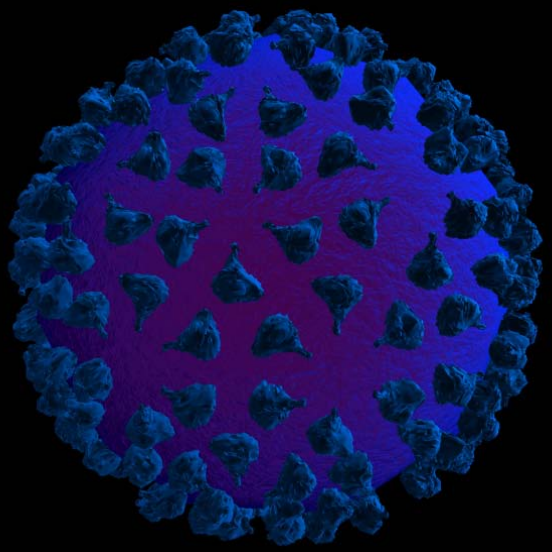
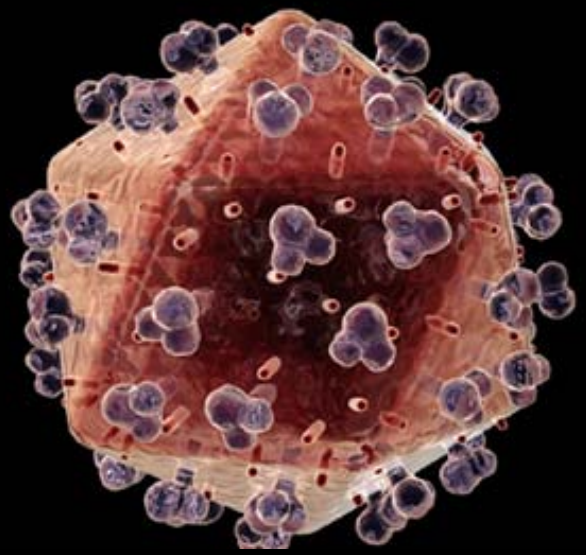
Evolution of Robustness

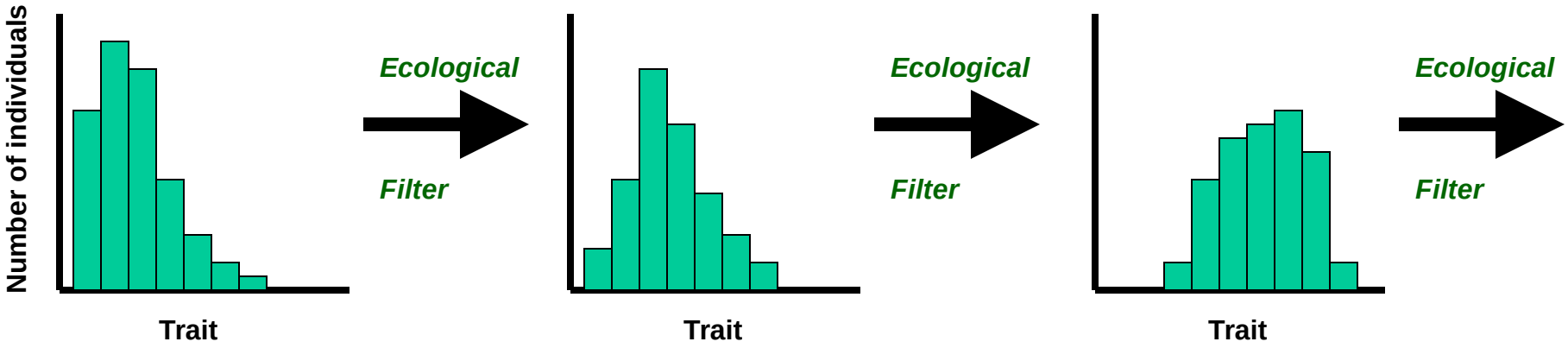




Paul E. Turner
Department of Ecology & Evolutionary Biology,
and Graduate Program in Microbiology
Yale University

Experimental evolution

Studies of evolution-in-action using model organisms

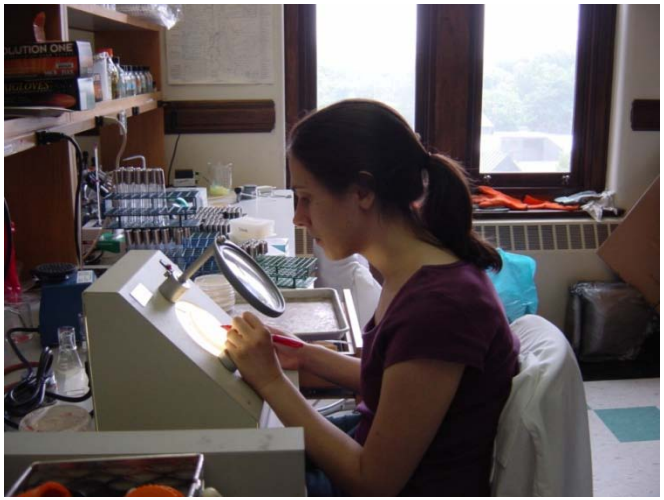


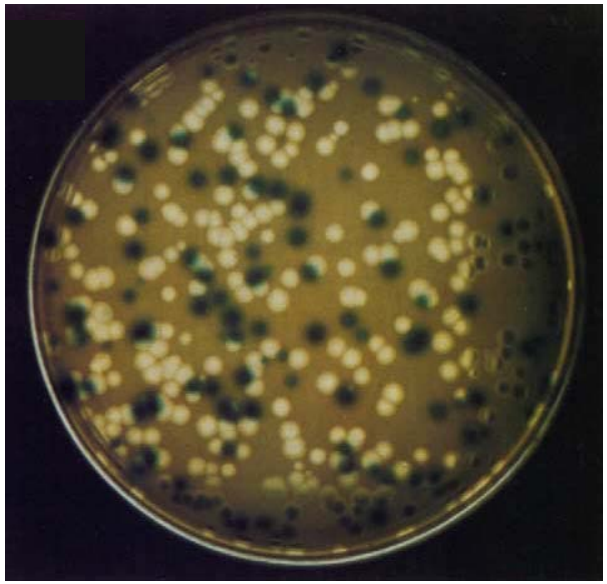
PHENOTYPE – GENOTYPE ASSOCIATIONS

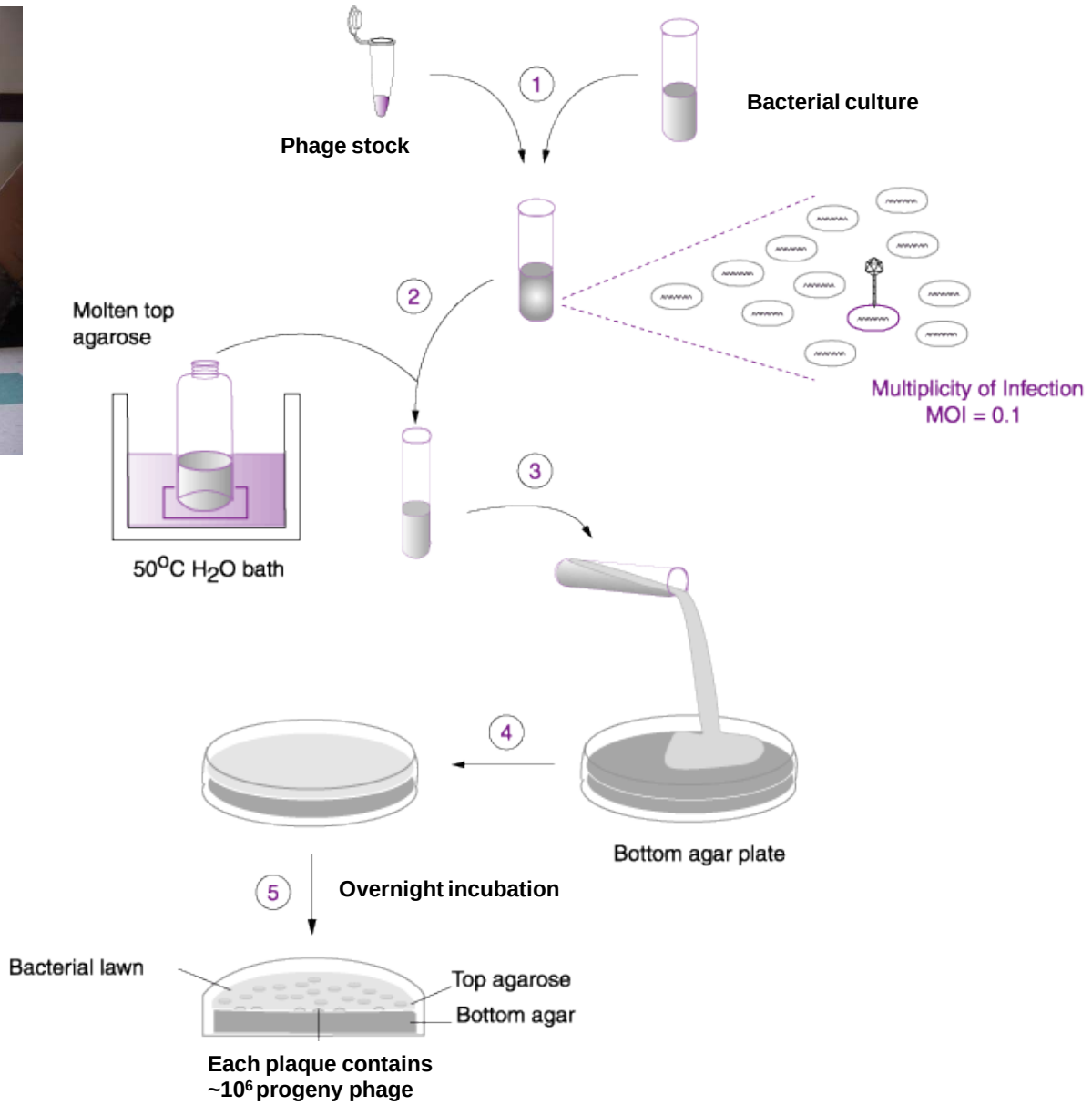
Generation *t* **Generation *t* + *1*** **Generation *t* + *2***



