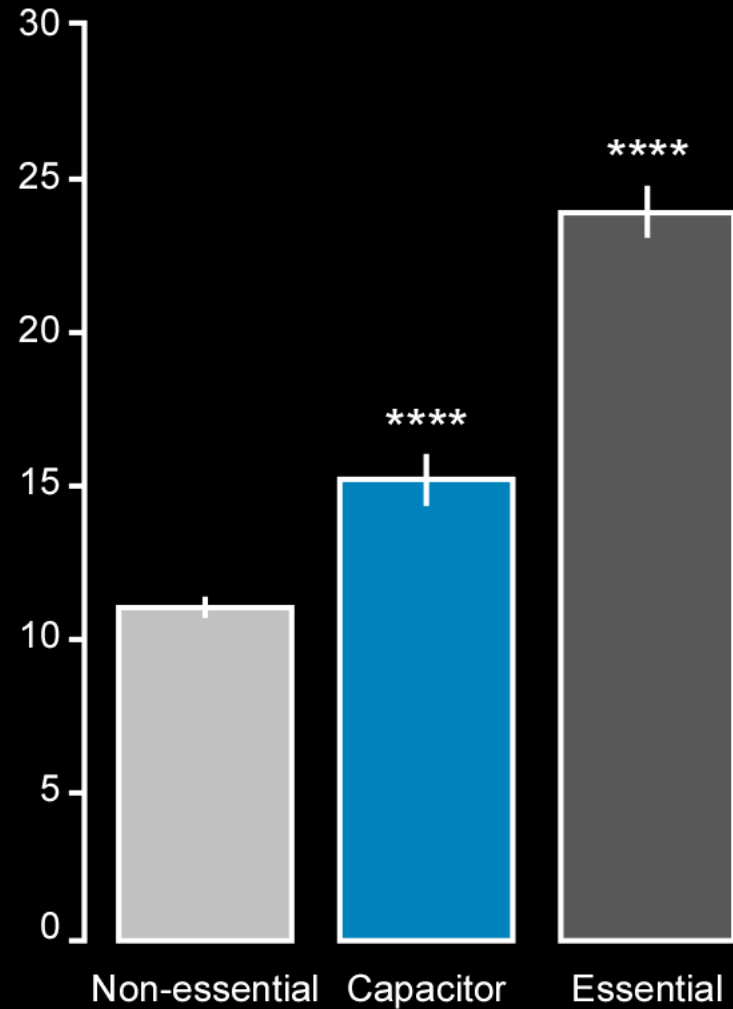
* Bonferroni corrected

PCs are hubs of genetic and physical interaction networks

Synthetic-lethal interactions



Protein-protein interactions



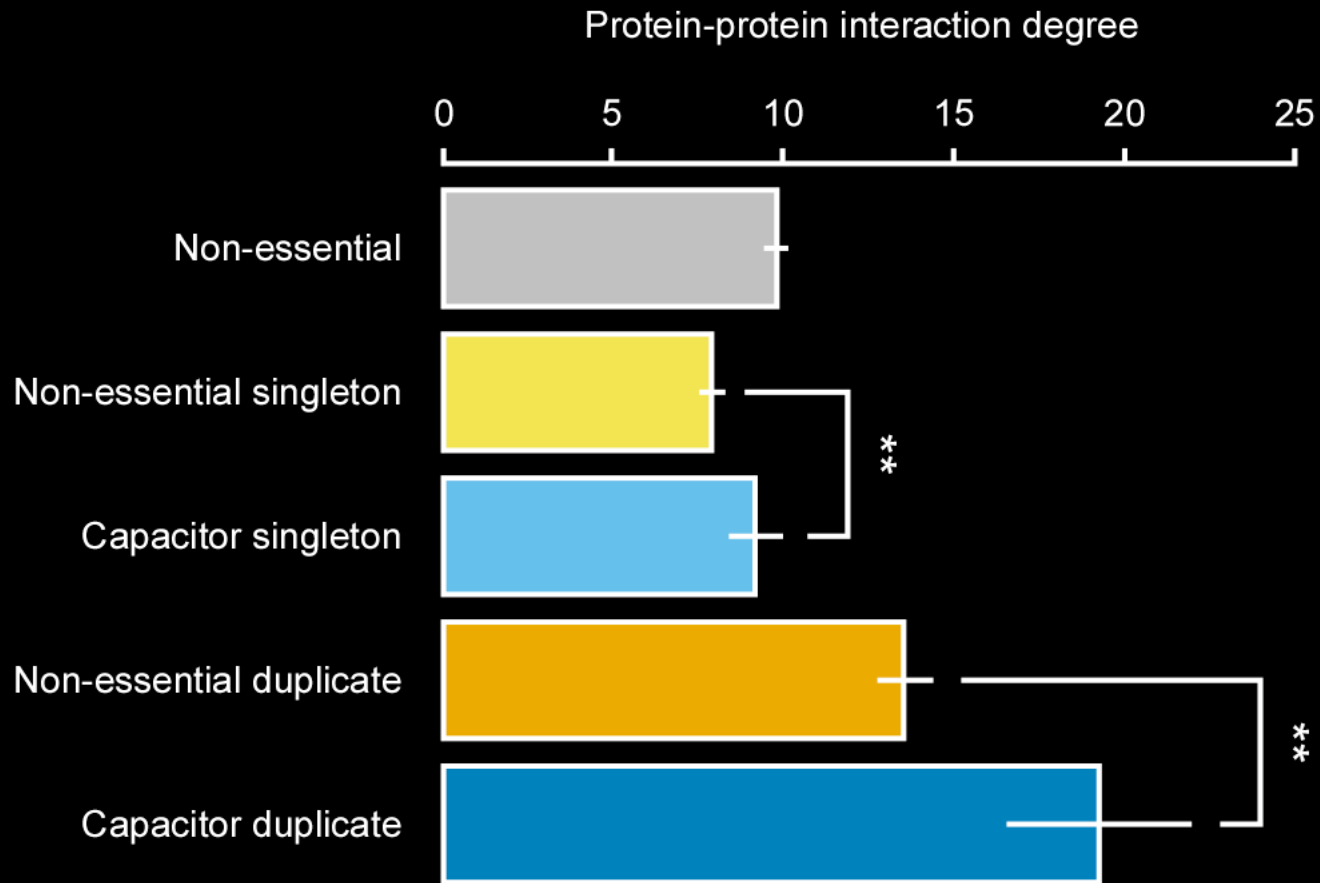
data from: The BioGrid (<http://www.thebiogrid.org/>)

