

Single-cell experiments that invite statistical physics in genetics

Gael.Yvert@ens-lyon.fr

CNRS

Ecole Normale Supérieure de Lyon, France

XXth Century: The Molecular Biology Revolution

Heredity/Evolution

Biochemistry

Physiology

XXth Century: The Molecular Biology Revolution

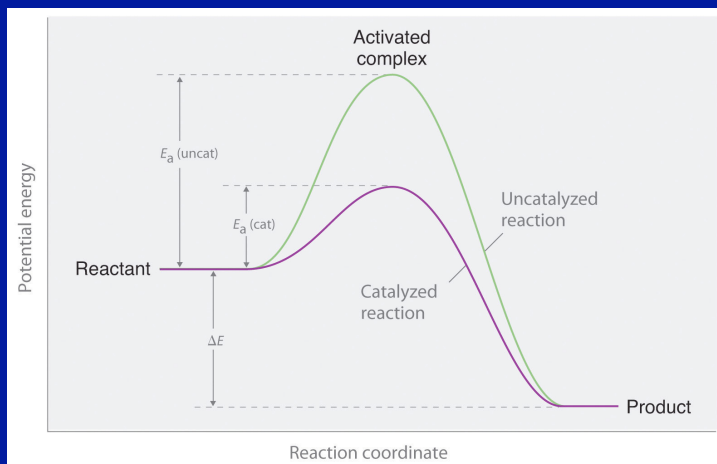
Heredity/Evolution

Enzymes

Biochemistry

Catalysis

Physiology



XXth Century: The Molecular Biology Revolution

DNA

Heredity/Evolution

Genetic code



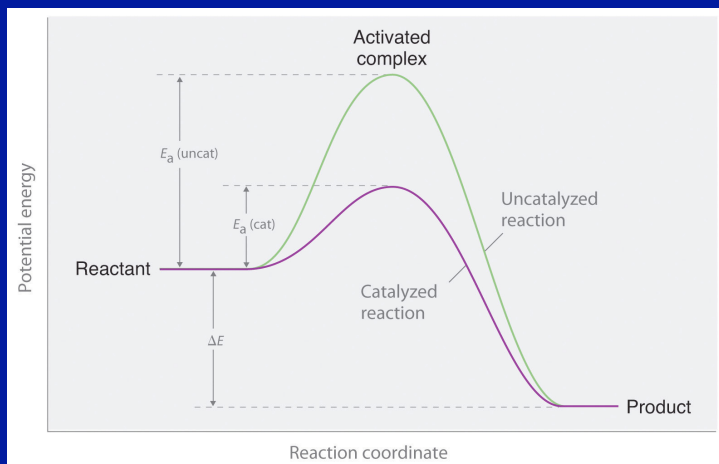
Enzymes

Biochemistry

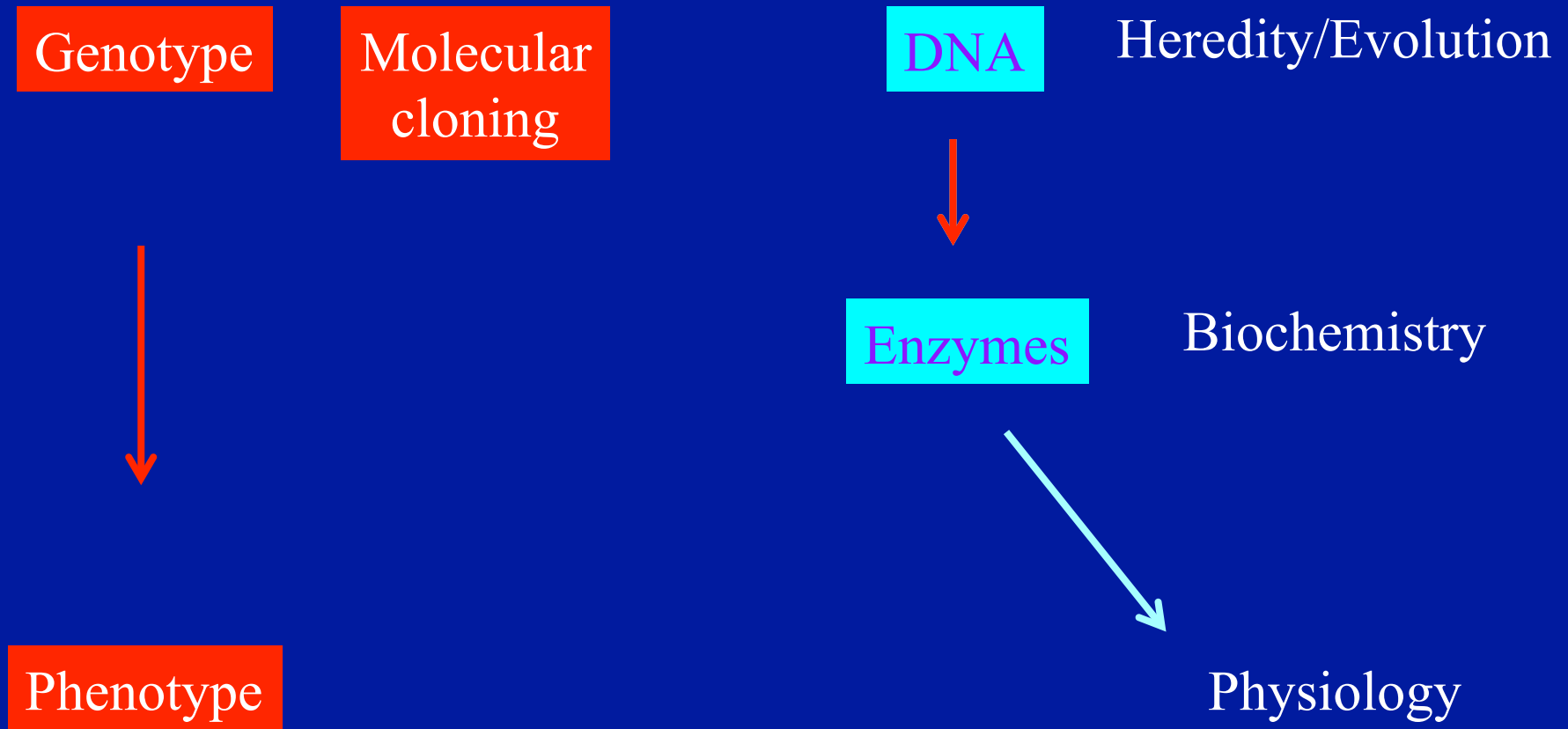
Catalysis



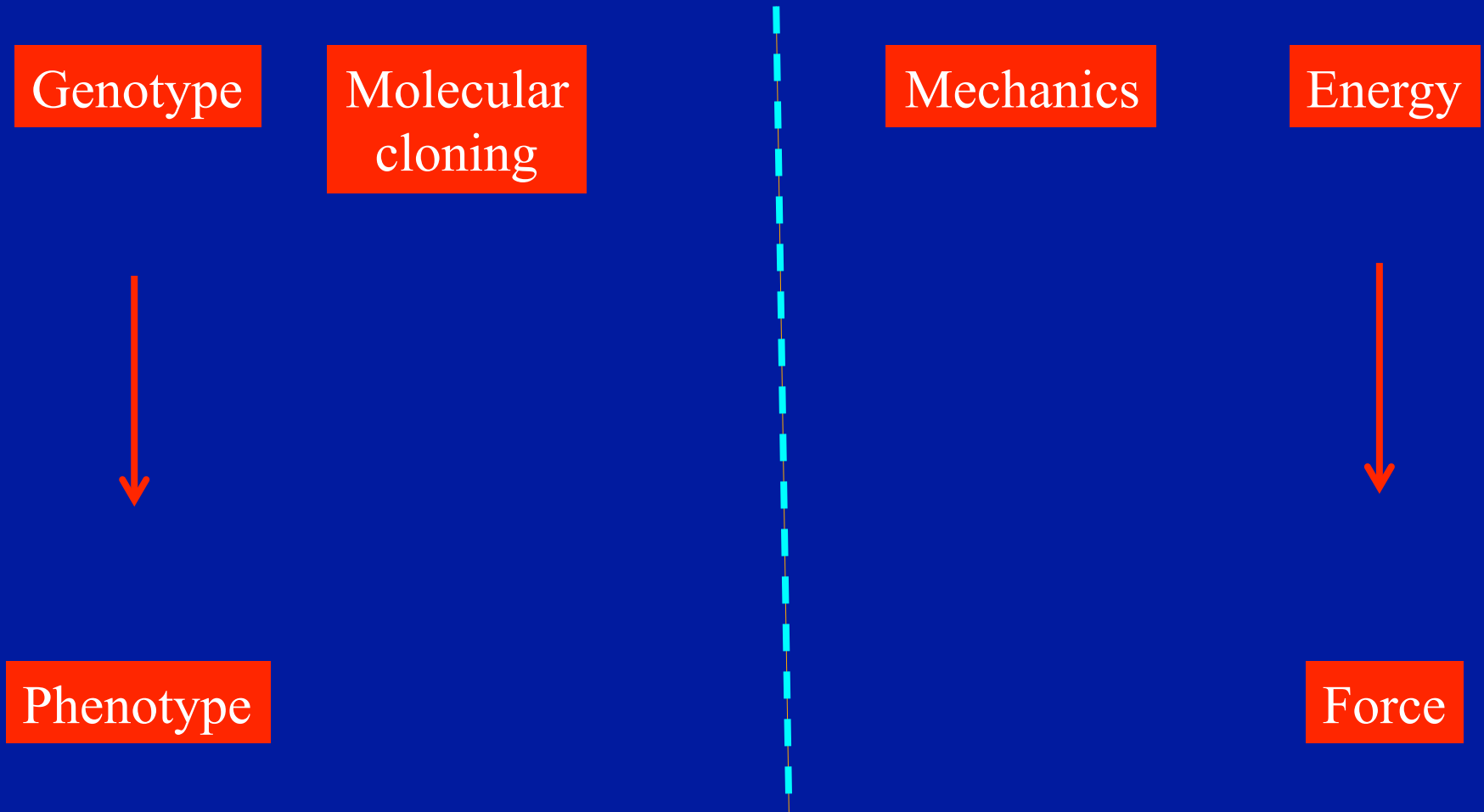
Physiology



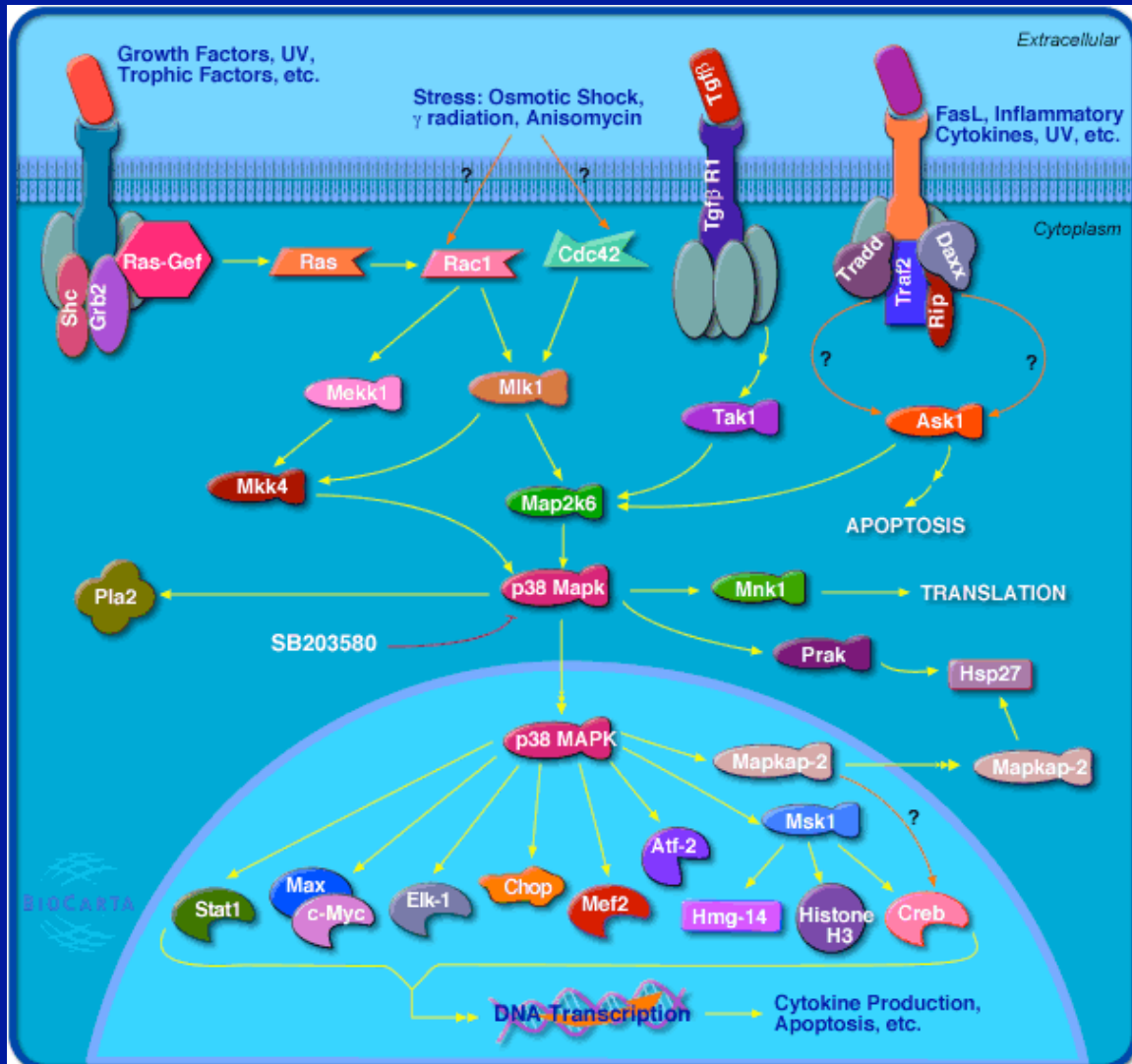
XXth Century: The Molecular Biology Revolution