Traditional study of viruses in the lab





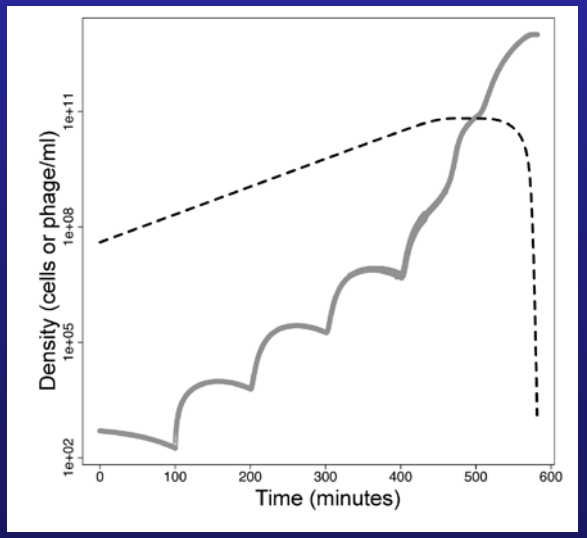


Novel methods for studying virus growth

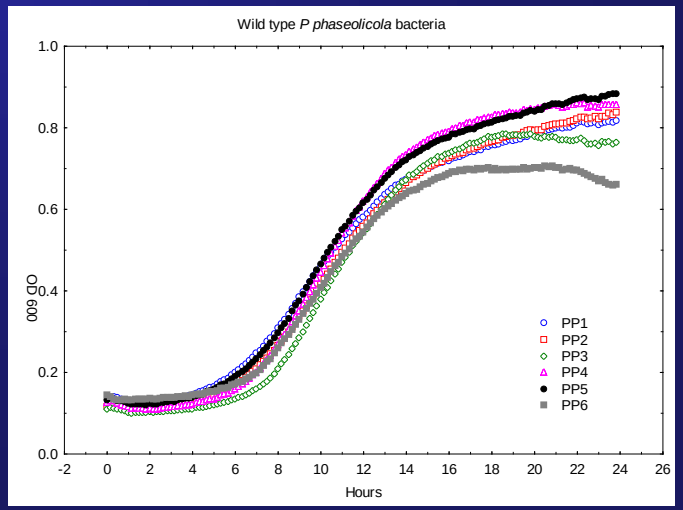
Liquid-handling robot

+

Microplate reader



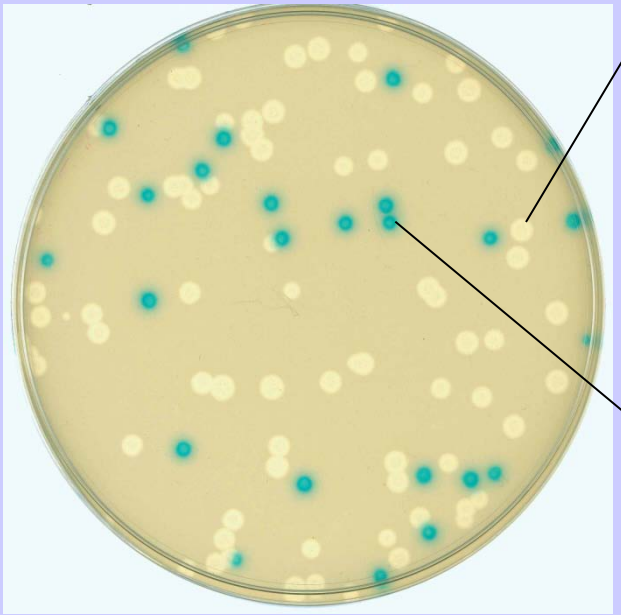
Inferred virus growth

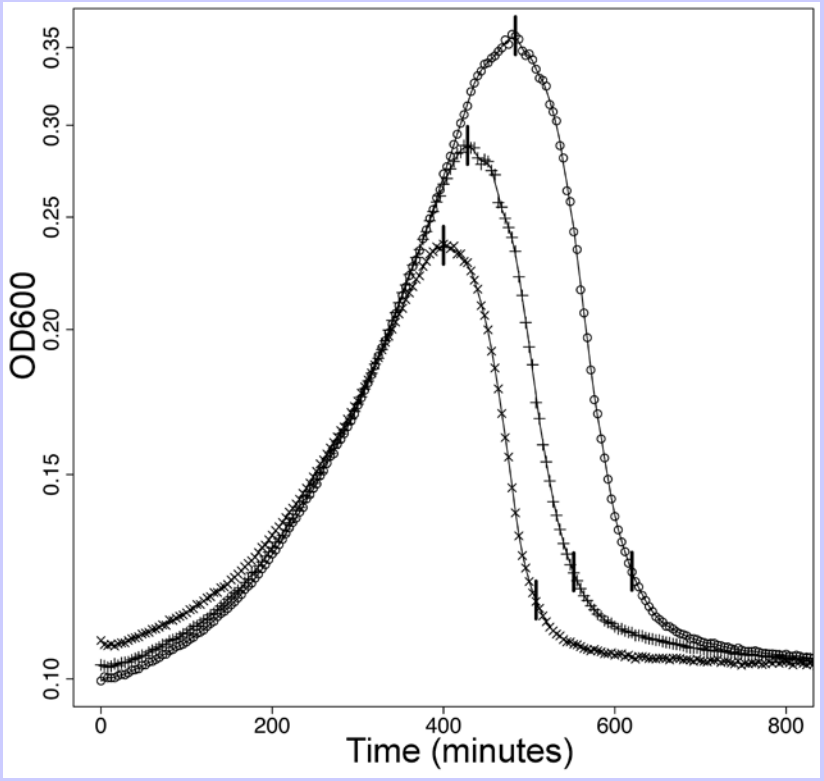


Bacteria growth curves

A New Method for Measuring Virus Fitness

Phage too small to count directly.
Measure phage fitness by tracking host?





Traditional method:
Grow two strains on one plate and count plaques.

Problems: Time consuming,
Small sample size

New method:
Measure growth curves of infected hosts in liquid.
Strong host growth means lower phage fitness

A New Method for Measuring Virus Fitness

Phage too small to count directly.
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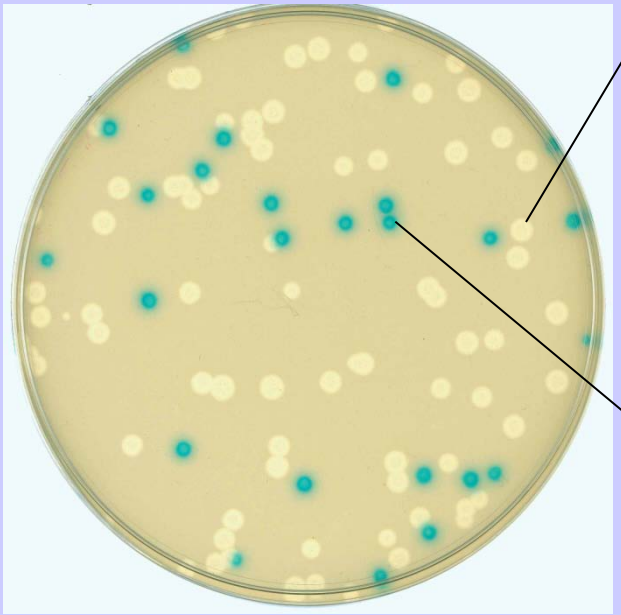

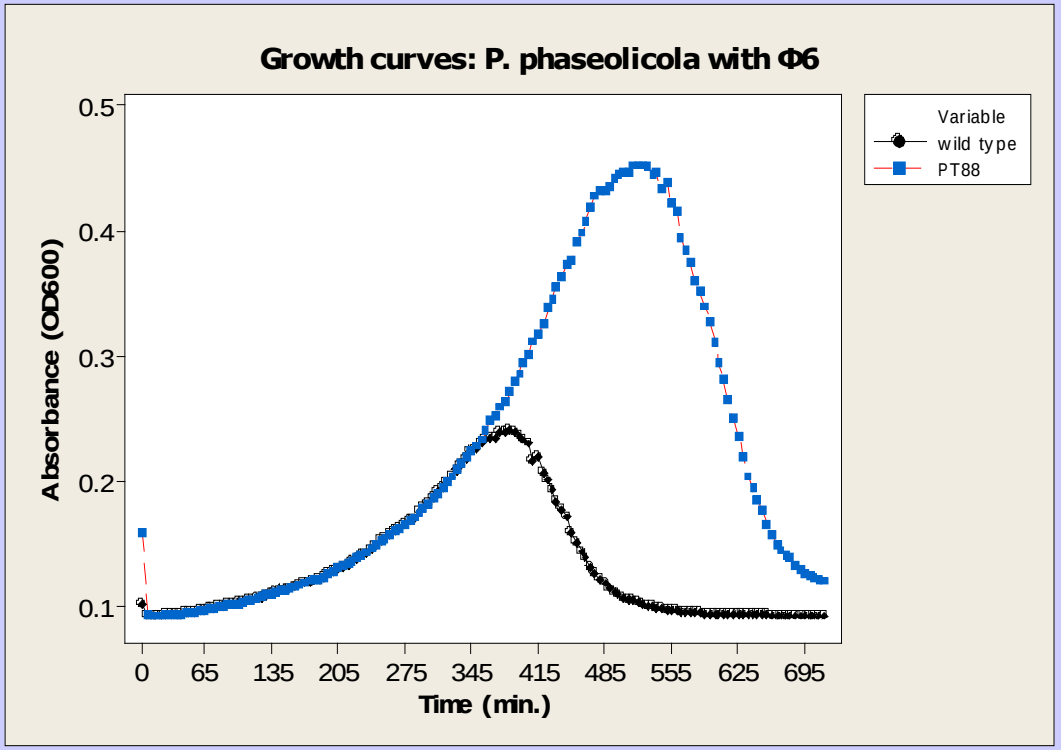

Traditional method:
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Problems: Time consuming,
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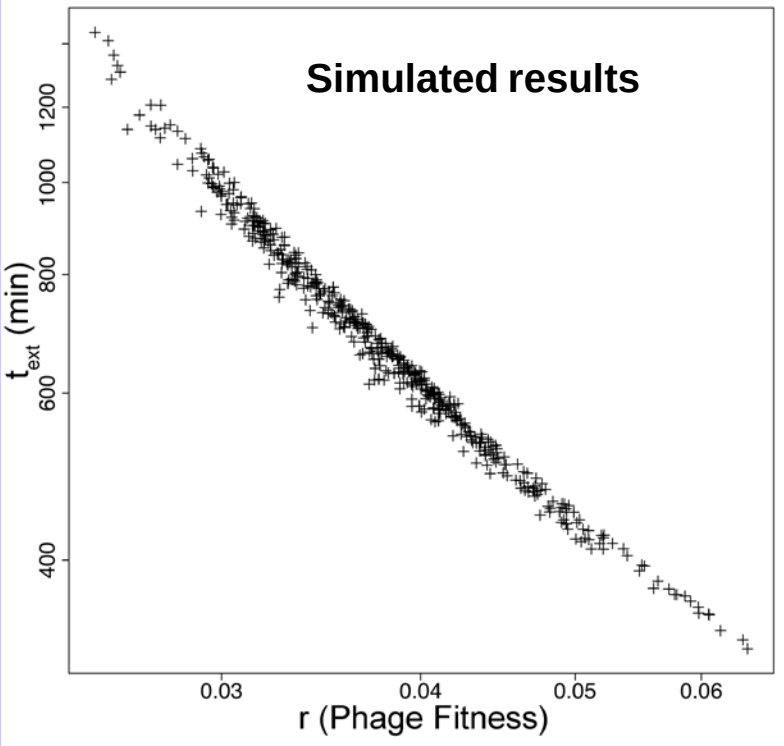
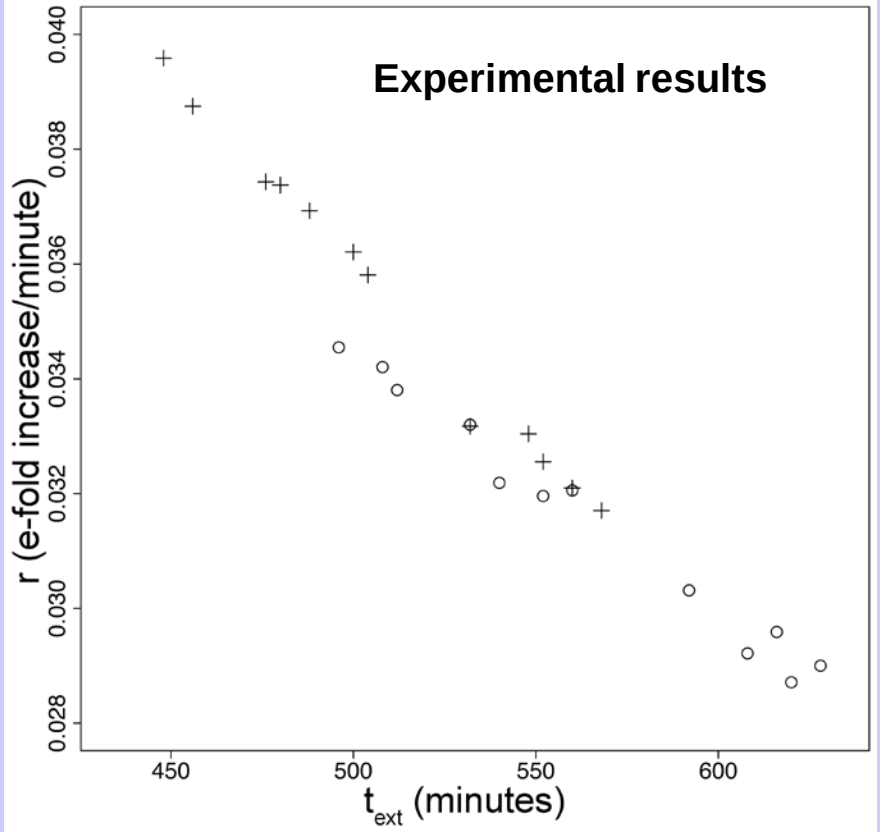
New method:
Measure growth curves of infected hosts in liquid.
Strong host growth means lower phage fitness