**** $P < 10^{-10}$ (Wilcoxon)

PCs tend to be encoded by singleton genes

	Singleton	Duplicate
Essential	577	148
Capacitor	211	118
Other non-essential	1587	1159

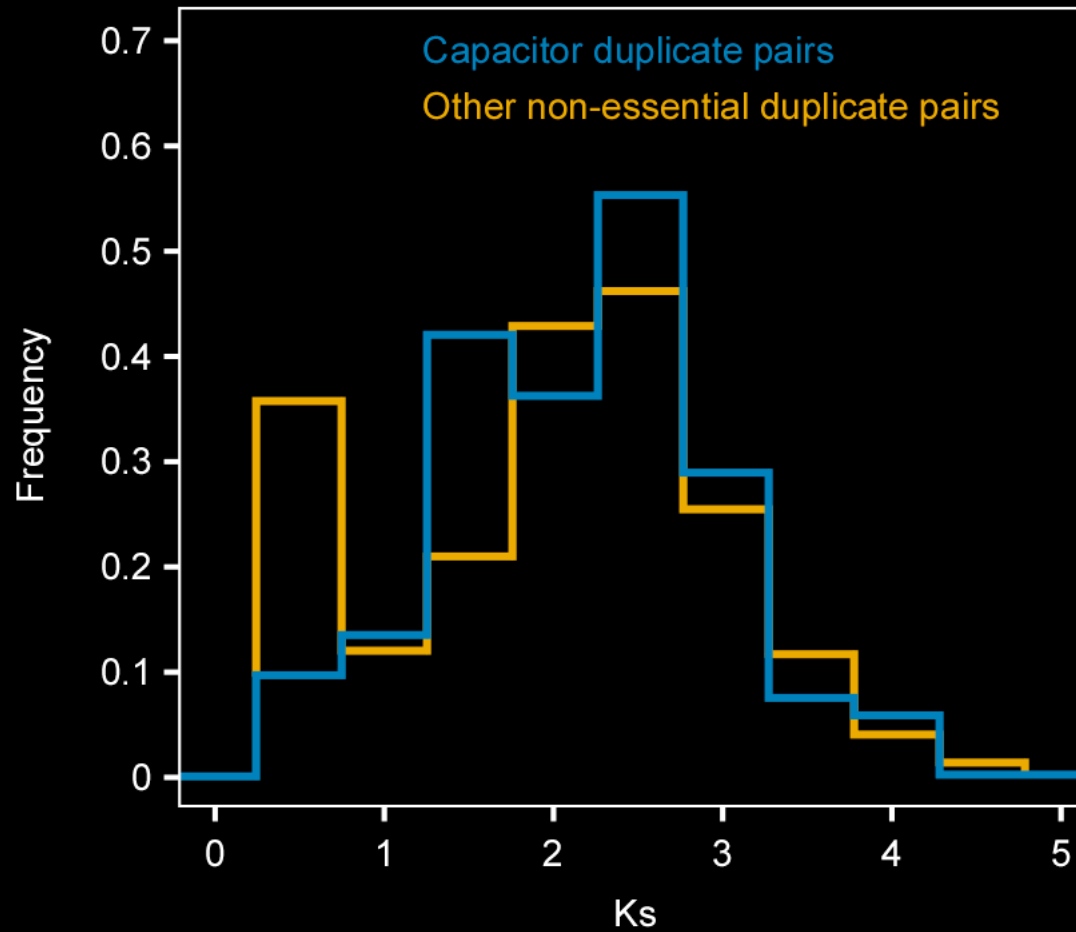
PC duplicates are protein-protein interaction hubs (but PC singletons are not)