XXth Century: The Molecular Biology Revolution



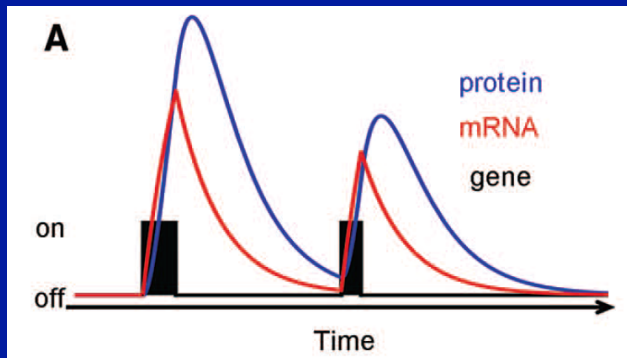
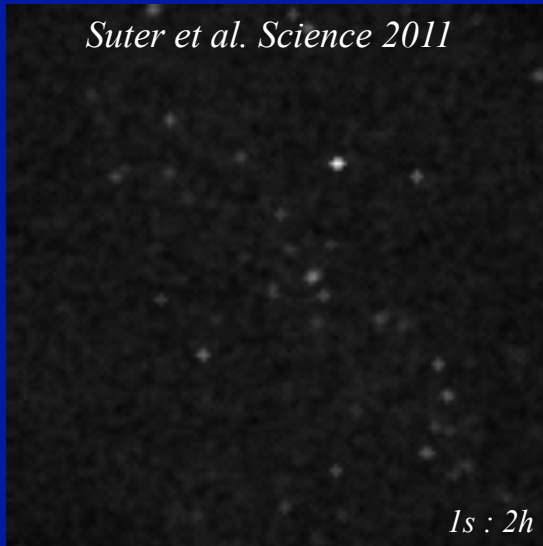
Molecular Biology



Powerful
but 'Newtonian'

Limits of current genetics

1. Gene expression is discrete and dynamic



DNA

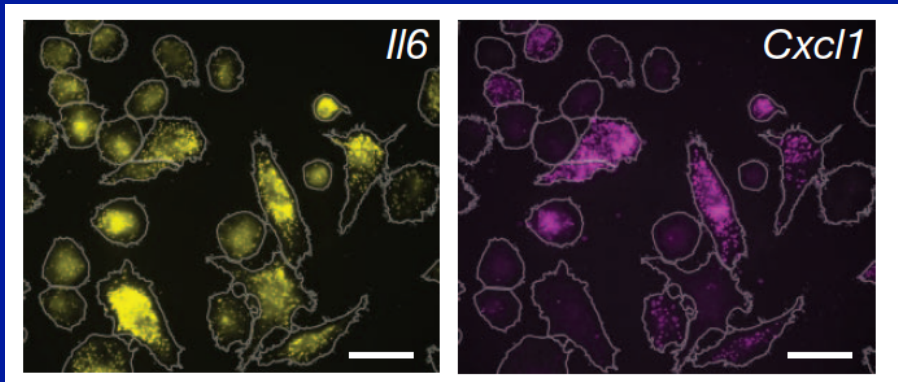


Enzymes

When exactly ?

Limits of current genetics

2. Gene expression varies across cells



Shalek et al. Nature 2013

DNA



Enzymes

When exactly ?
In which cells ?

Limits of current genetics

3. Gene expression makes ‘errors’

Transcriptional misincorporation: $4 \cdot 10^{-6}$
Gout et al. PNAS 2013

Splicing errors: $10^{-6} - 10^{-2}$
Fox-Walsh et al. PNAS 2009

Translation misincorporation: 10^{-4}
Drummond & Wilke Nat. Rev. Gen. 2009

Frameshift: 10^{-5}
Curran & Yarus PNAS 1986

Premature termination: $10^{-4} - 10^{-3}$
Drummond & Wilke Nat. Rev. Gen. 2009

Post-translation modification: UNKNOWN

Misfolding: UNKNOWN

DNA



Enzymes

When exactly ?
In which cells ?
What enzyme exactly?

Limits of current genetics

4. So many cells !

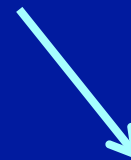


DNA



Enzymes

When exactly ?
In which cells ?
What enzyme exactly?



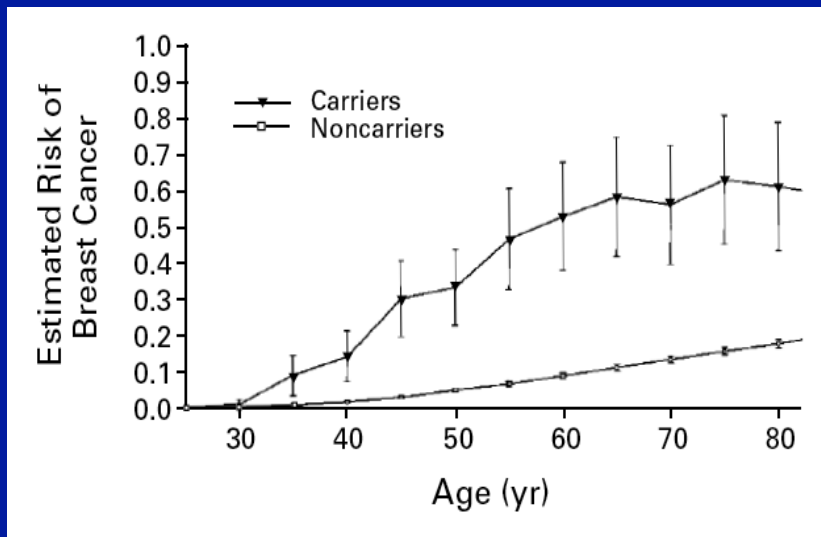
Via which cells ?

Physiology

Limits of current genetics

5. Incomplete penetrance

BRCA mutations



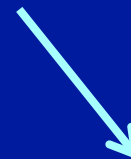
Struwing et al. New Engl. J. Med 1997

DNA



Enzymes

When exactly ?
In which cells ?
What enzyme exactly?

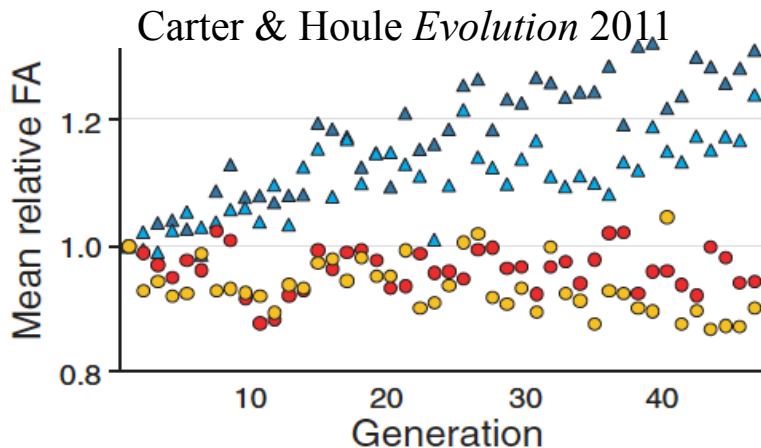


Via which cells ?
Not always !

Physiology

Limits of current genetics

6. Fluctuating asymmetry



DNA



Enzymes

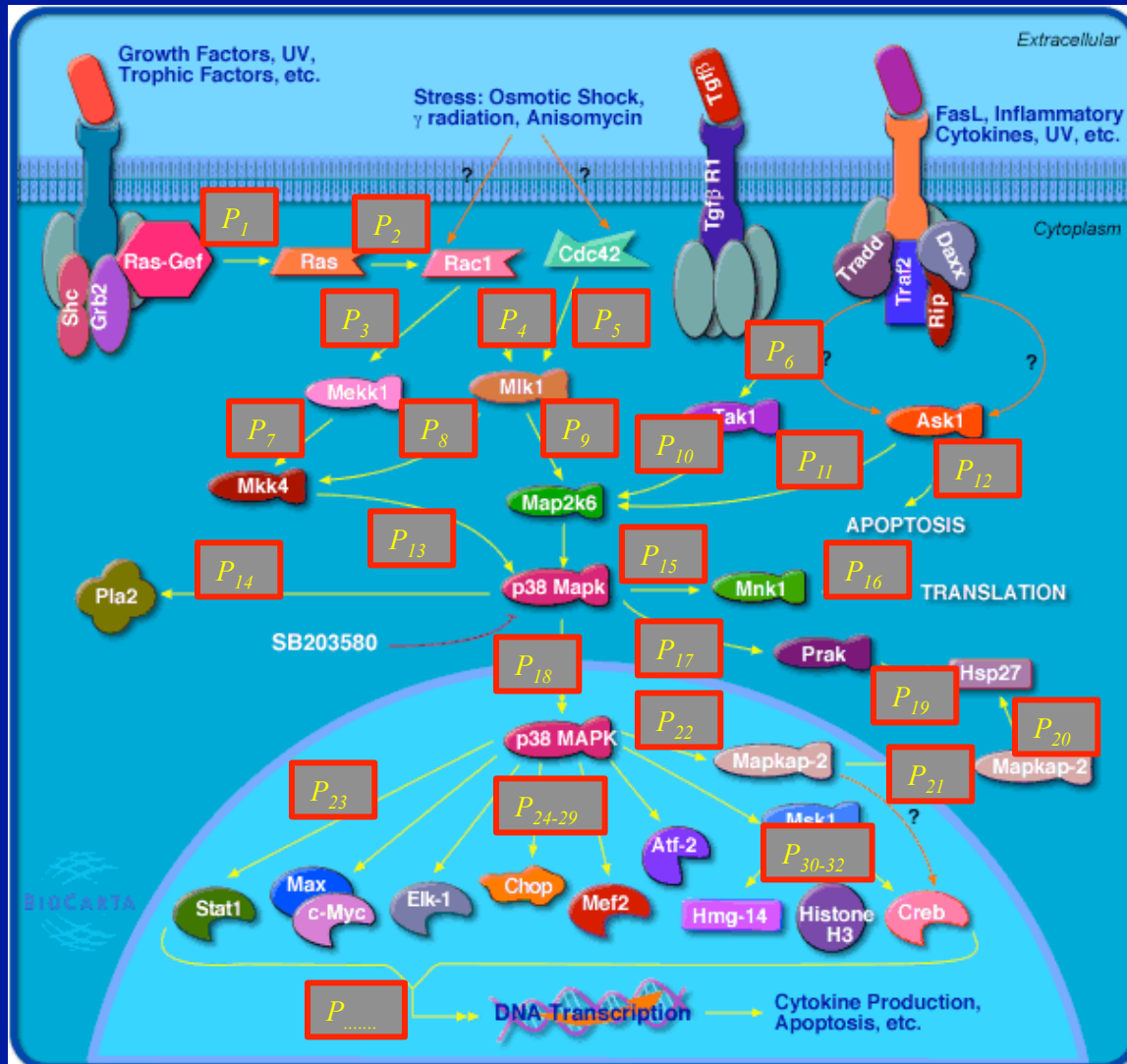
When exactly ?
In which cells ?
What enzyme exactly?