High throughput measures of virus fitness



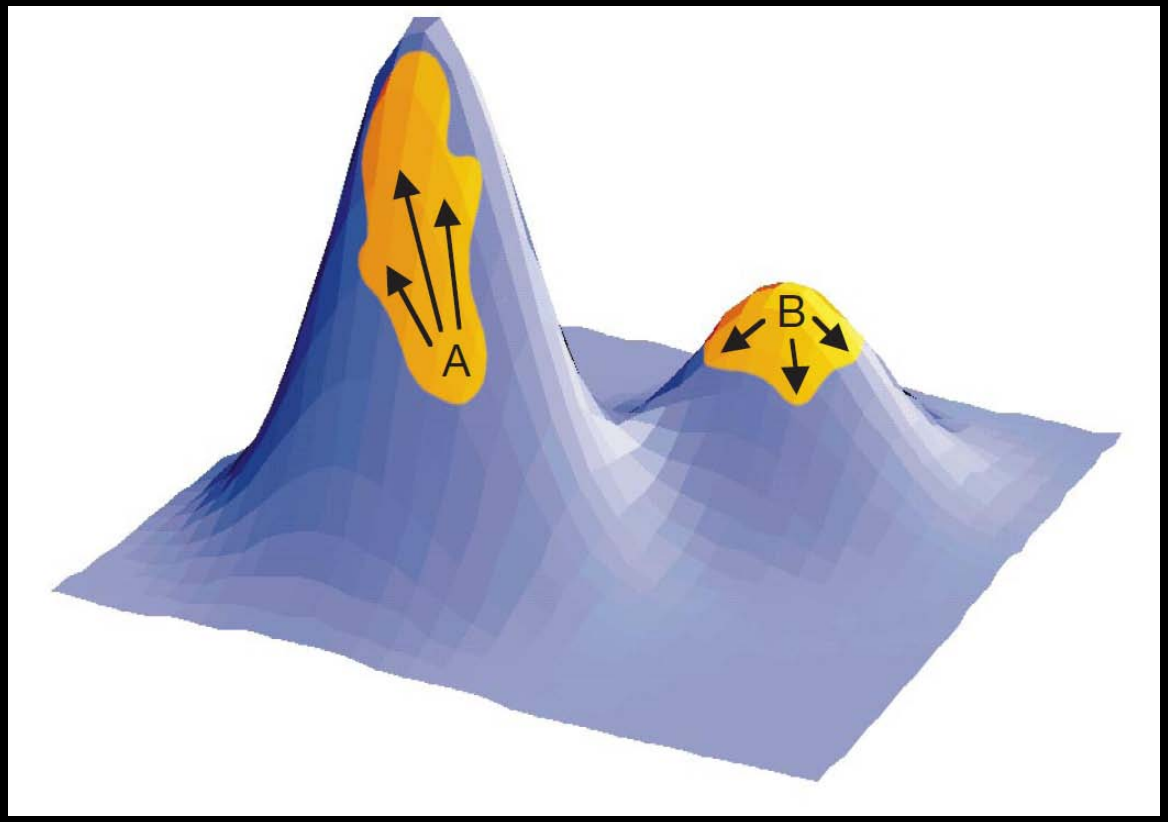
Simulations show that peak time correlates with growth rate



Empirical results show similar correlation

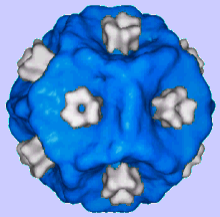
Turner et al. (submitted)

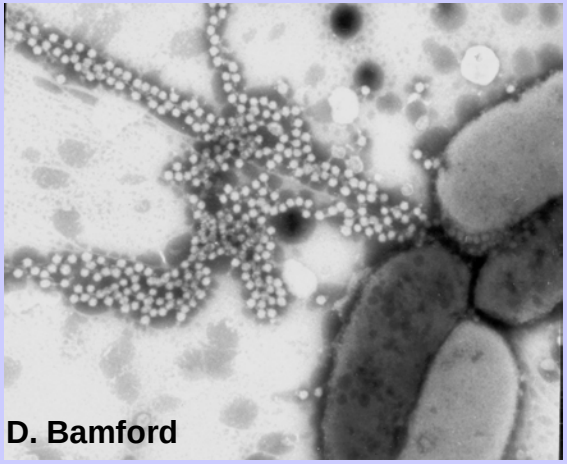
Evolution of Robustness in RNA Viruses

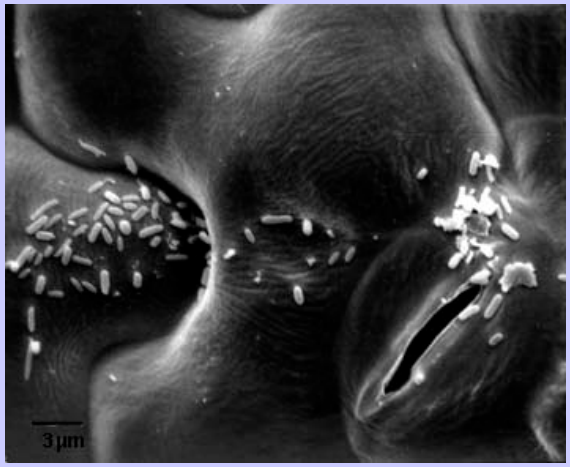


Evolution of Sex
Robustness
Evolvability

Model: Bacteriophage ϕ6



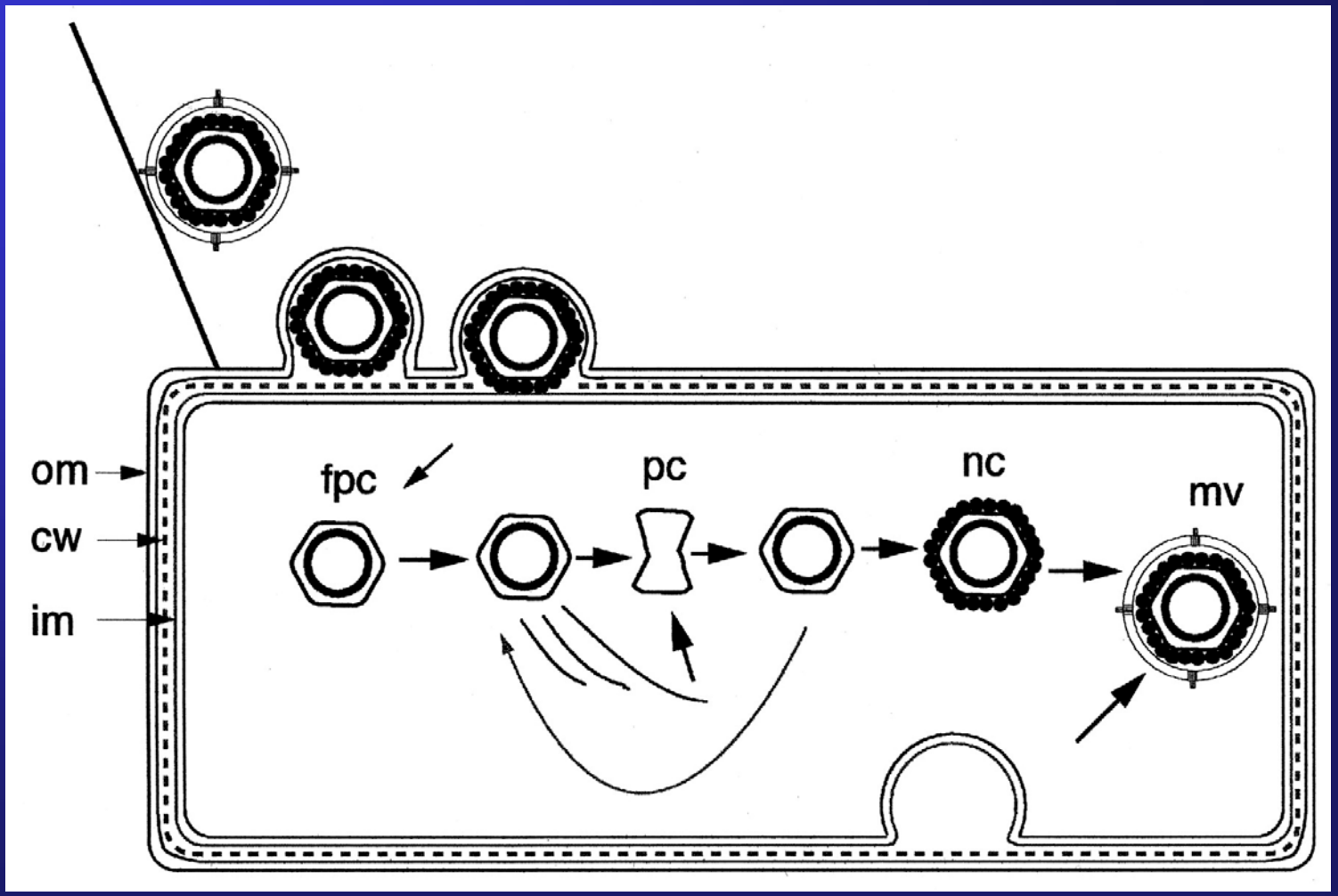




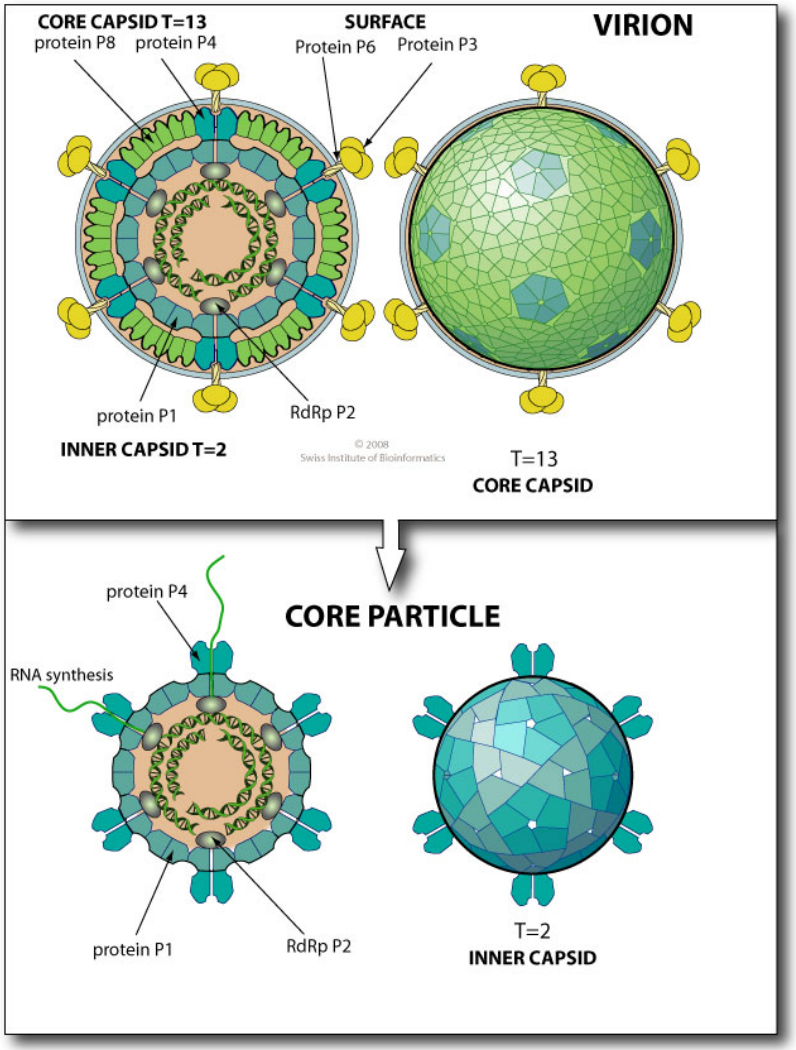
- ~13 kb dsRNA genome
- 3 segments/particle
- $0.01 < U < 0.1$

- lipid coated
- phyto-pathogenic Pseudomonas hosts
- lytic life cycle

Phage ϕ6 replication cycle

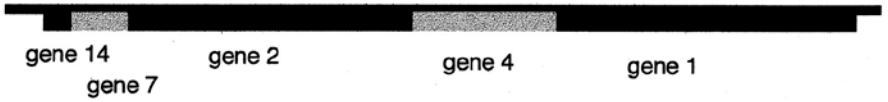


Phage ϕ6 genetics



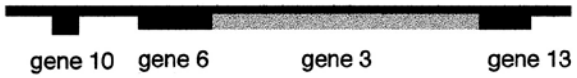
segment L polymerase and packaging functions. *pac* sequence at 5' end.

6374 bp



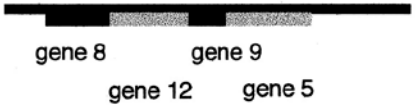
segment M membrane proteins including host specificity gene 3. *pac* sequence at 5' end.