data from: The BioGrid (<http://www.thebiogrid.org/>)
Kafri et al., 2008, *PNAS* 105:1243

** $P < 10^{-3}$ (Wilcoxon)

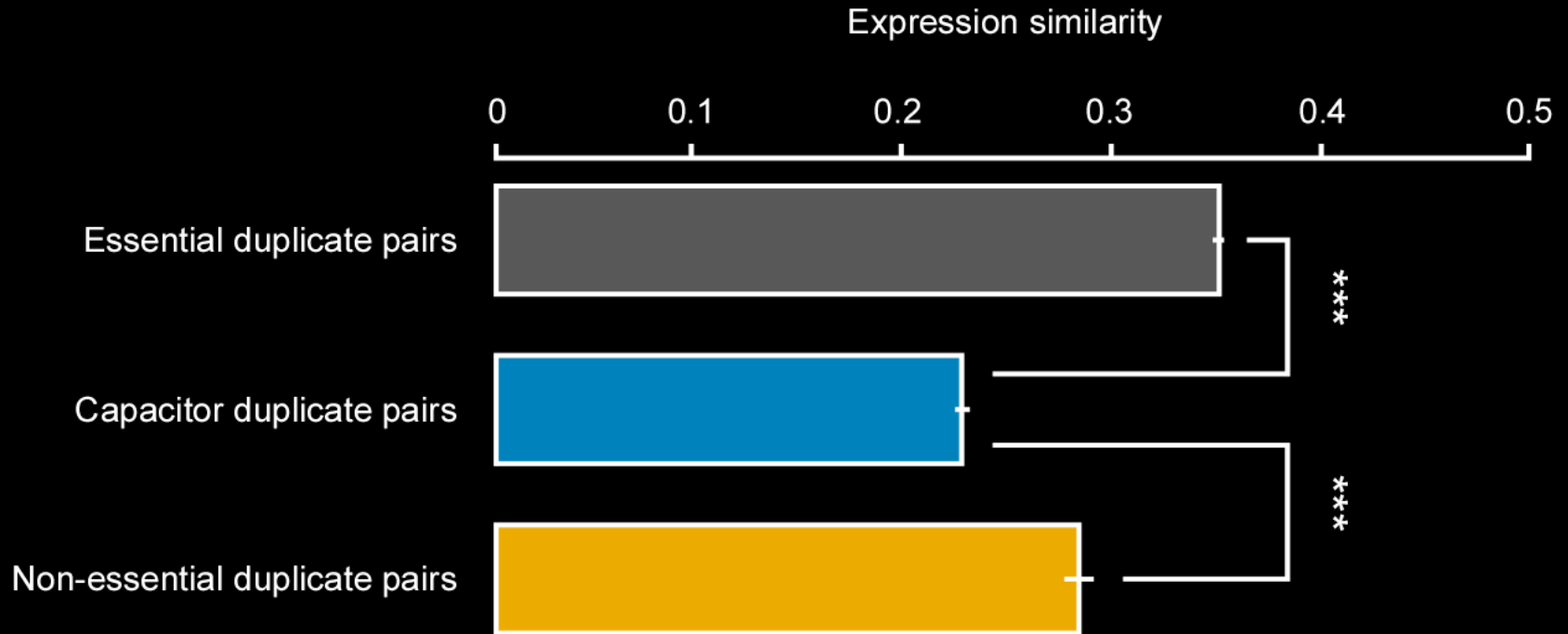
PCs tend to derive from more ancient duplication events