Via which cells ?
Not always !

Physiology

All probabilistic ?



So many cells:



- A challenge: forget exhaustive description



- An opportunity: snapshots of probability laws

Classical
Genetics

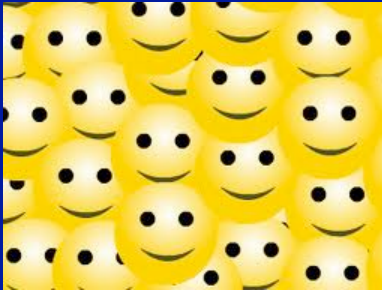


~~Statistical
Genetics~~

‘Particle’
Genetics

‘Particle Genetics’

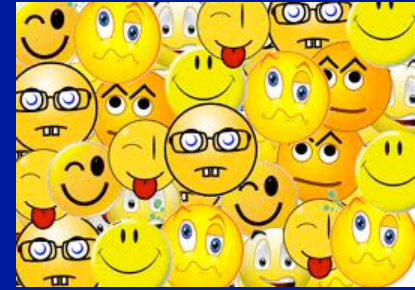
Genotype A



Genotype B

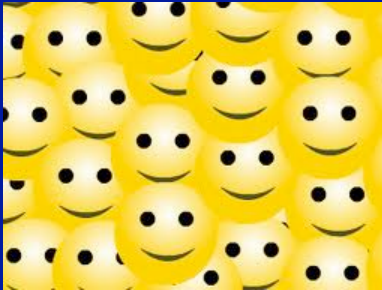


Genotype C



‘Particle Genetics’

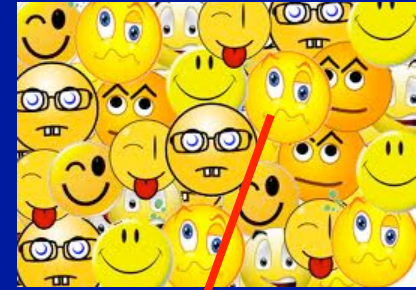
Genotype A



Genotype B



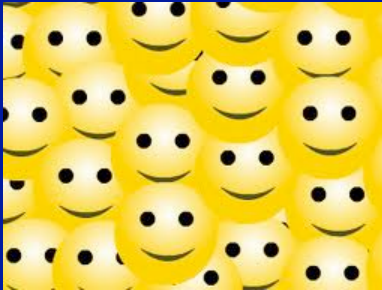
Genotype C



Disease...

‘Particle Genetics’

Genotype A

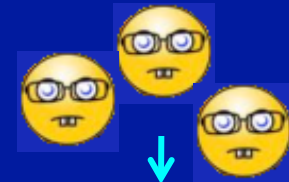
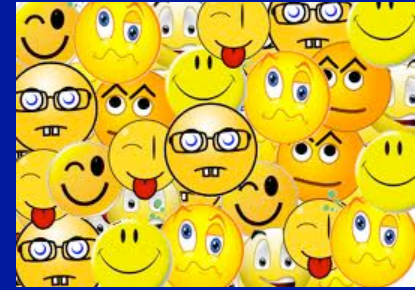


Genotype B



Environmental crisis

Genotype C



« Bet-Hedging »

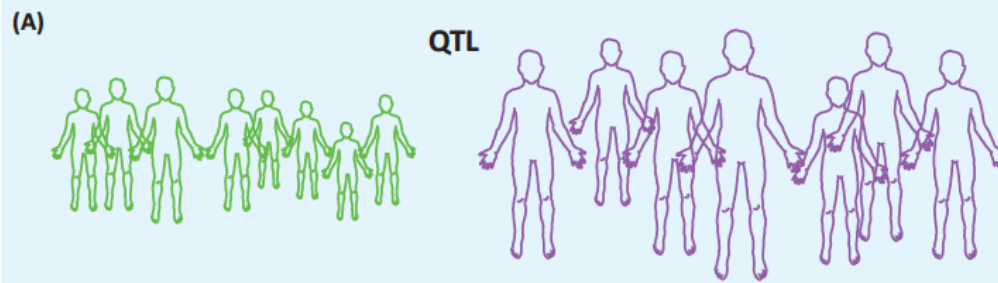
Viral latency (Finzi 1999, Weinberger 2008)

Antibiotic resistance (Balaban 2004)

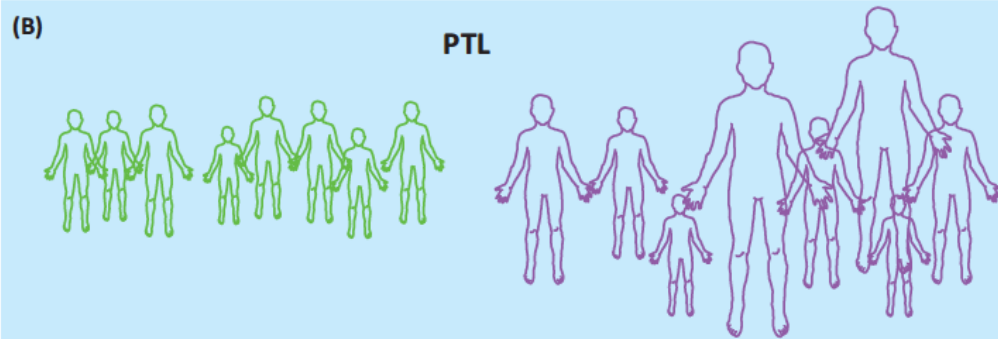


From classical to particle genetics

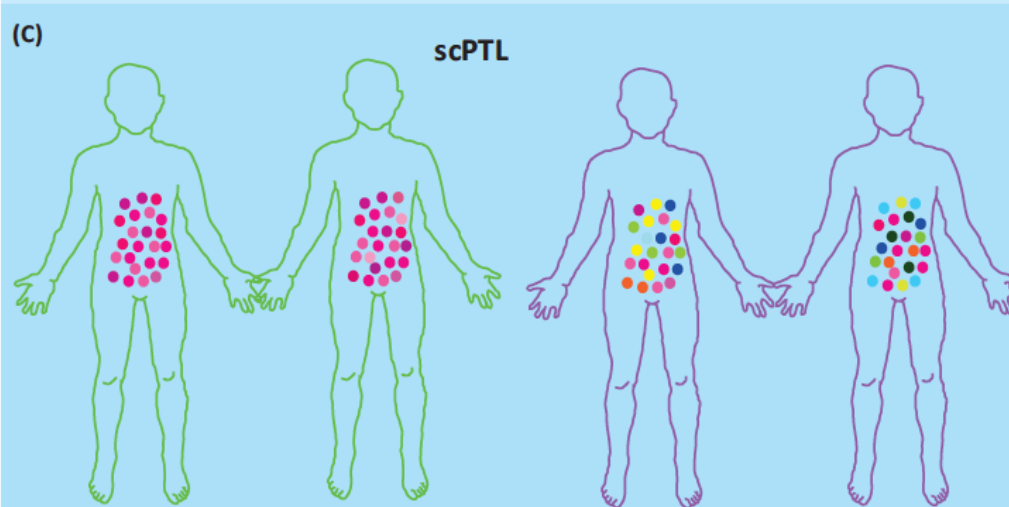
Quantitative
Trait Locus



Probabilistic
Trait Locus



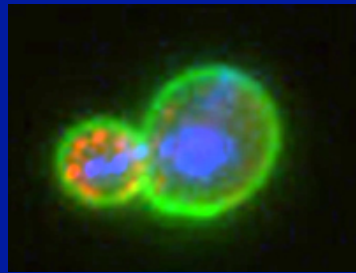
Single-cell
Probabilistic
Trait Locus



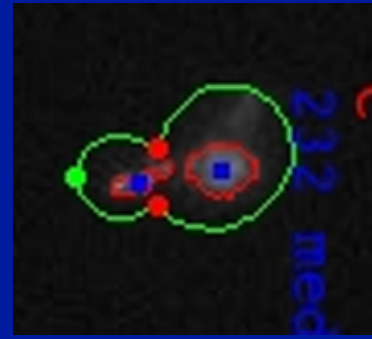
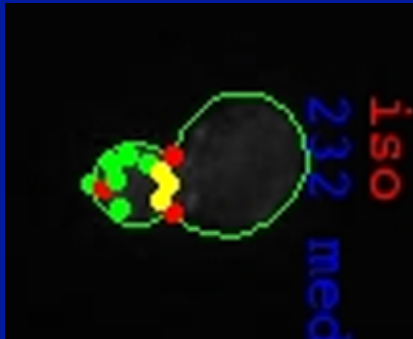
Single-cell experiments that invite statistical physics in genetics

- Phenotypic cell-cell variation differs among natural populations
- scPTL of molecular traits

Single-cell traits

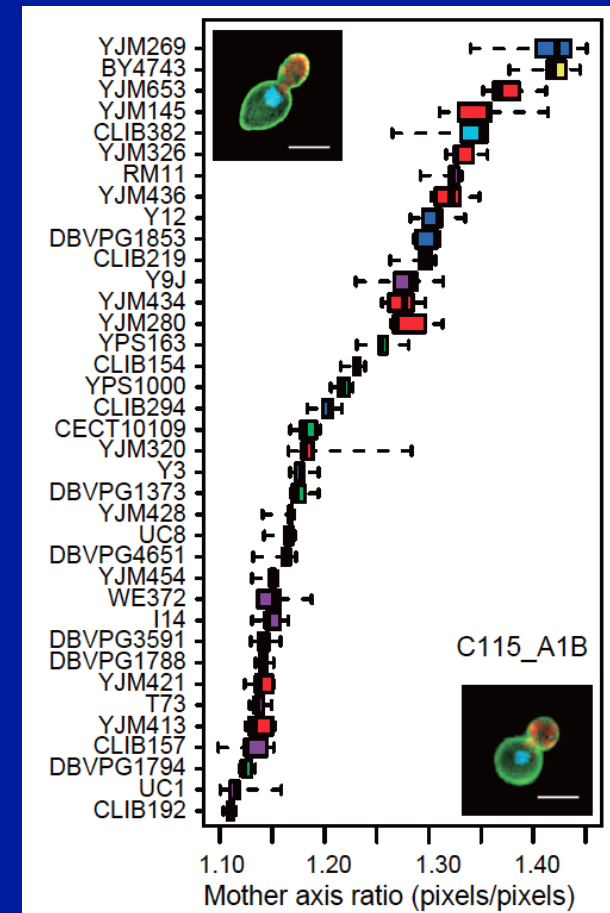
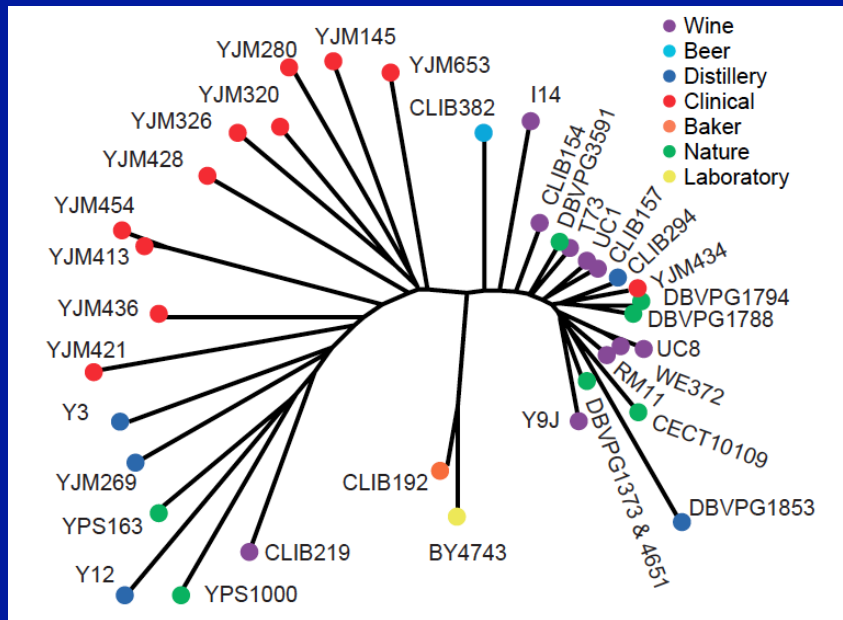