4063 bp

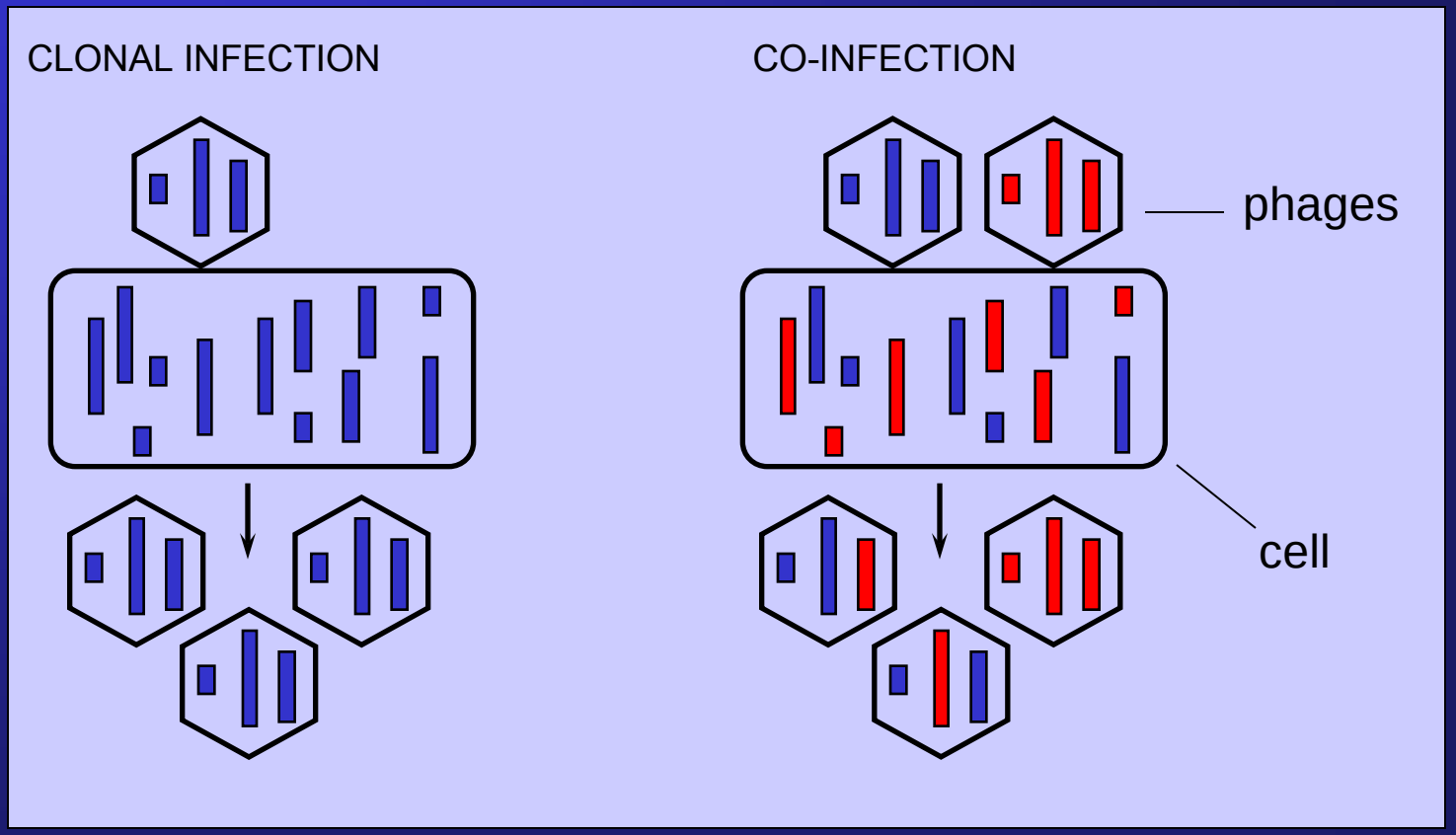


segment S nucleocapsid shell P8, ns protein P12, membrane protein P9, lysin P5. *pac* sequence at 5' end.

2948 bp

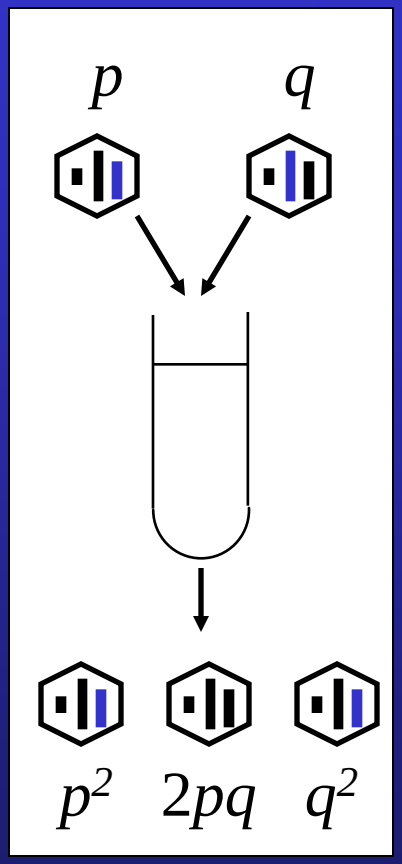


Infection modes of phage ϕ6



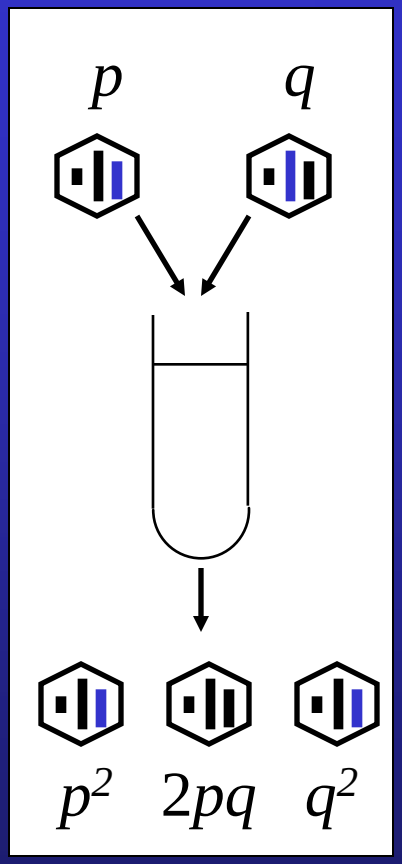
- “sex” via segment reassortment
- no recombination (3-locus population genetics)

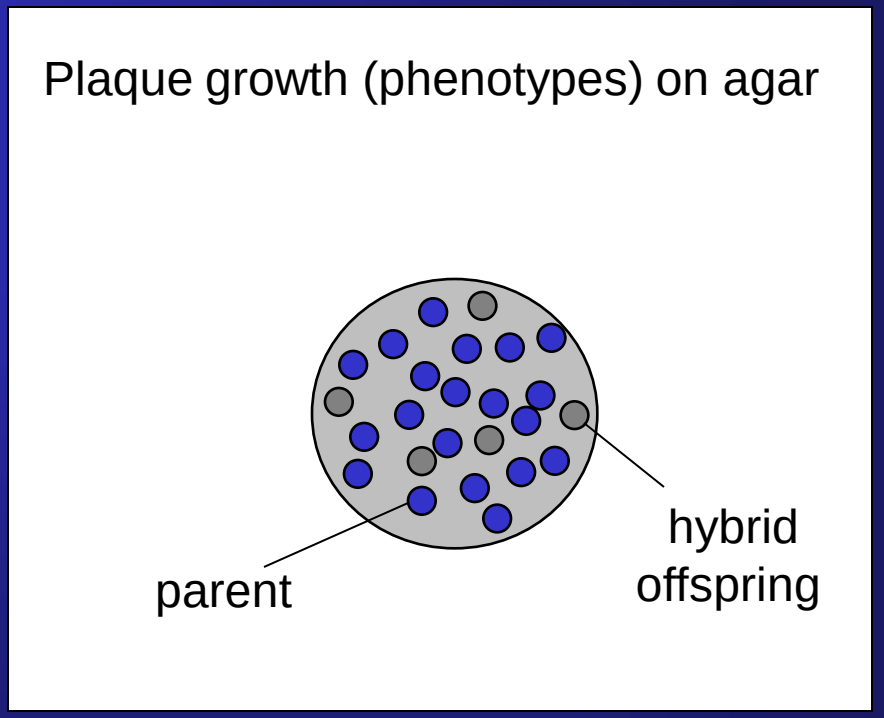
ϕ6 hybridization in the lab



Turner et al. 1999, *J Virology*

ϕ6 hybridization in the lab





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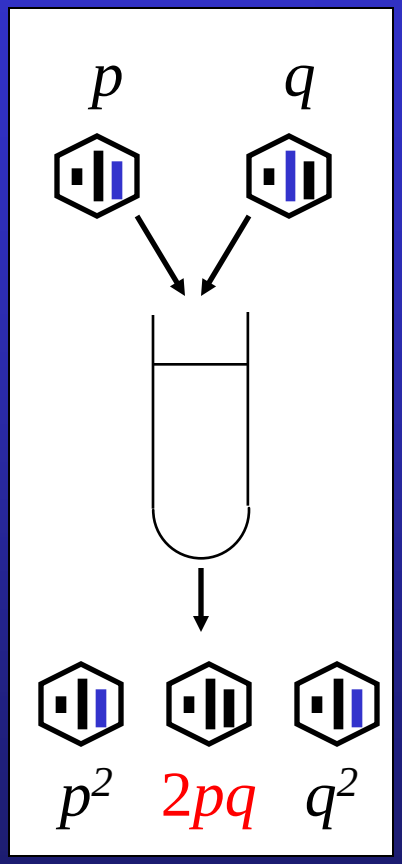
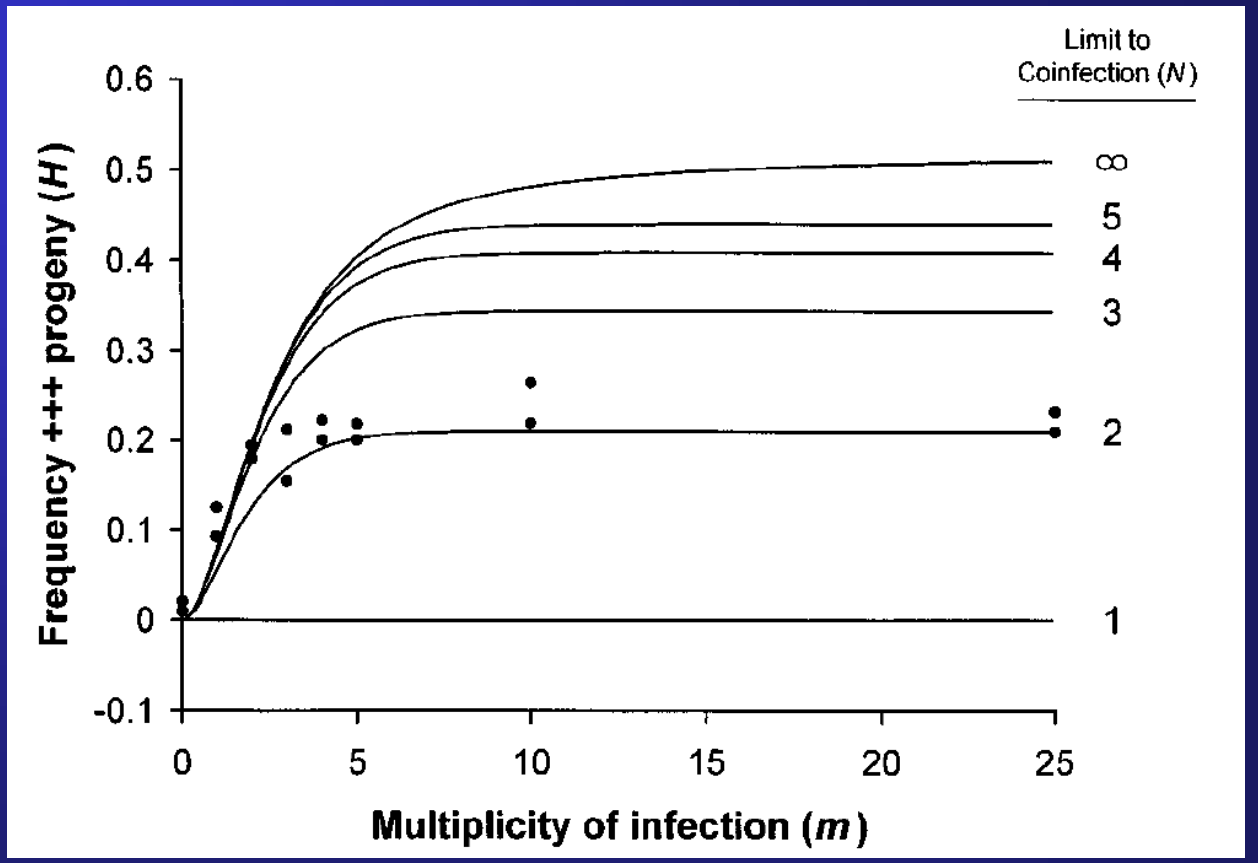




Turner et al. 1999, *J Virology*

Is sex beneficial in evolving populations of viruses?