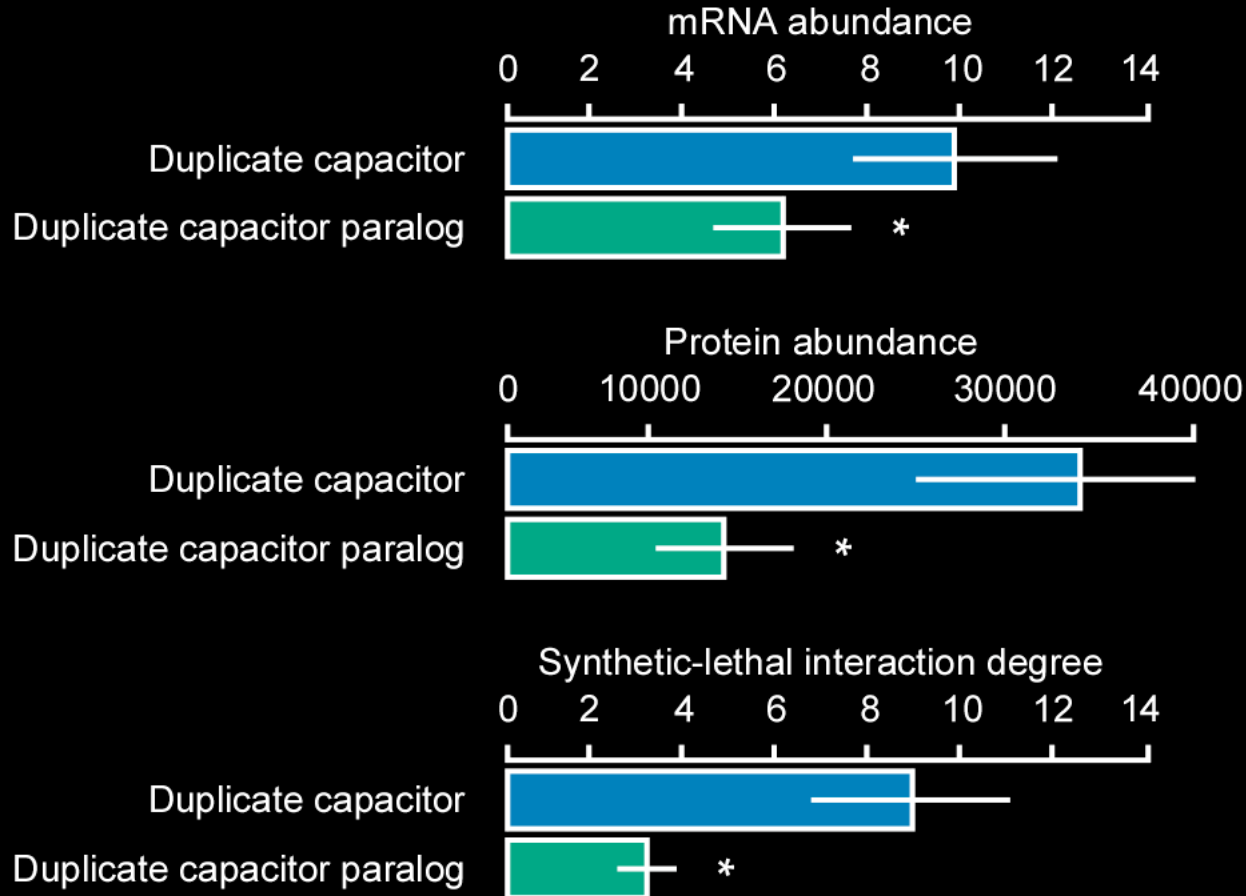
data from: Kafri et al., 2008, *PNAS* 105:1243

$P < 0.01$ (ANCOVA with expression level)