Collaboration with
Y. Ohya, U. of Tokyo



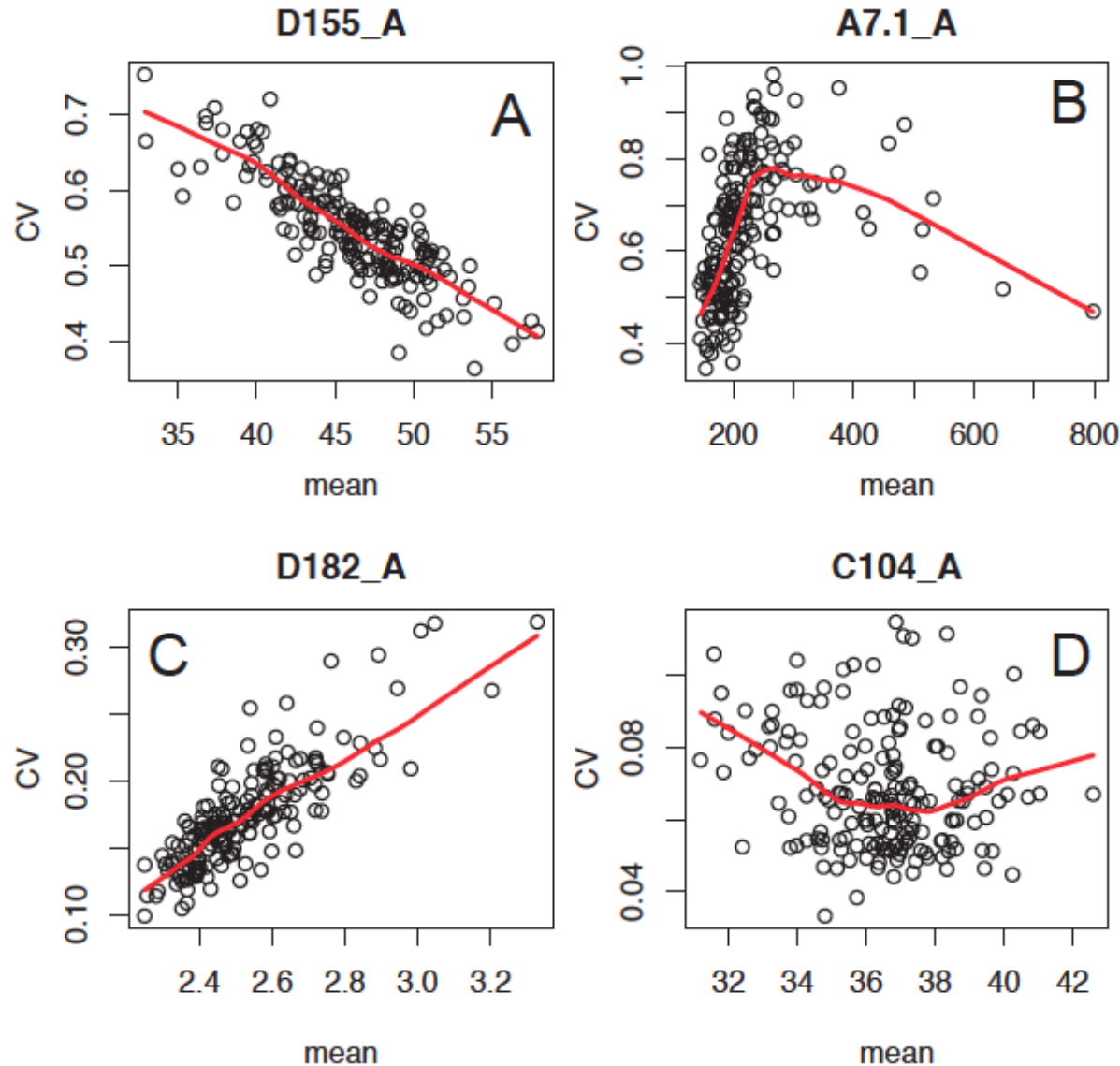
220 parameters = 'Traits'

Morphological diversity of *S. cerevisiae* cells



Each strain:
5 indep cultures
200 cells profiled per culture

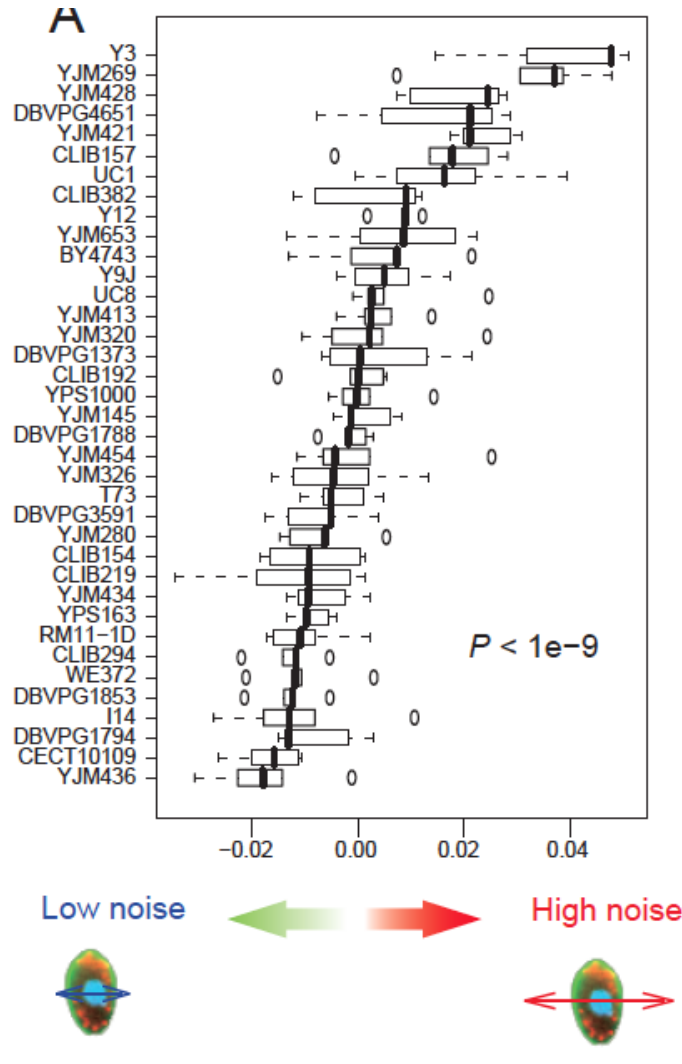
Variability vs. mean dependence



Residuals from
lowess regression
-> decoupling

Natural diversity of trait variability

Cell-to-cell variability of short axis length



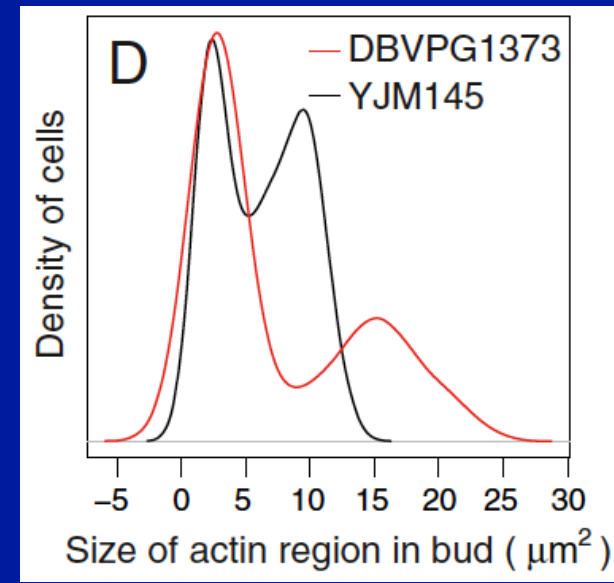
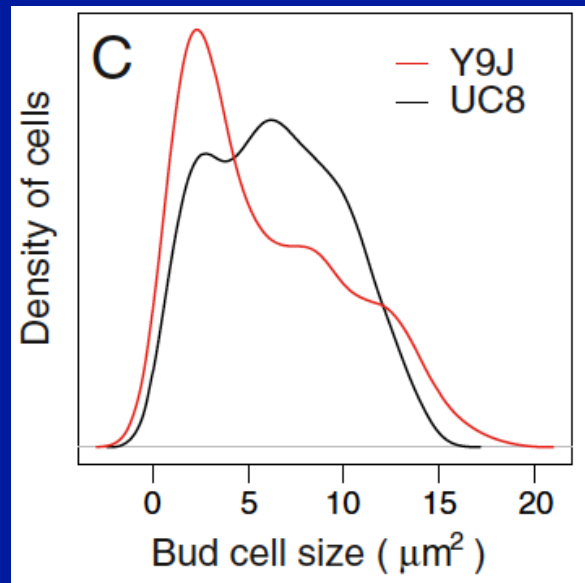
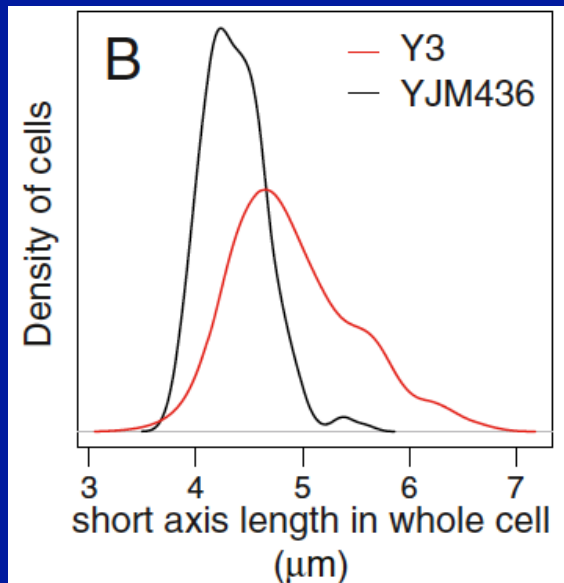
5 indep estimates of variability
per strain



Strain effect ?

Significant for
76 of 220 traits !

Natural diversity of trait variability



→ The genotype changes the probability to observe a given trait value in a given cell

Single-cell experiments that invite statistical physics in genetics

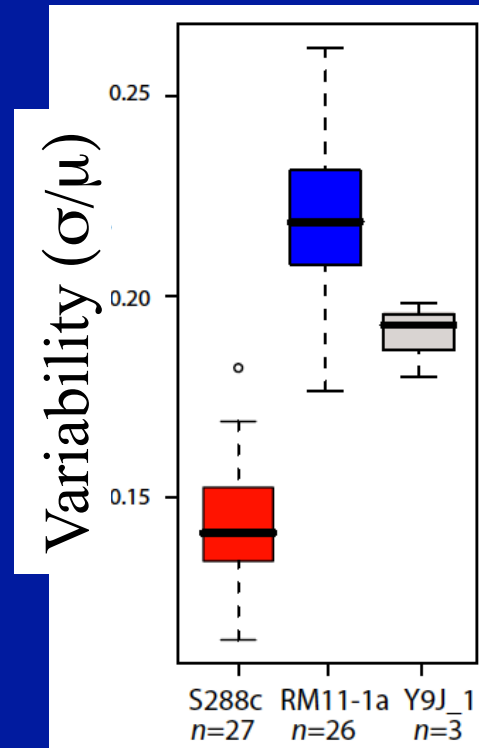
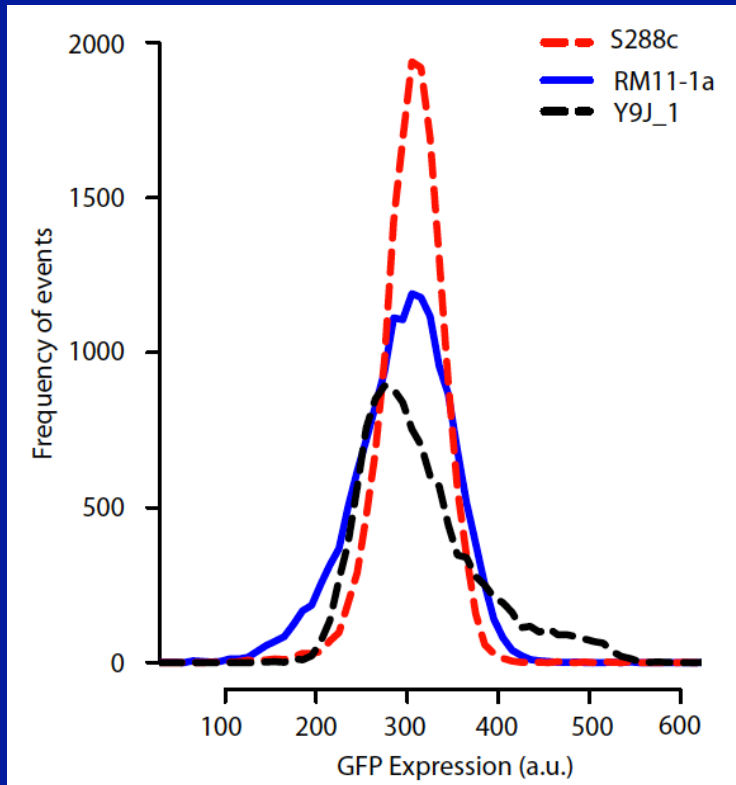
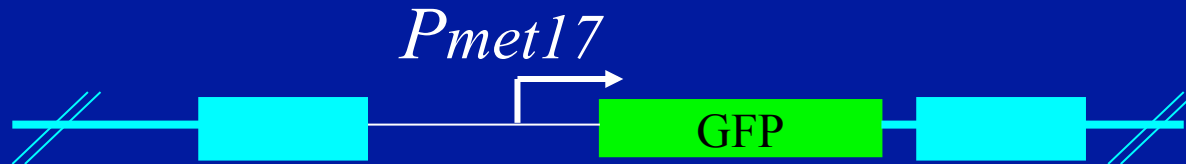
- Phenotypic cell-cell variation differs among natural populations
- scPTL of molecular traits