Prediction: *Sex promotes mixis (linkage equilibrium)*

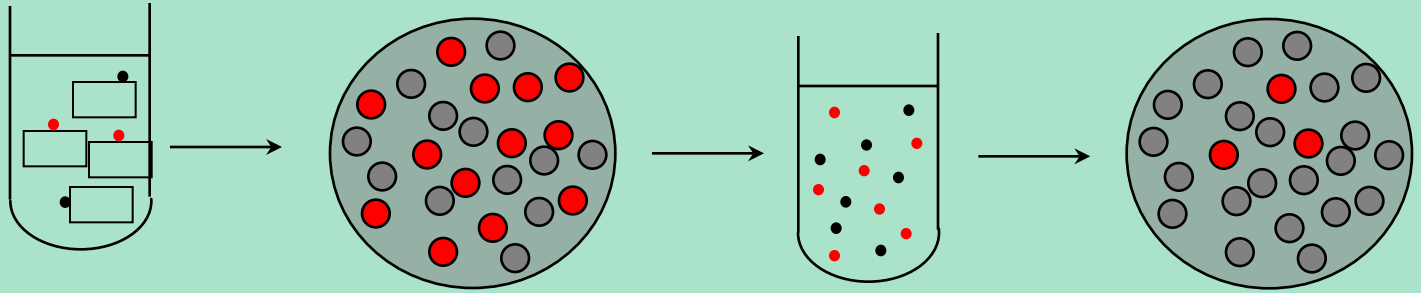
Brings together good alleles (directional selection)

Tears apart bad alleles (combat mutational load)

Assumption: *mixis is useful*

Gauging phenotypic success: Fitness assay

ϕ_1 vs ϕ_2

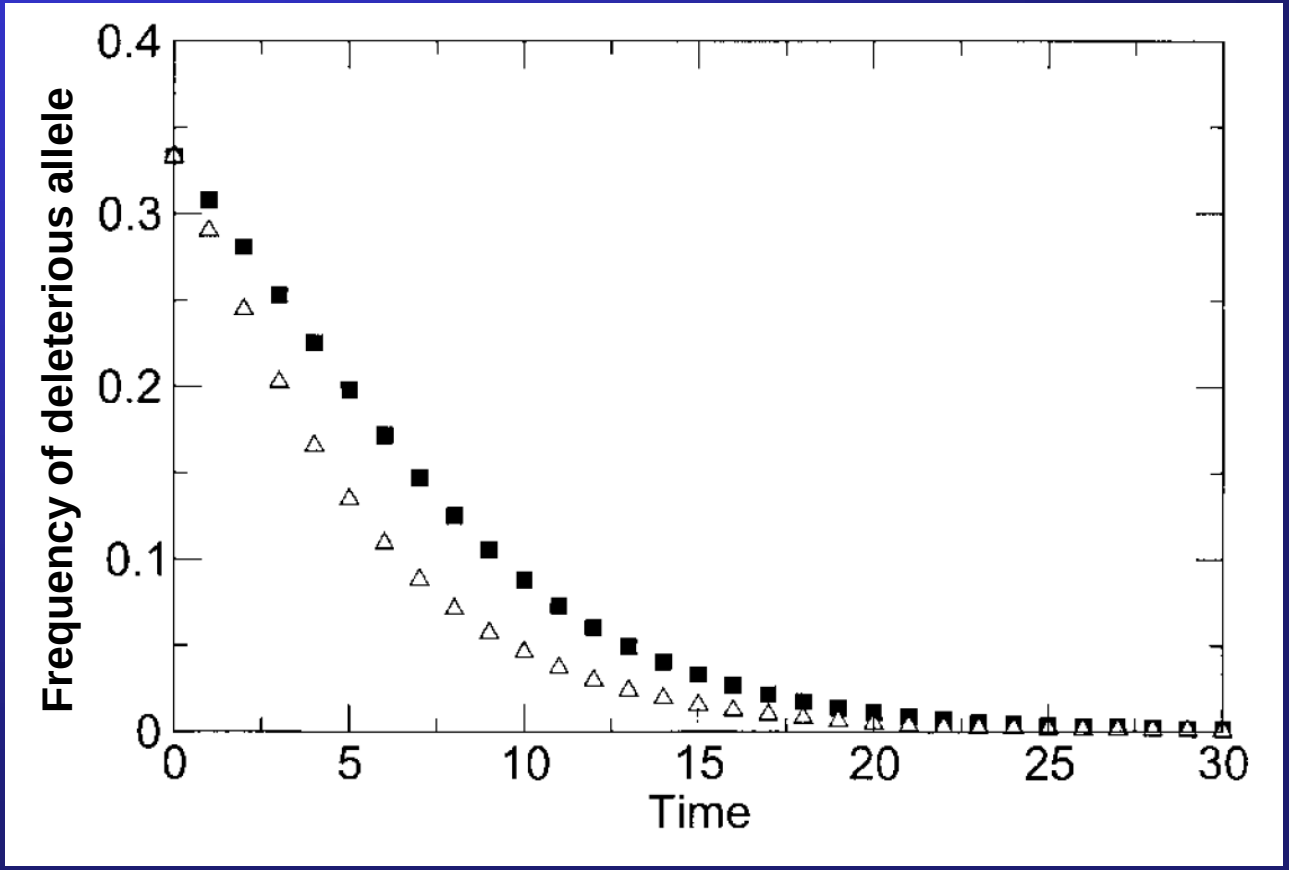


$R_0 = N_1/N_2$

$R_1 = N_1/N_2$

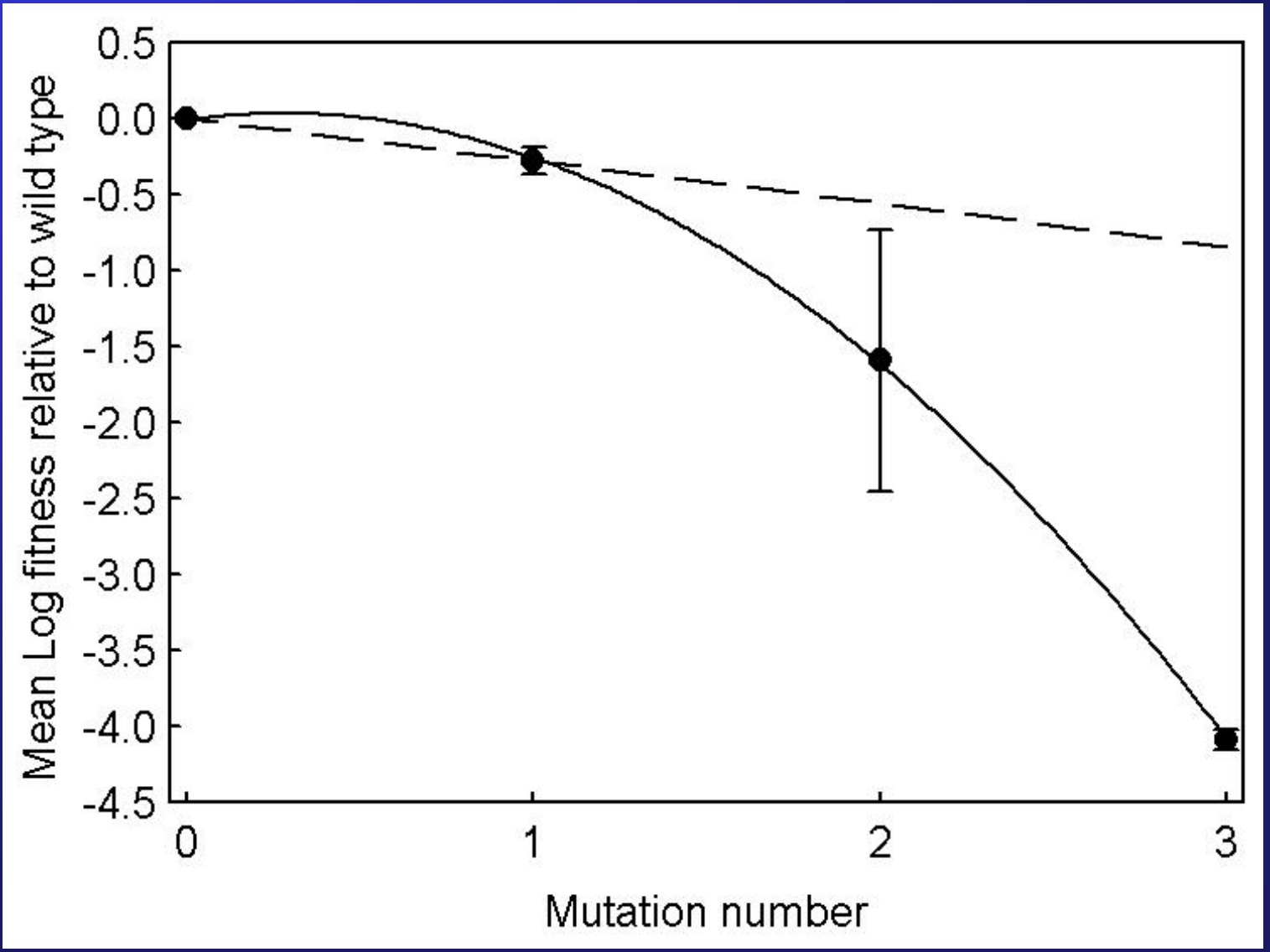
Fitness (W) = R_1/R_0

General theory on evolutionary advantage of sex



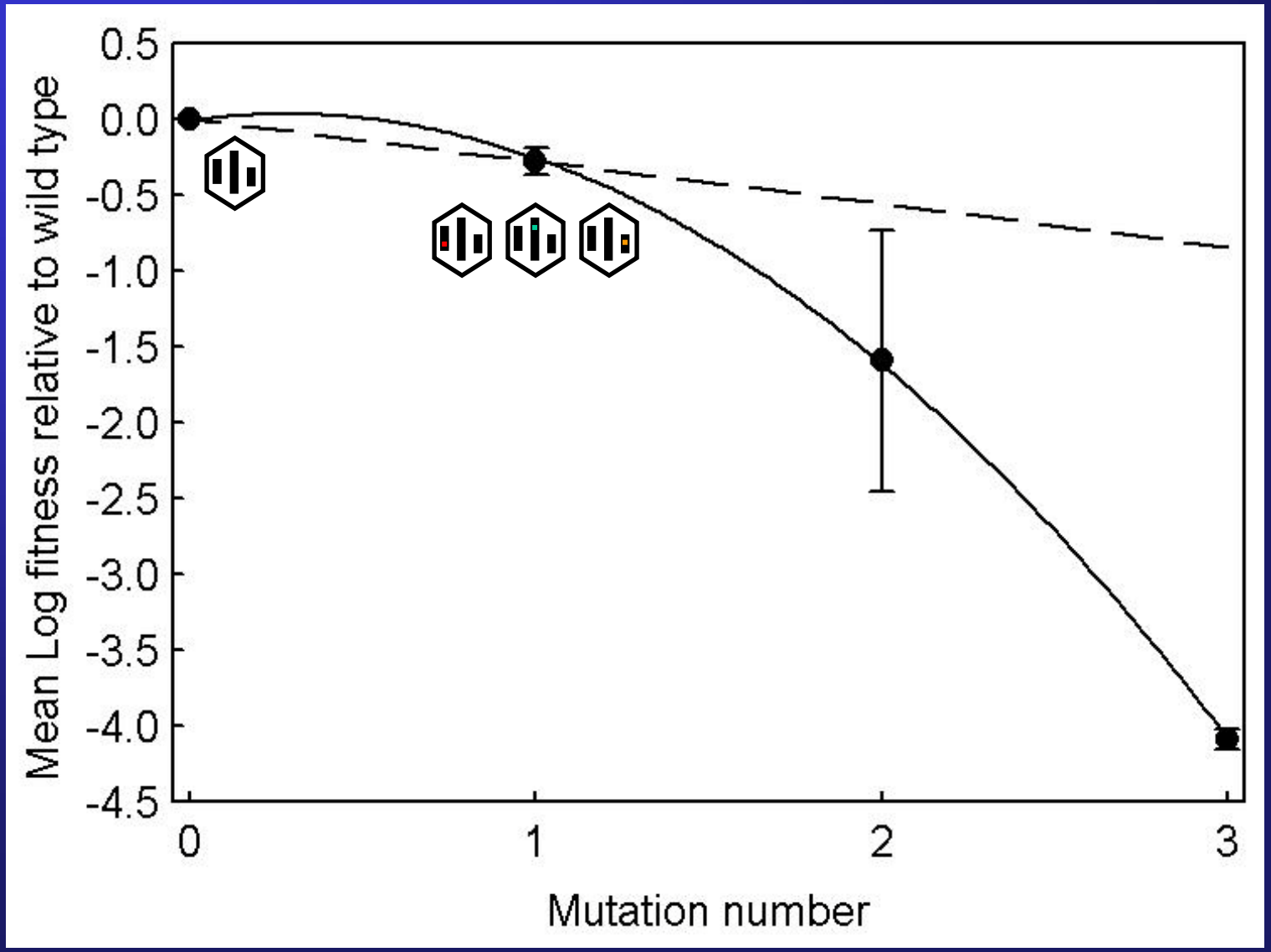
Froissart et al. 2004, *Genetics*

Known mutational load: negative epistasis



Turner et al. 2009 (in Garland & Rose: *Experimental Evolution*)