PC expression patterns differ greatly from those of their paralogs



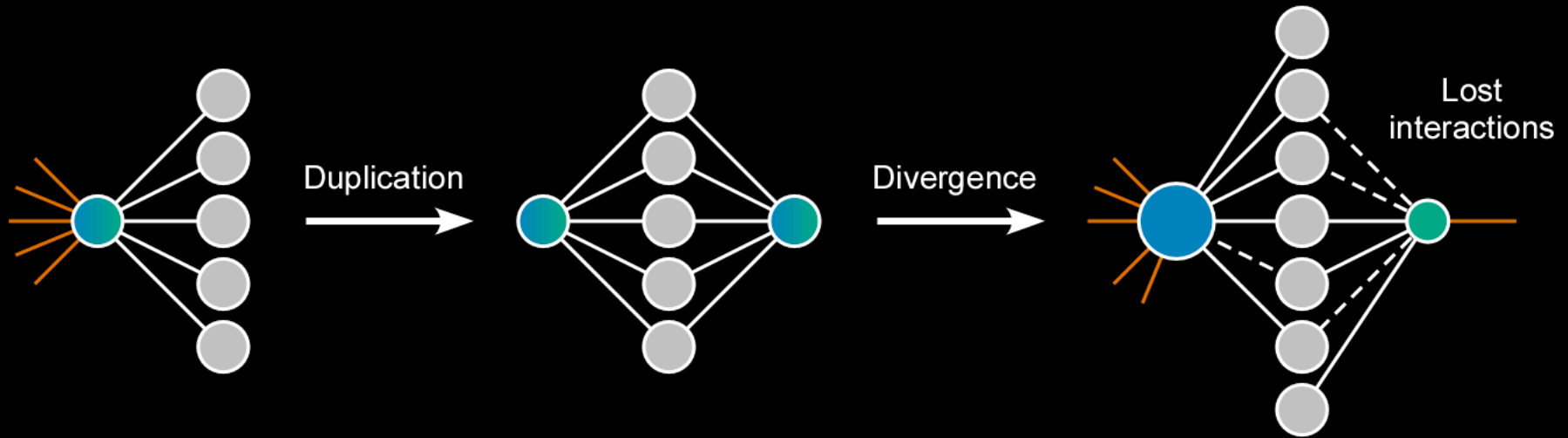
PCs are more highly expressed and have more genetic interactions than their paralogs



data from: Kafri et al., 2008, *PNAS* 105:1243
Drummond et al., 2006, *Mol Biol Evol* 23:327
Holstege et al., 1998, *Cell* 95:717
The BioGrid (<http://www.thebiogrid.org/>)

* $P < 0.05$ (Wilcoxon)