DNA

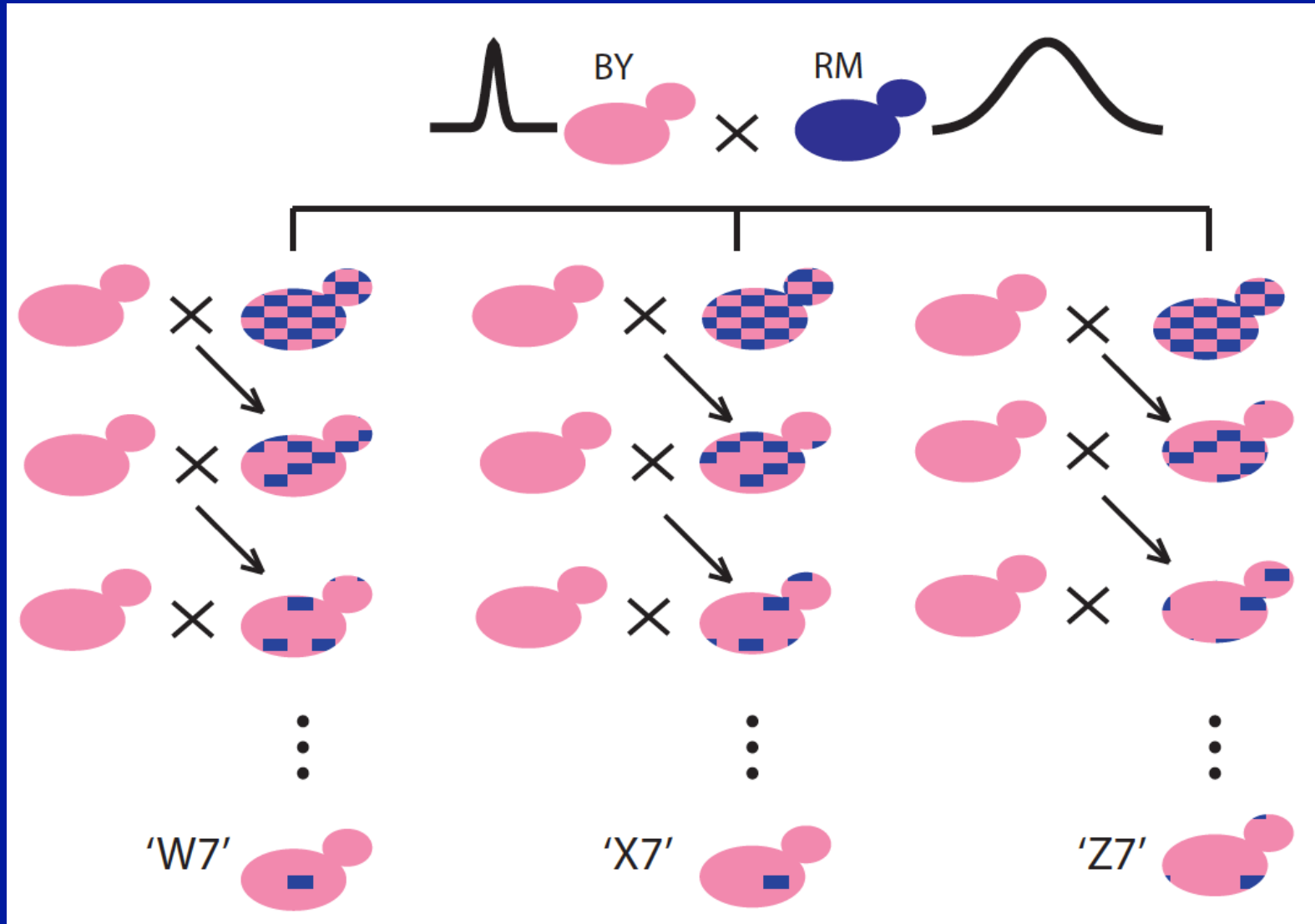


Enzymes

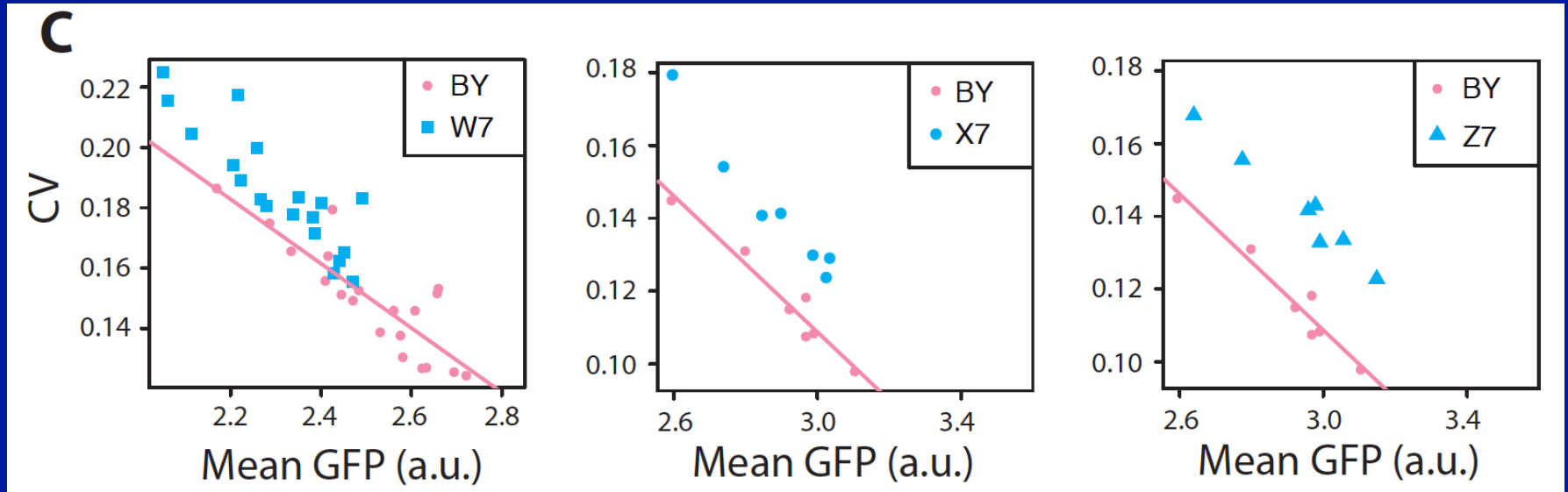
Flow cytometry



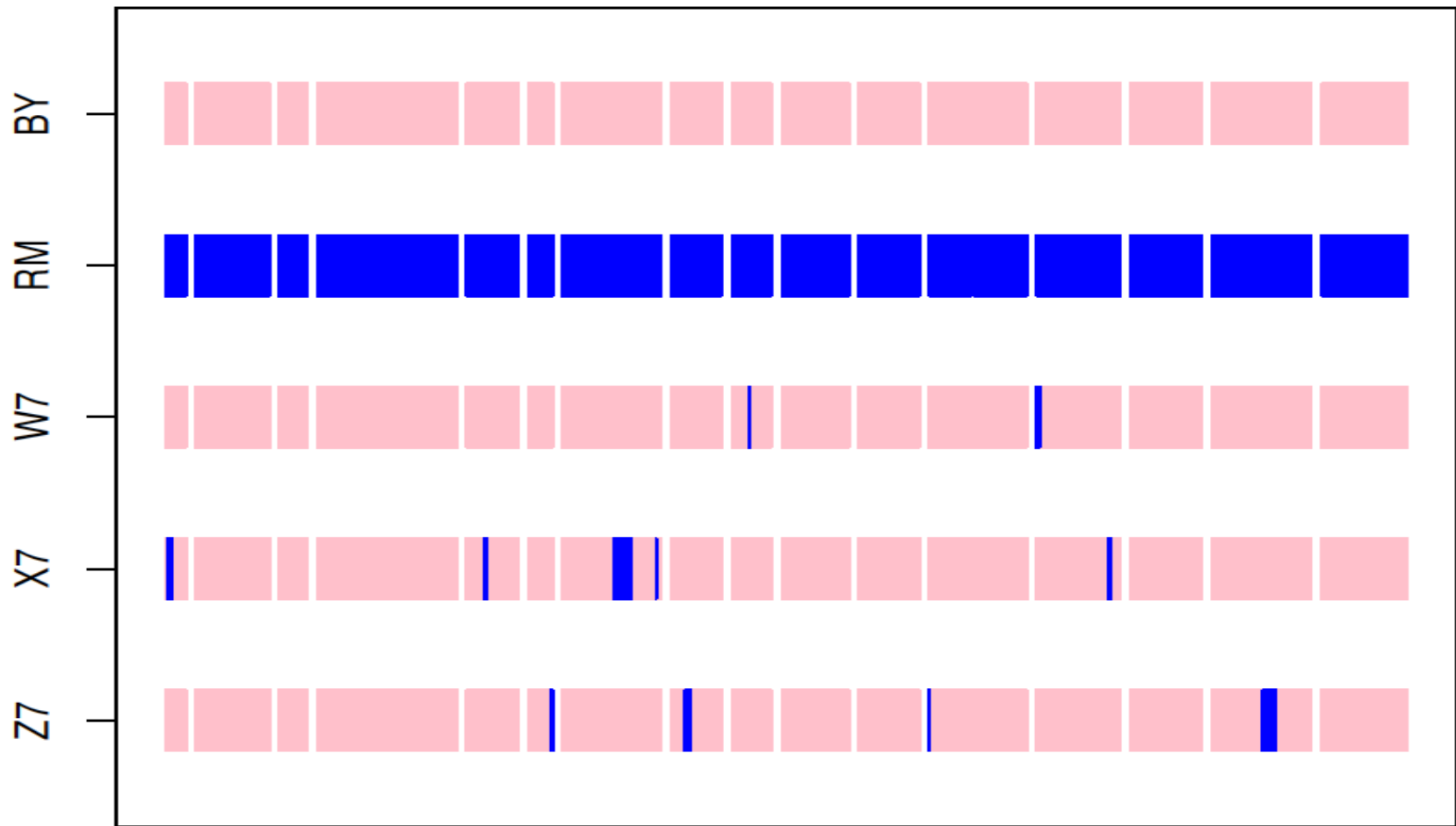
Mapping by introgression



Introgression of high variance

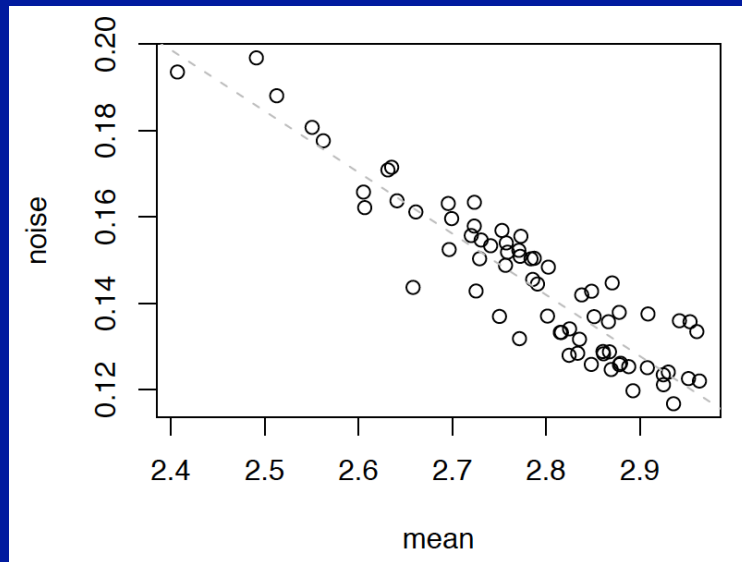
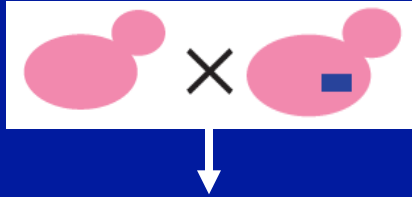