Known mutational load: negative epistasis



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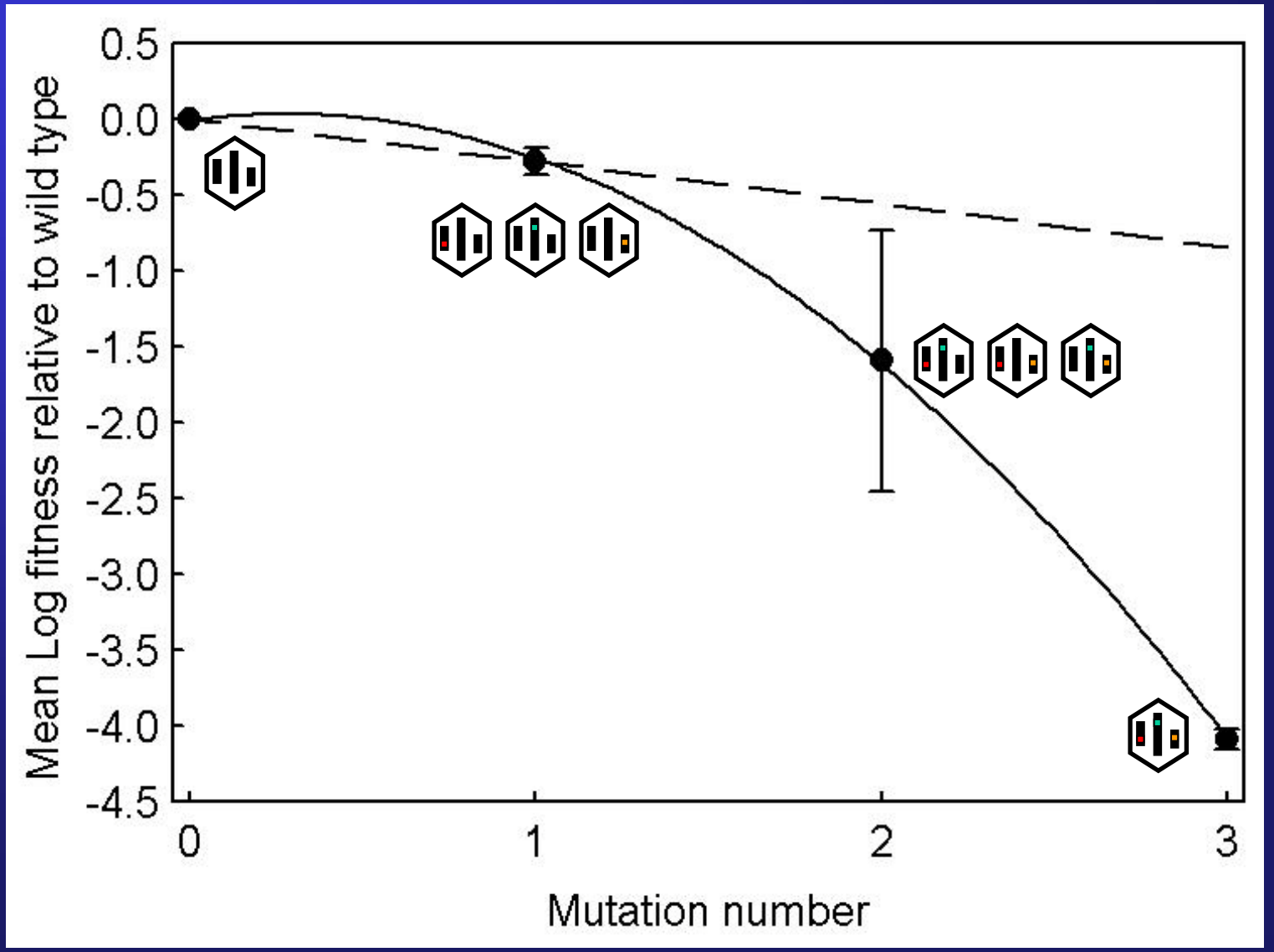
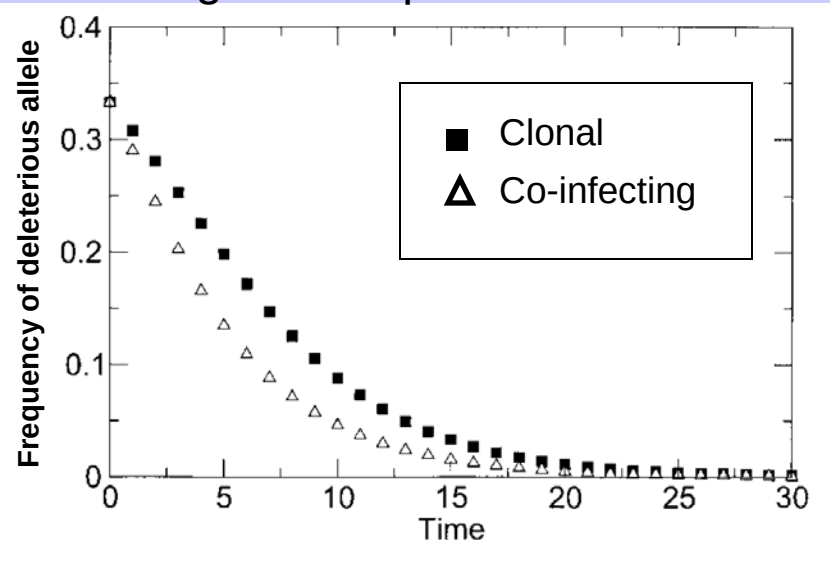


Turner et al. 2009 (in Garland & Rose: *Experimental Evolution*)

Testing advantage of sex in combating load

Advantage of sex prediction:



Froissart et al. 2004 *Genetics*

Testing advantage of sex in combating load

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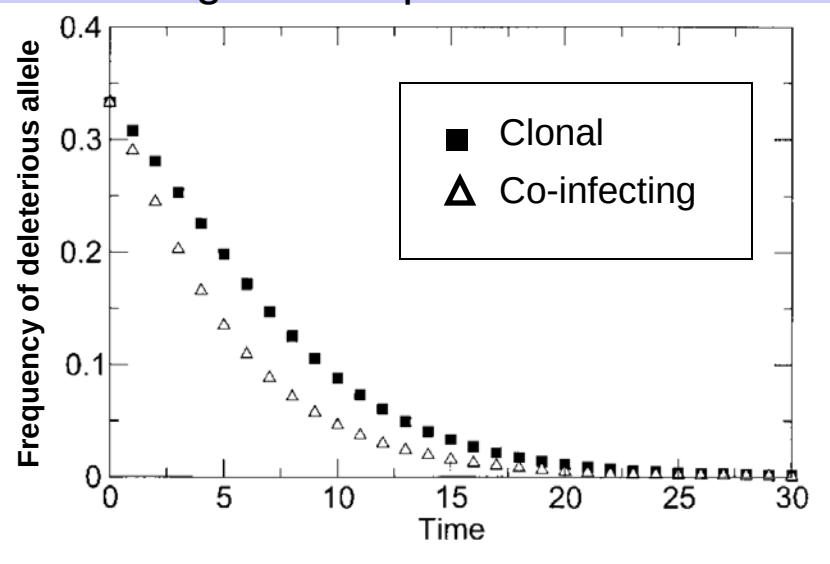
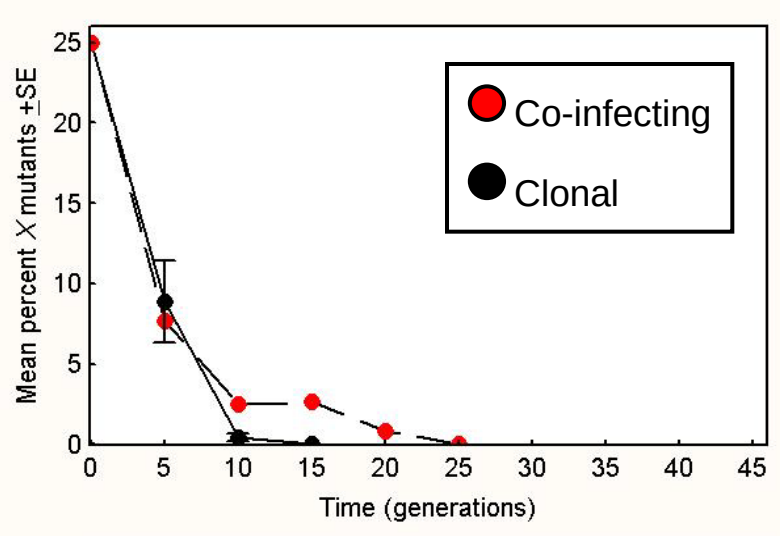
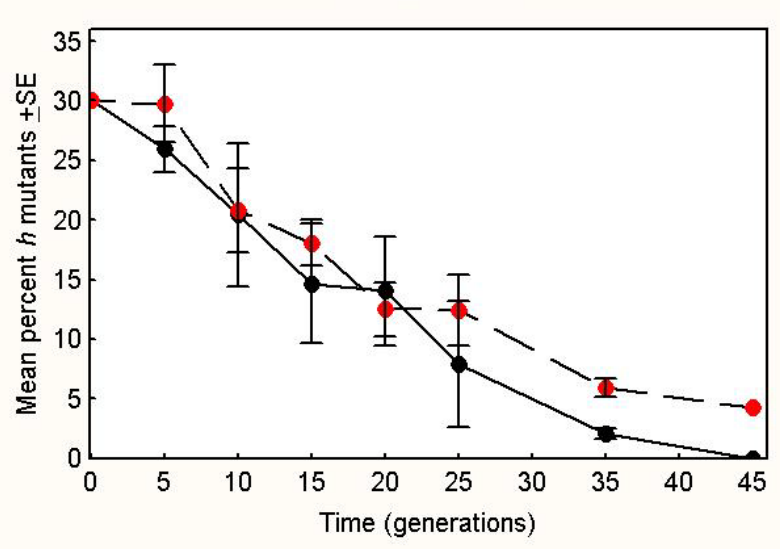
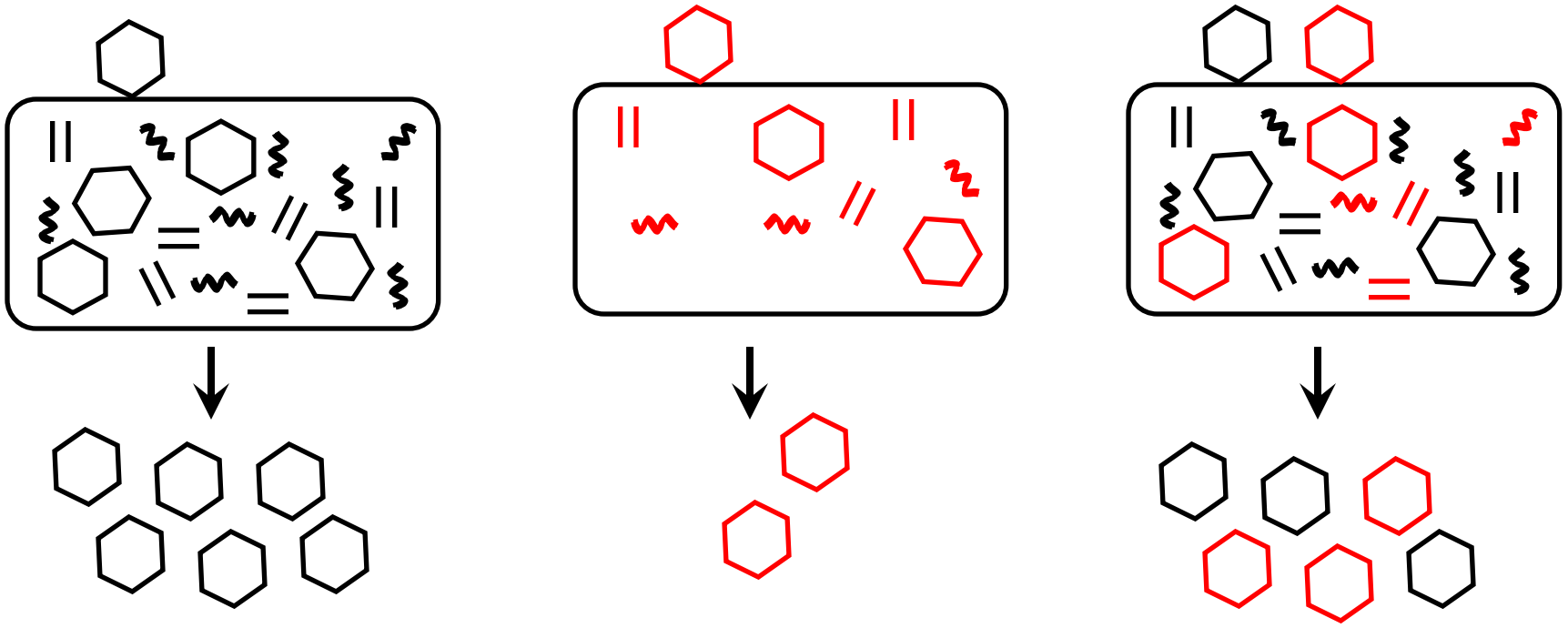