Model of duplicate capacitor formation



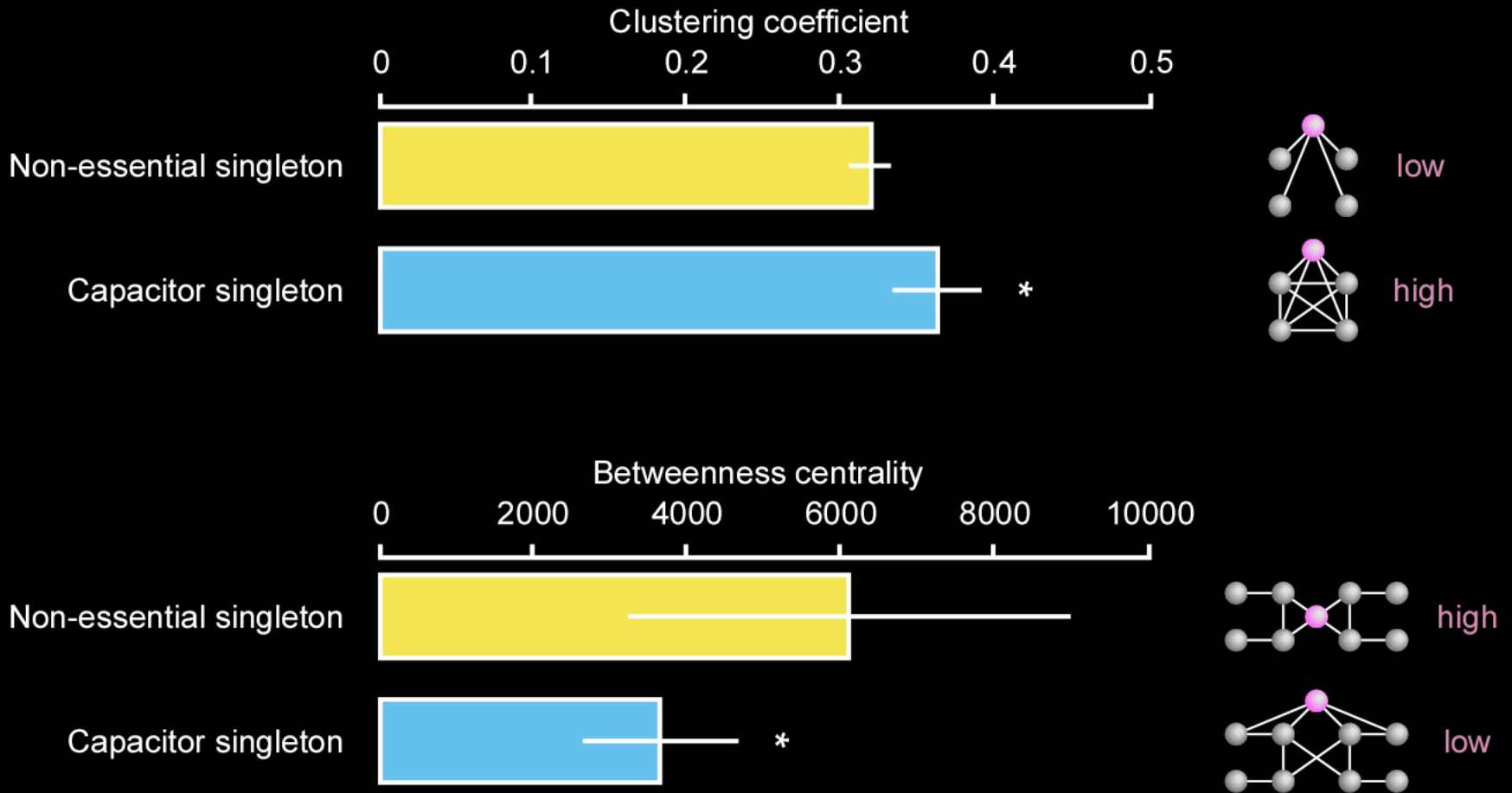
— Synthetic-lethal interaction

— Protein-protein interaction

Capacitor

Capacitor paralog

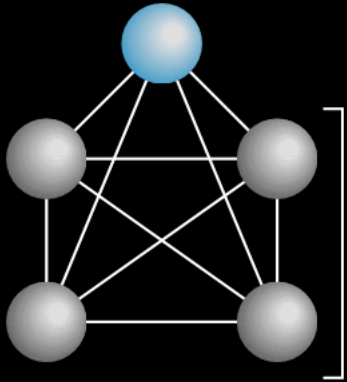
Capacitor singletons tend to be in highly interconnected modules



data from: Kafri et al., 2008, *PNAS* 105:1243
The BioGrid (<http://www.thebiogrid.org/>)

* $P < 0.05$ (Wilcoxon)

Capacitor singletons have important protein-interaction partners



Compared with the immediate neighbors of other non-essential singletons, immediate neighbors of capacitor singletons:

- are more likely to be essential
- tend to have more synthetic-lethal interactions
- tend to cause greater decreases in growth rate when deleted
- tend to have higher phenotypic potential (tend to be capacitors)

Network hubs buffer microenvironmental/stochastic variation

Analysis of variation in cell morphology identified ~333 gene products that buffer environmental fluctuations

These gene products participate in diverse processes and are enriched in processes central to cellular function (chromosome organization, RNA elongation, protein modification, stress response)