Whole-genome re-sequencing

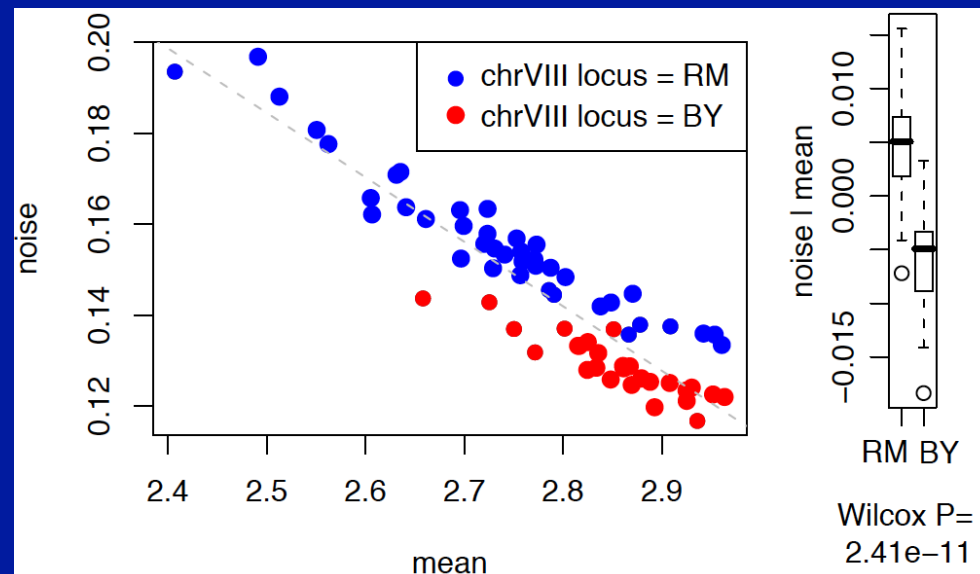
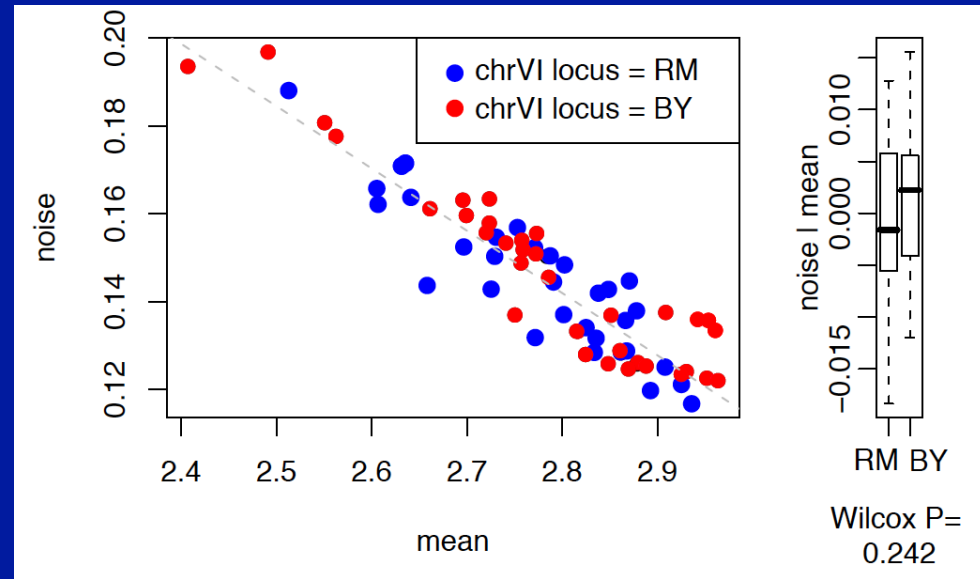