Froissart et al. 2004 *Genetics*

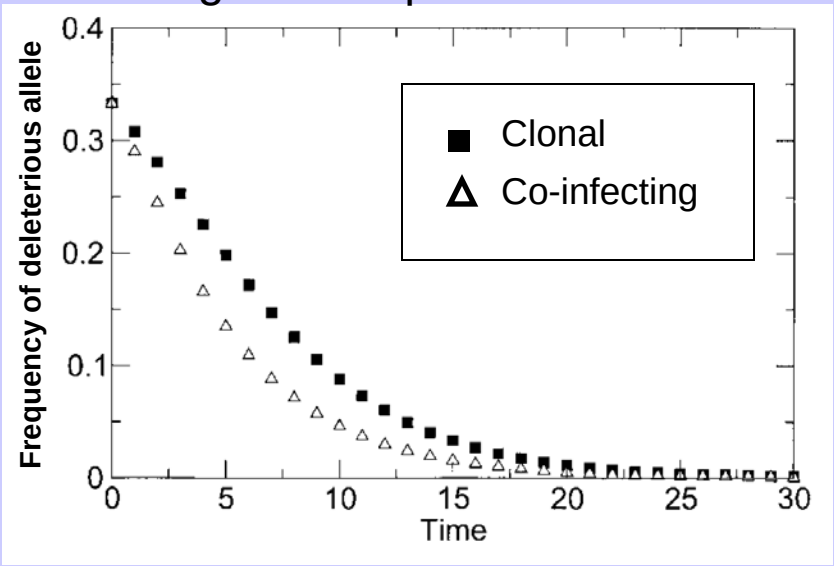
Mechanism: Complementation

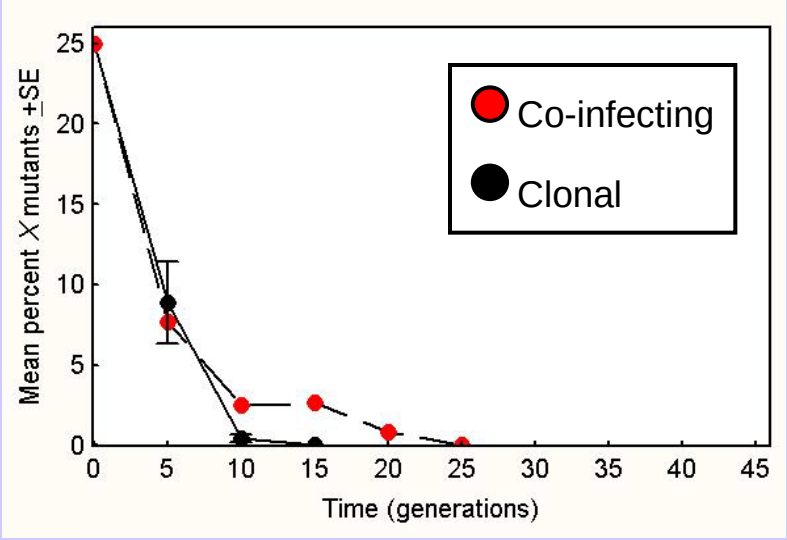


- Buffers mutational effects
- Selectable trait (Turner and Chao 1999, *Nature*)

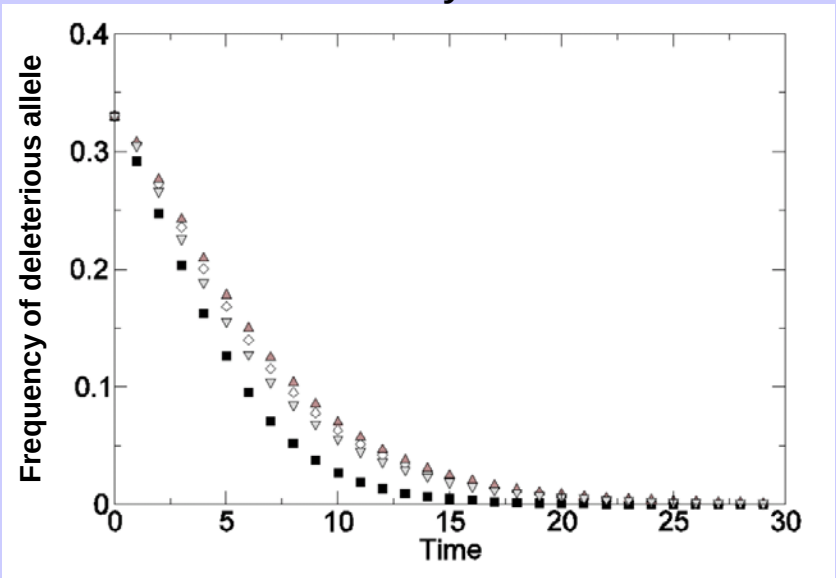
Effect of complementation on combating load

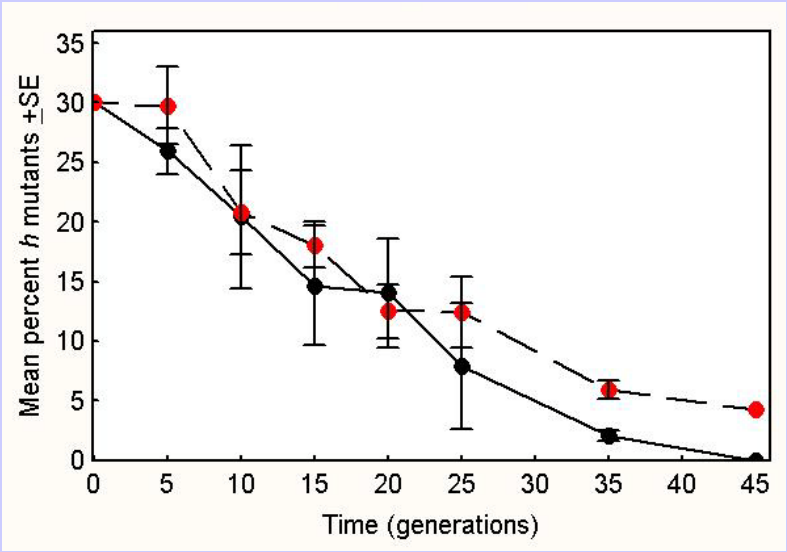
Advantage of sex prediction:





Refinement of theory:



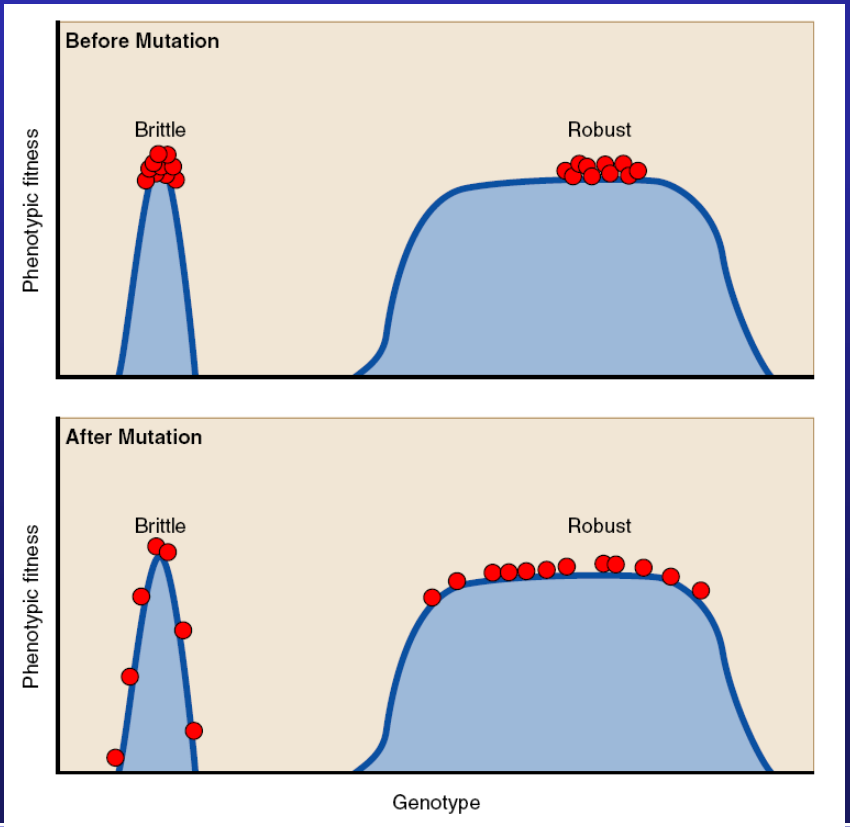


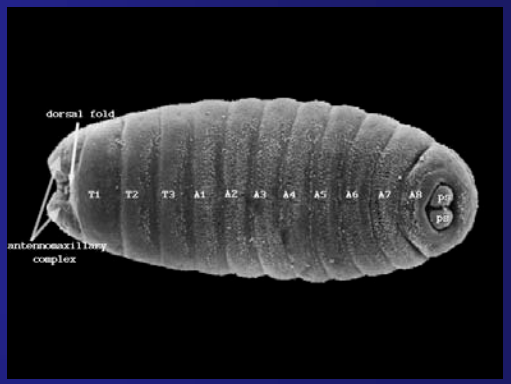
Froissart et al. 2004 *Genetics*

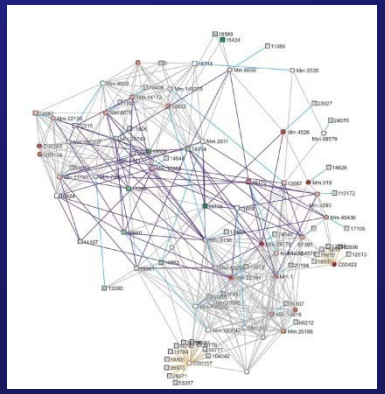
Mutational Robustness

Phenotypic constancy in face of mutational change

Critical for evolution: *Natural selection is fueled by phenotypically expressed genetic variation*