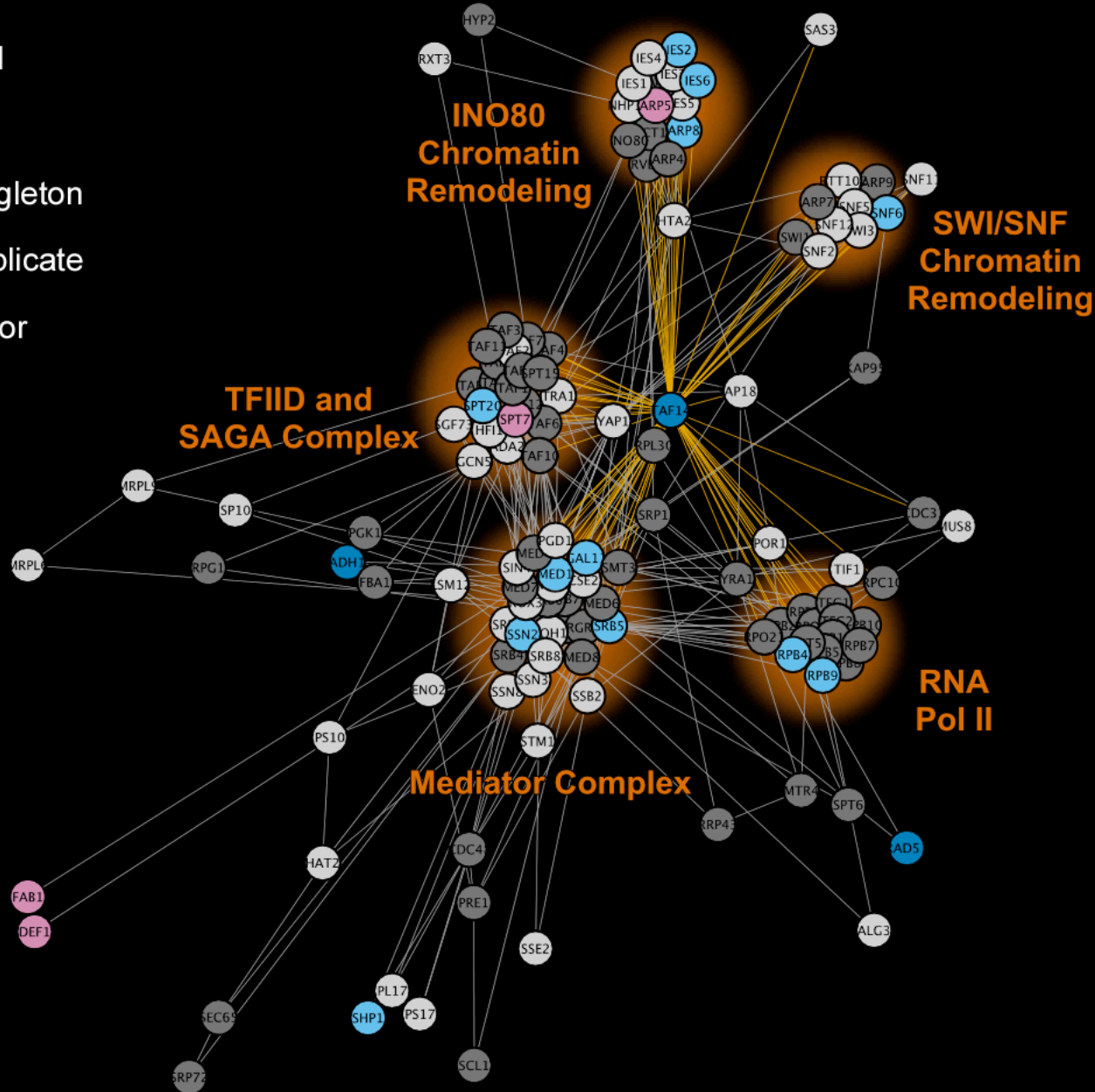
These gene products tend to have many genetic interactions

Those with a paralog tend to have many protein interactions and to have diverged considerably from the paralog