Validation of Probabilistic Trait Locus

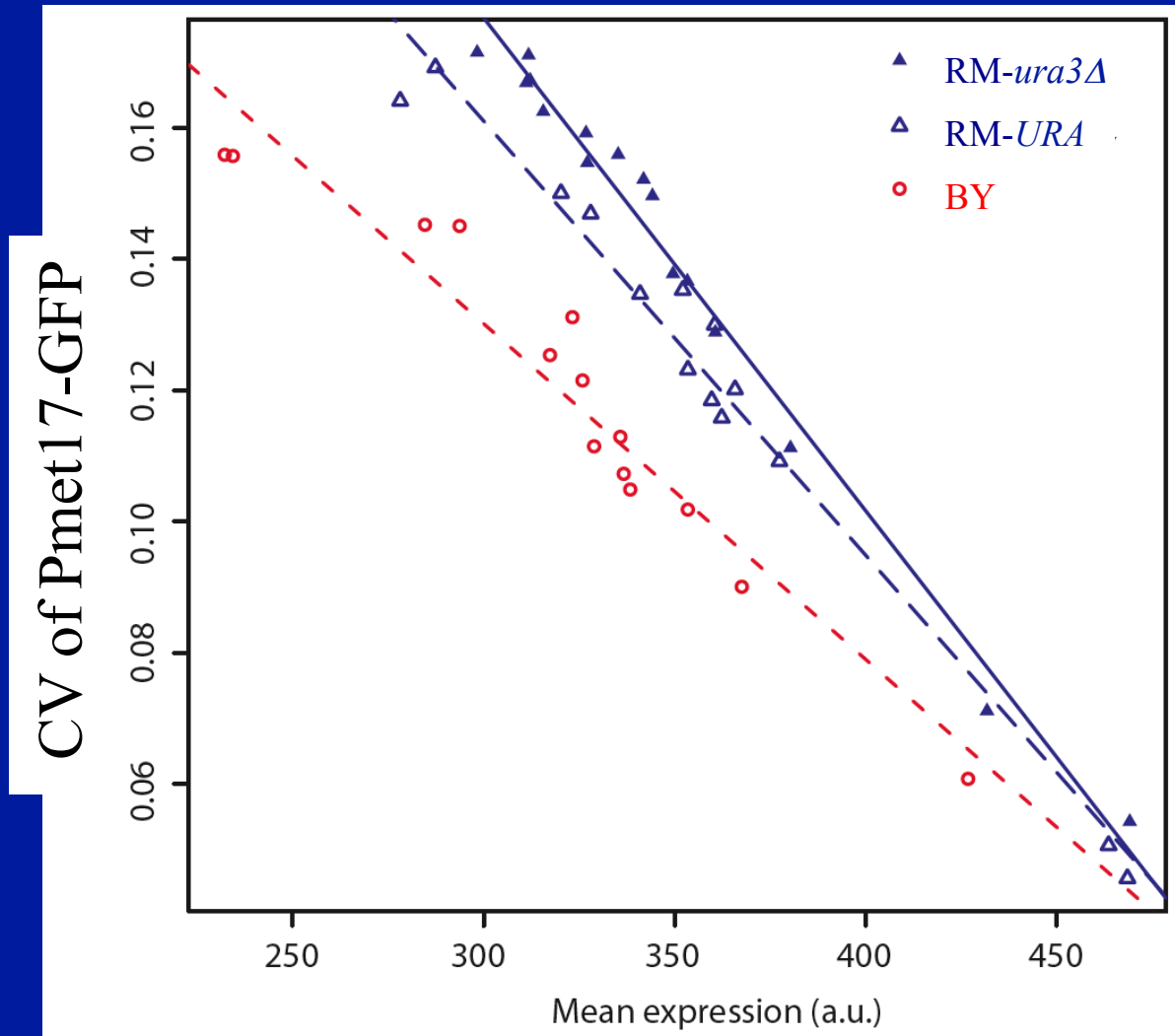
One last backcross



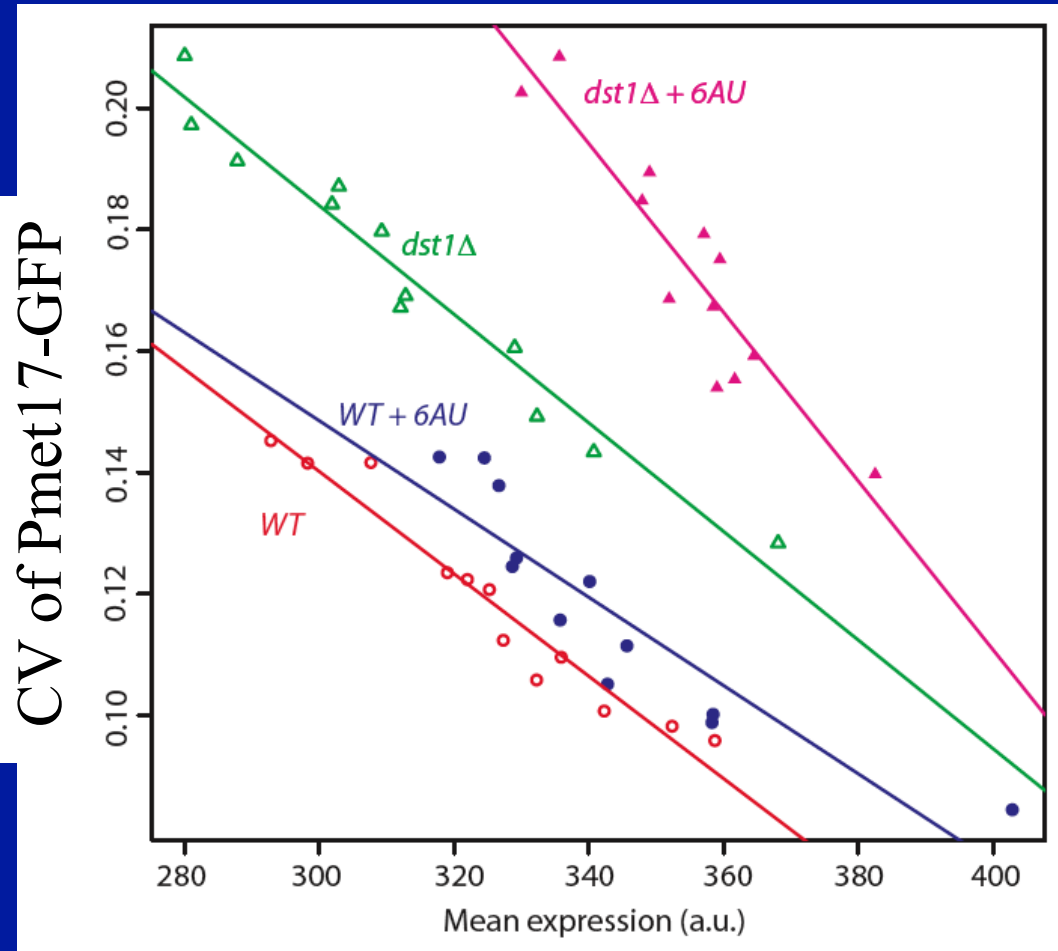
6 loci validated



ura3 is a PTL



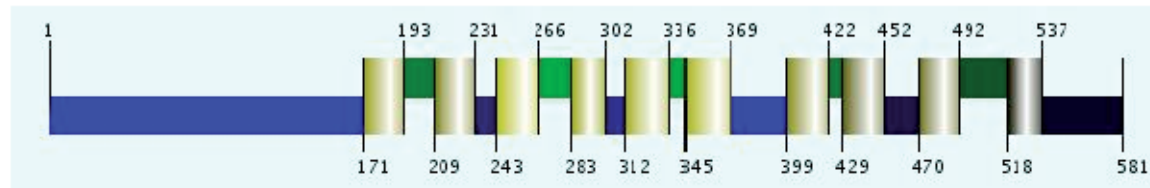
Transcriptional elongation impairment



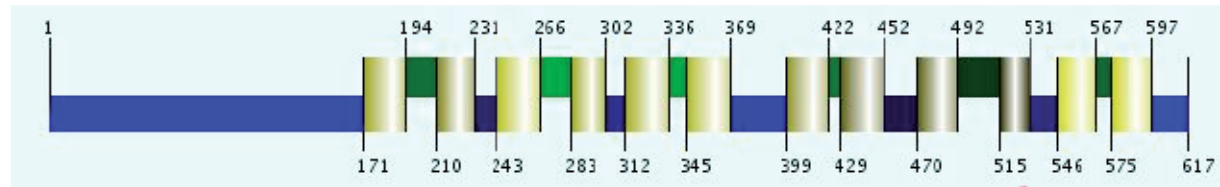
Amplifies an
intrinsic source of
noise

ERC1 gene is also a scPTL

BY



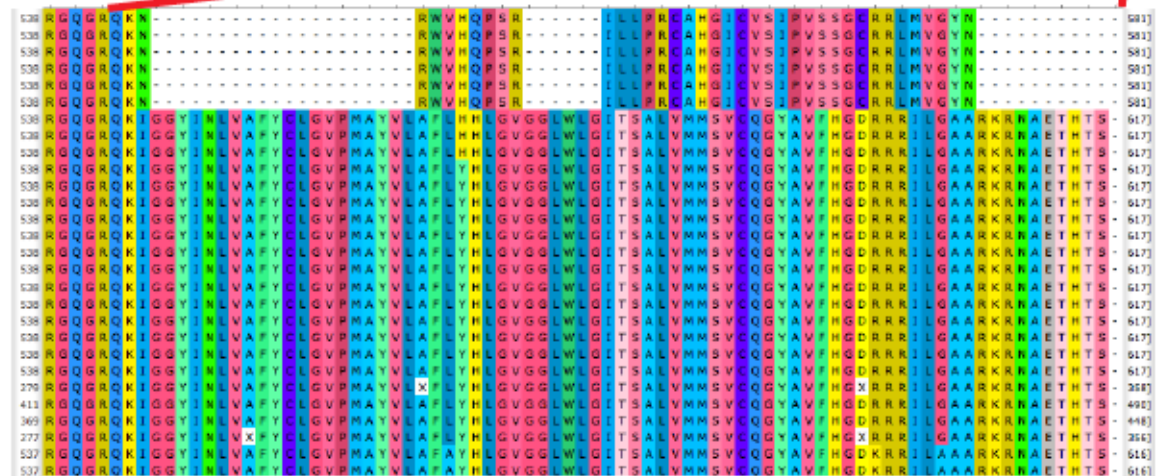
RM



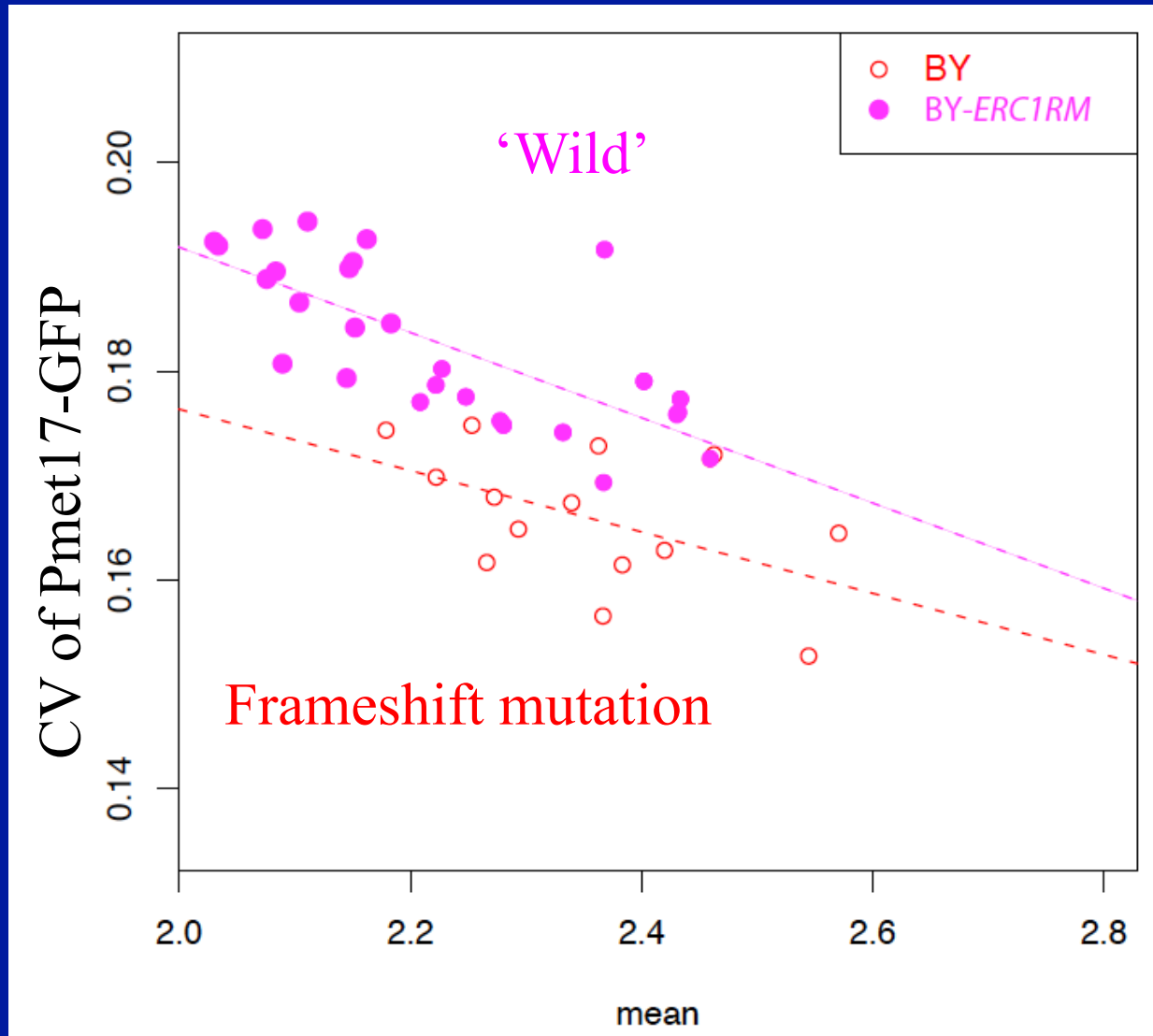
lab strains

wild strains

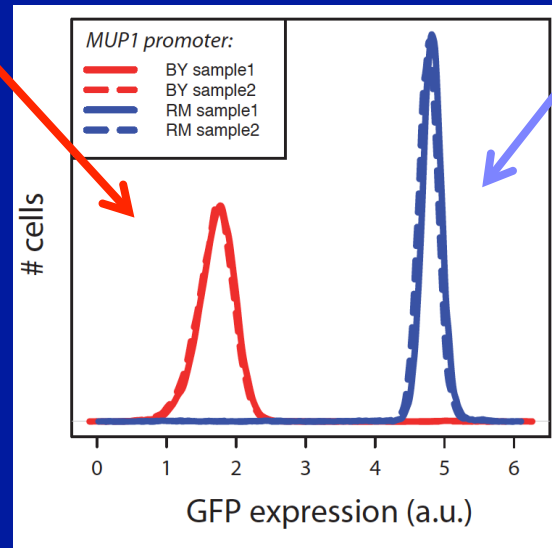
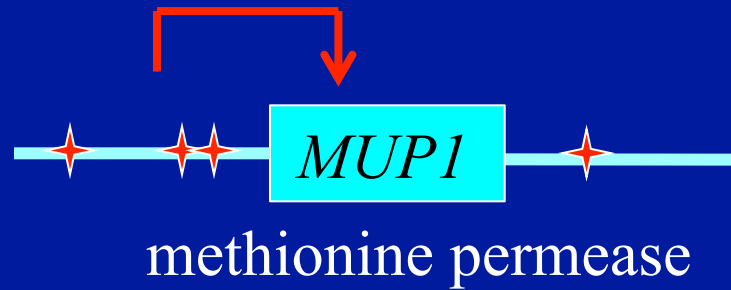
S. bayanus

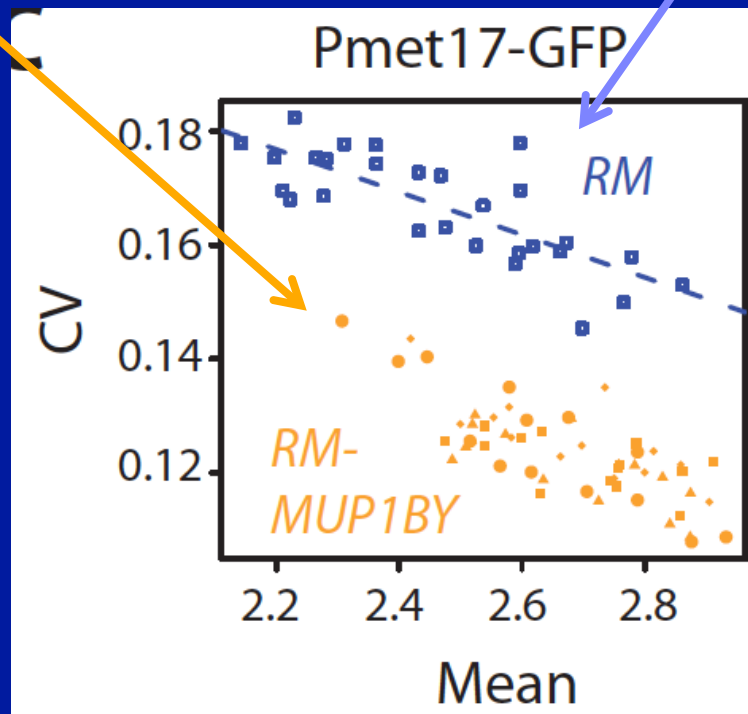
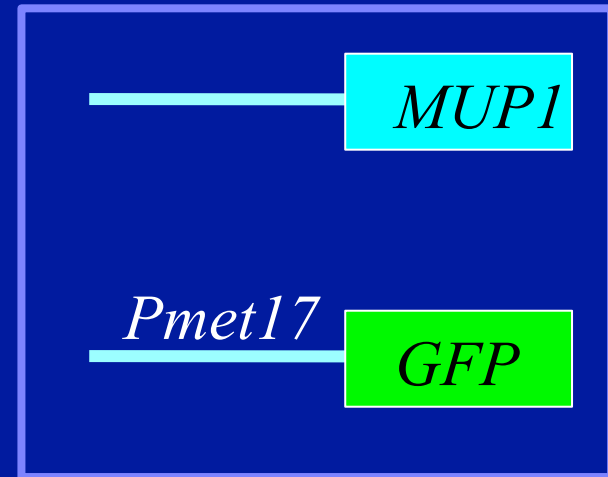
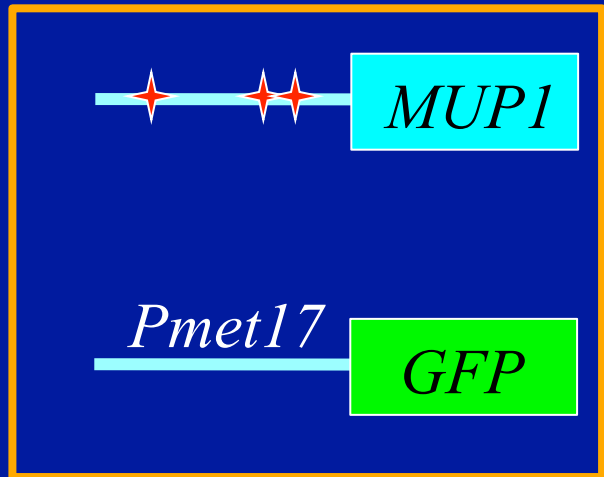


Allele Replacement of *ERC1*

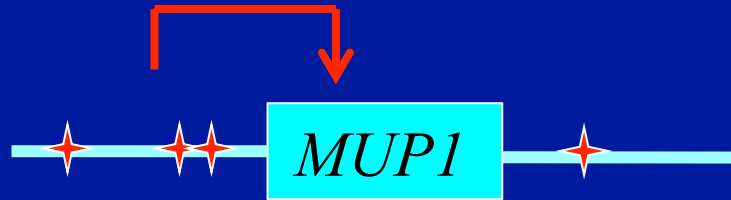


Change level in all cells (deterministic)





Change level in all cells (deterministic)

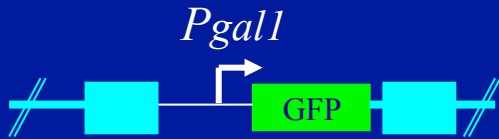


Change variance
among cells
(probabilistic)



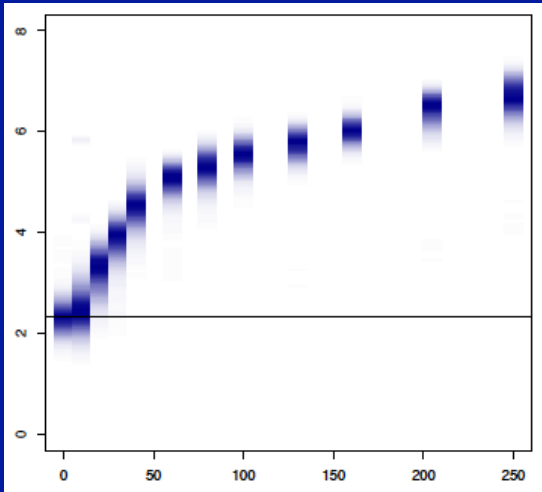
Not only the variance!