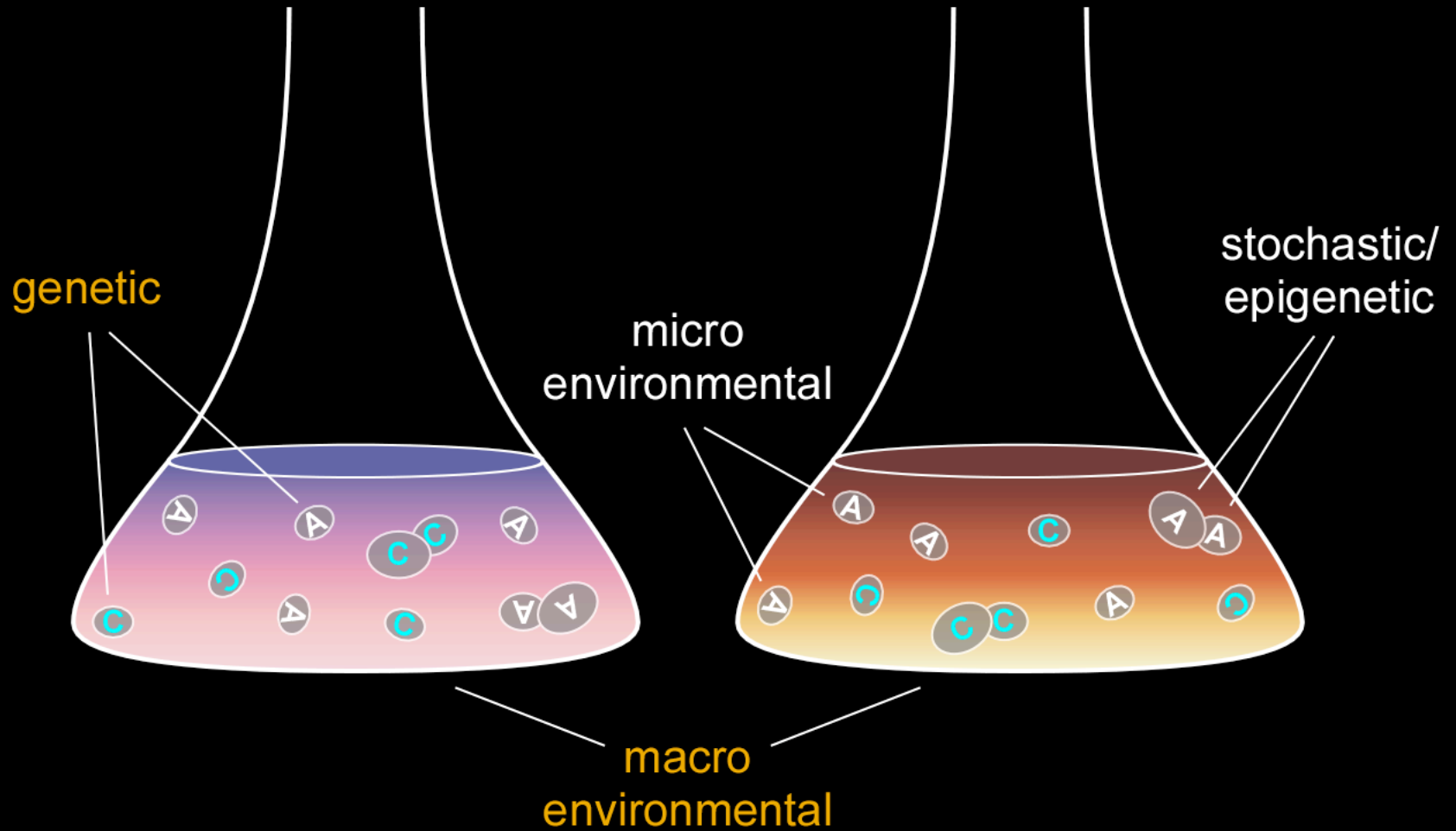
Those without a paralog tend to be part of highly interconnected protein modules, the other members of which tend to be important

Capacitors in the network neighborhood of the Mediator complex

- Non-essential
- Essential
- Capacitor singleton
- Capacitor duplicate
- Other capacitor



Do the same mechanisms buffer different sources of variation?



Are PC knockouts potentially adaptive?

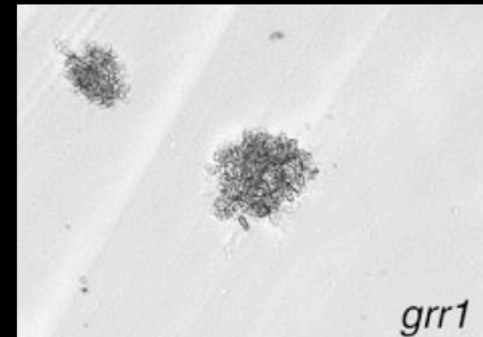
PC knockouts have no effect on growth rate in heterozygous diploids (can be carried in heterozygous condition until starvation/sporulation)

79% of PC knockouts have growth rates > 0.80 of wild-type

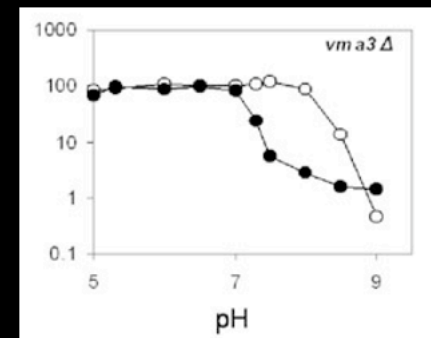
25% of PC knockouts have growth rates $>$ wild-type

12/27 knockouts that promote haploid invasive growth are PCs

4/6 knockouts that give a heterogeneity-dependent fitness advantage in toxic media are PCs

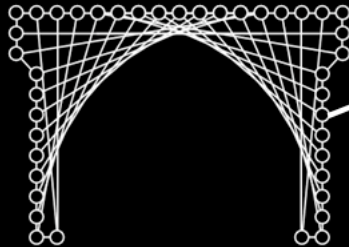
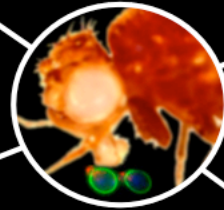


Palecek *et al.*, 2000, *Genetics* 156:1005



Bishop *et al.*, 2007, *Mol Microbiol* 63:507

Sasha
Levy



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