Kinetics of gene activation

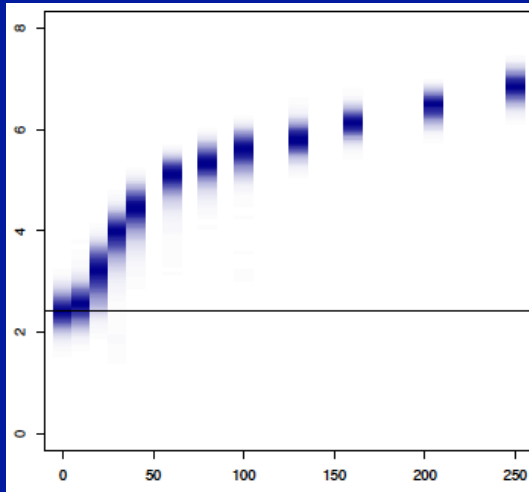


Independent replicates, one genetic background

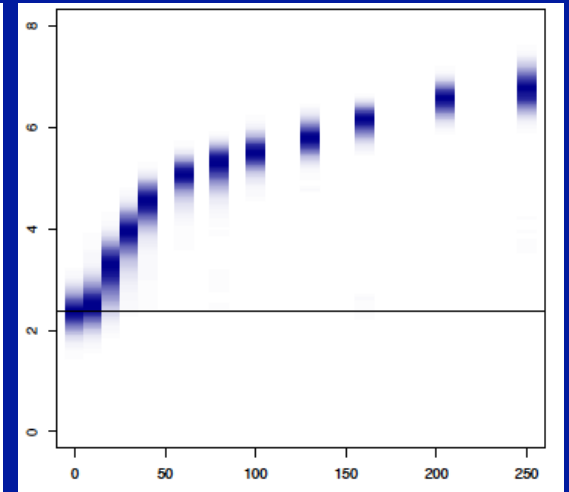
GFP



Time (sec)



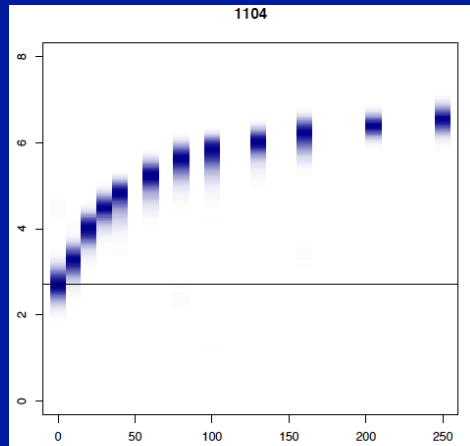
Time (sec)



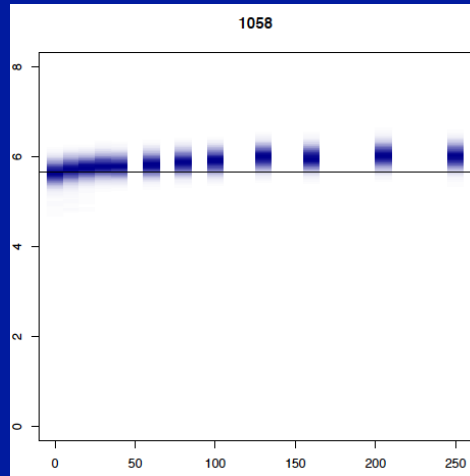
Time (sec)

Cell-cell variability

Strain 1

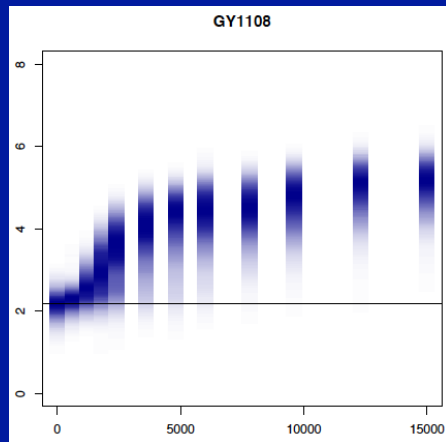


Strain 2

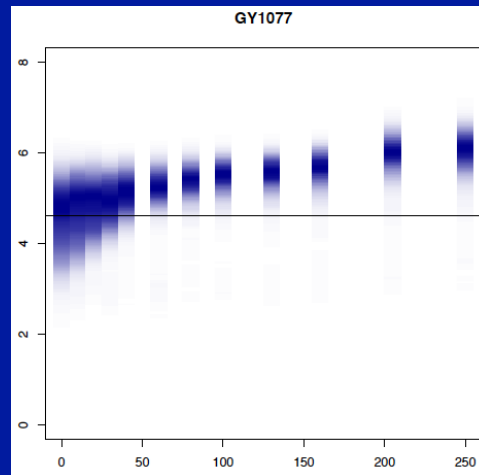


Low variance

Strain 3



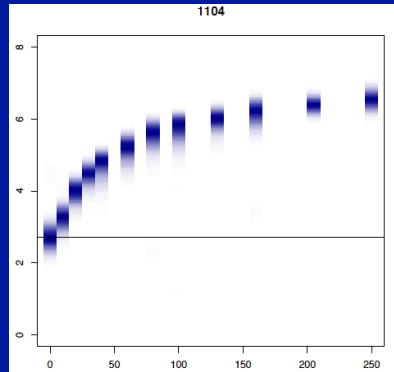
Strain 4



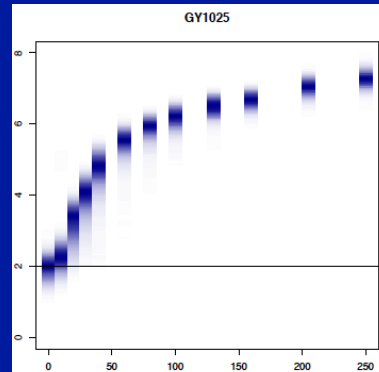
High variance

Activation

Strain 1

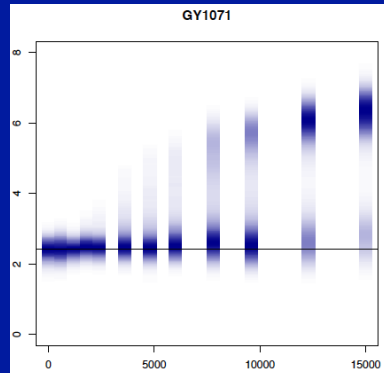


Strain 5

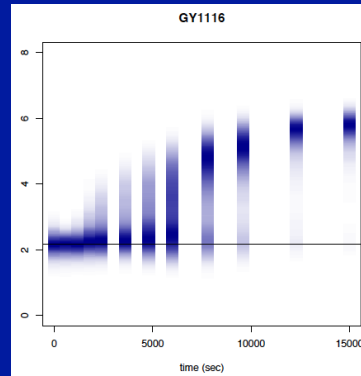


Gradual

Strain 6

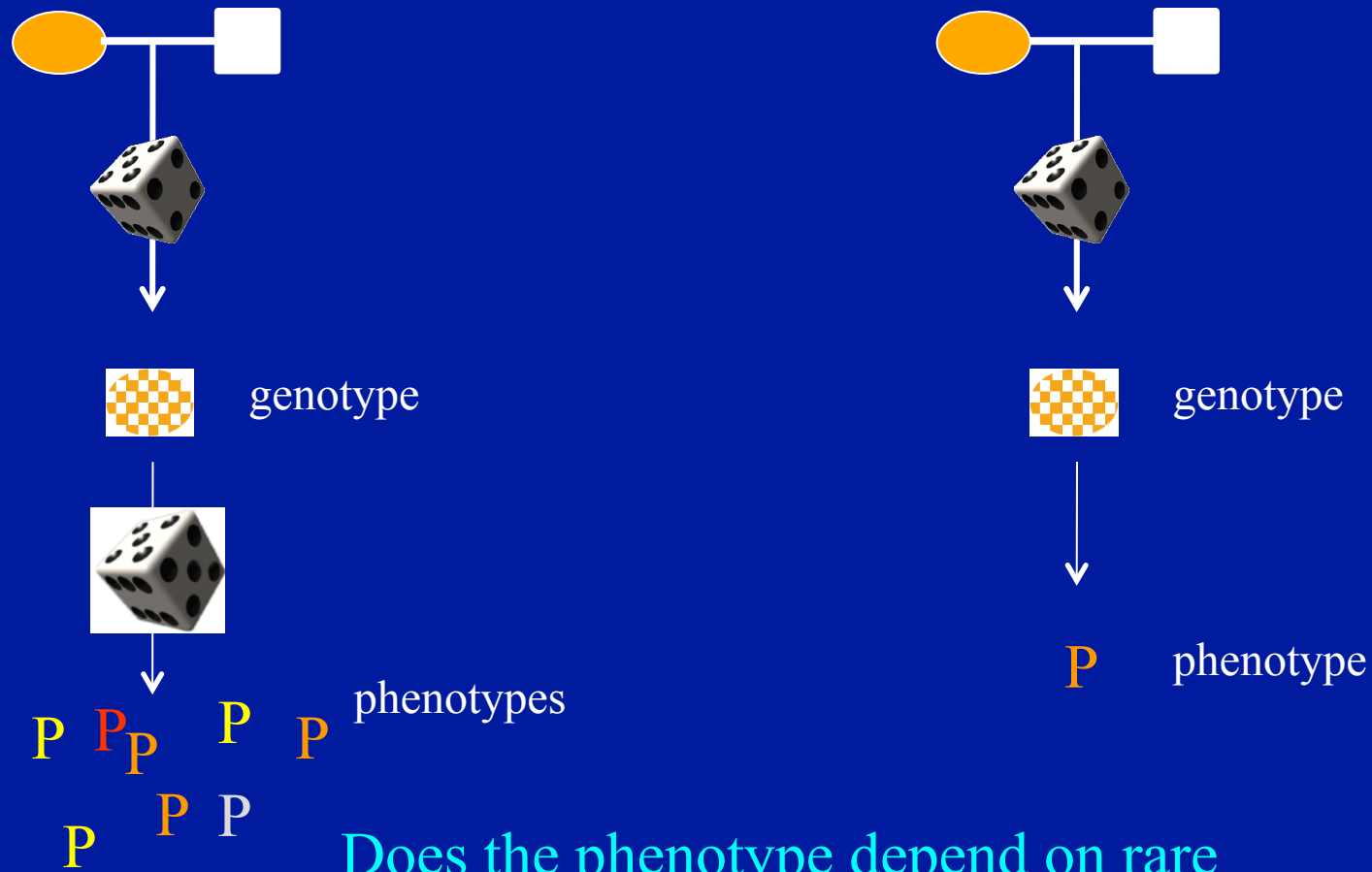


Strain 7



Switch

Particle vs. Classical Genetics



Does the phenotype depend on rare events, few cells ?



Thanks!

Steffen Fehrmann (Ph.D. Student)
Hélène Bottin (Tech.)

Andri Leonidou (Undergrad)
Juliet Ansel (Undergrad)
Marianne Entrevan (Undergrad)
Esther Mollereau (Tech.)

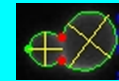


<http://www.ens-lyon.fr/LBMC/gisv/>



Physics:

Daniel Jost



U. Tokyo:

Shinsuke Ohnuki
Yoshikazu Ohya



EMBL:

Wu Wei
Lars Steinmetz