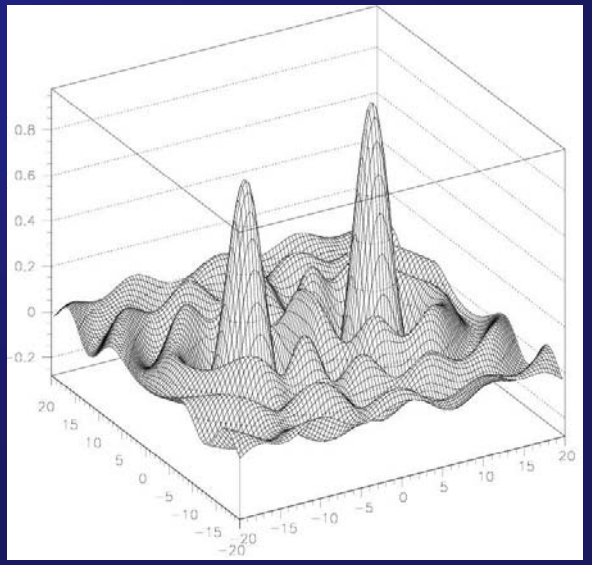


Mutational Robustness

Phenotypic constancy in face of mutational change

Critical for evolution: *Natural selection is fueled by phenotypically expressed genetic variation*

Abundant theory, few experiments

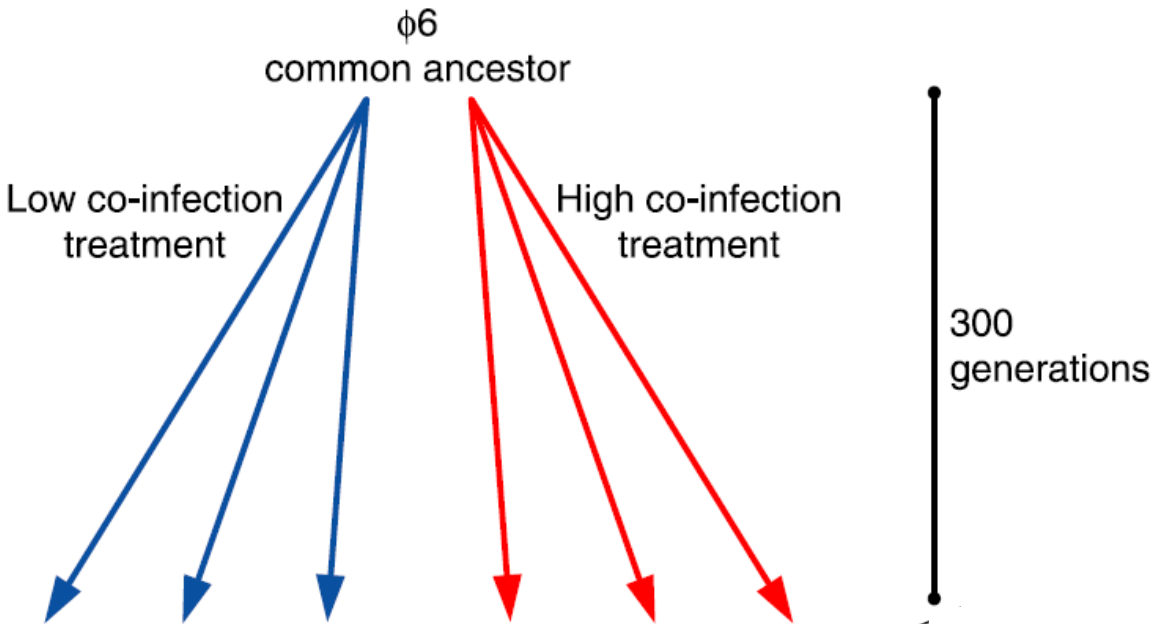


Co-infection and Robustness

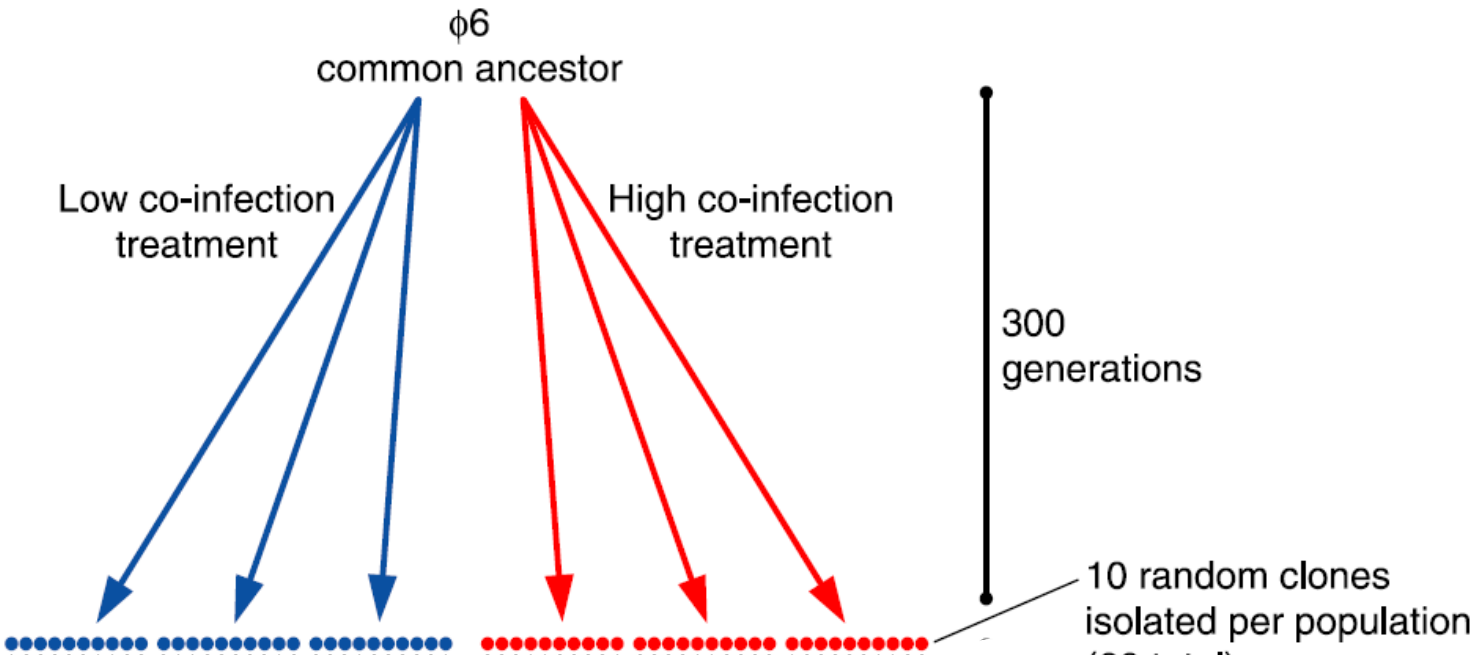
- Co-infection allows complementation
- Complementation is *built-in* robustness mechanism

THEREFORE, *Complementation (hence, co-infection) should weaken selection for individual robustness*

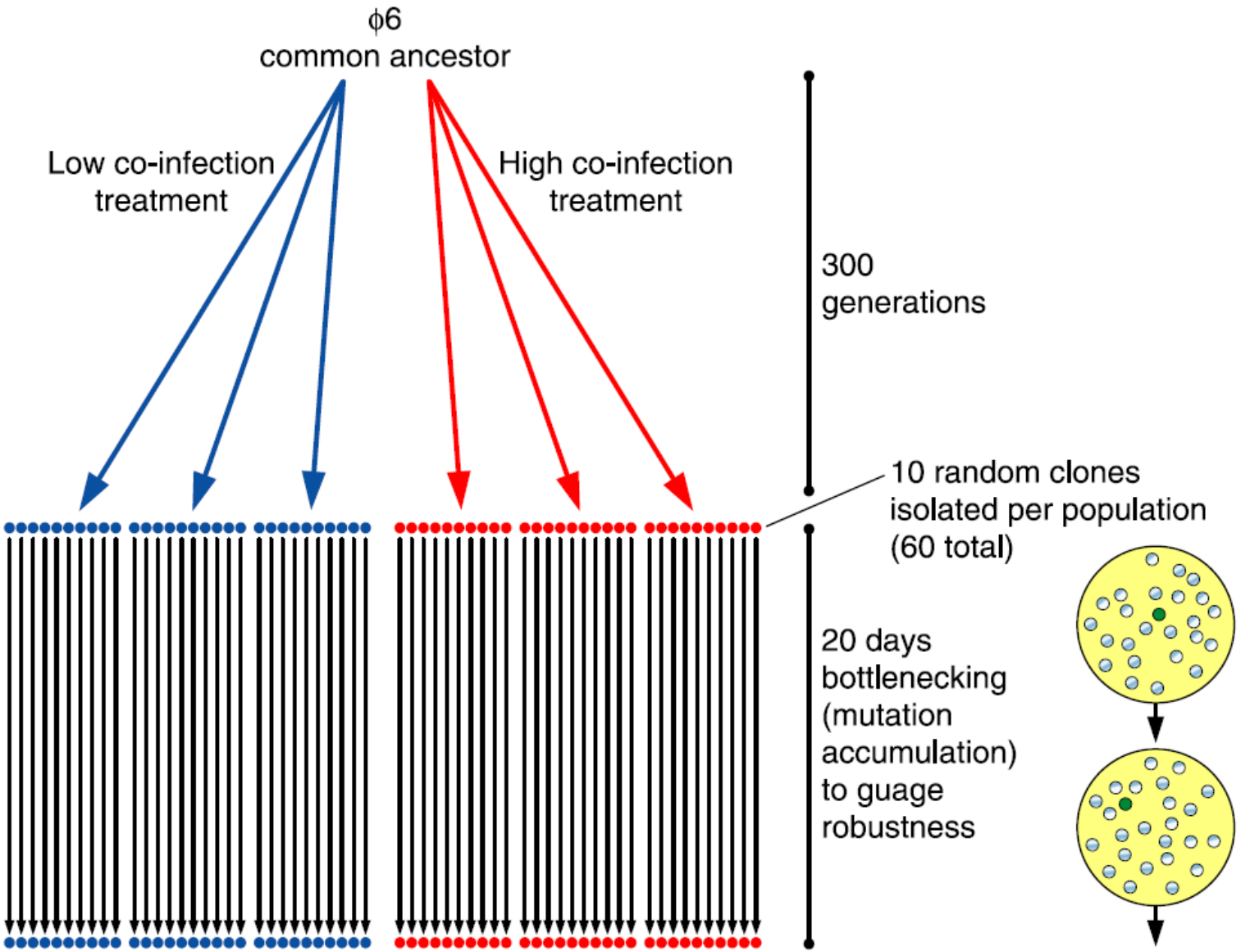
DESIGN: Does infection mode impact robustness?



DESIGN: Does infection mode impact robustness?



DESIGN: Does infection mode impact robustness?



Analysis

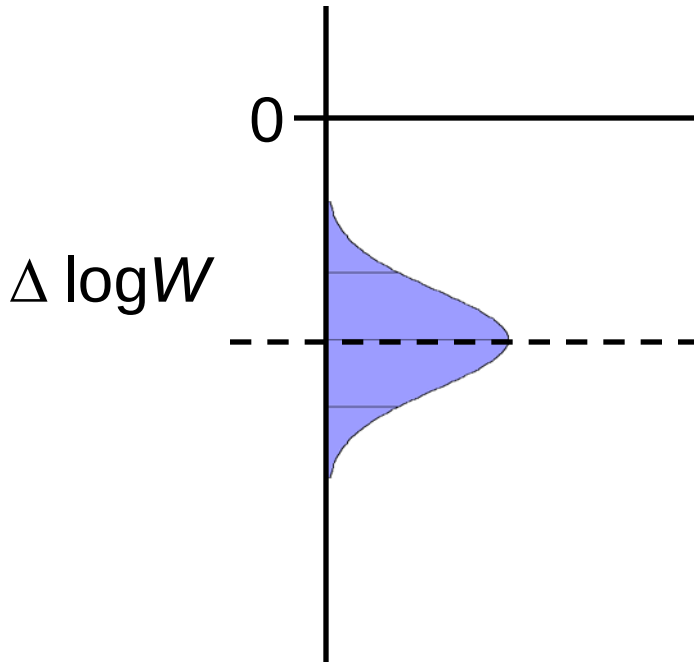
Measure fitness (W) for each lineage
before and after mutation accumulation

$$\Delta \log W = \log W_{\text{post-bottleneck}} - \log W_{\text{pre-bottleneck}}$$

Predictions:

$\text{Var}\ (\Delta \log W)_{\text{Clonal lineages}}$

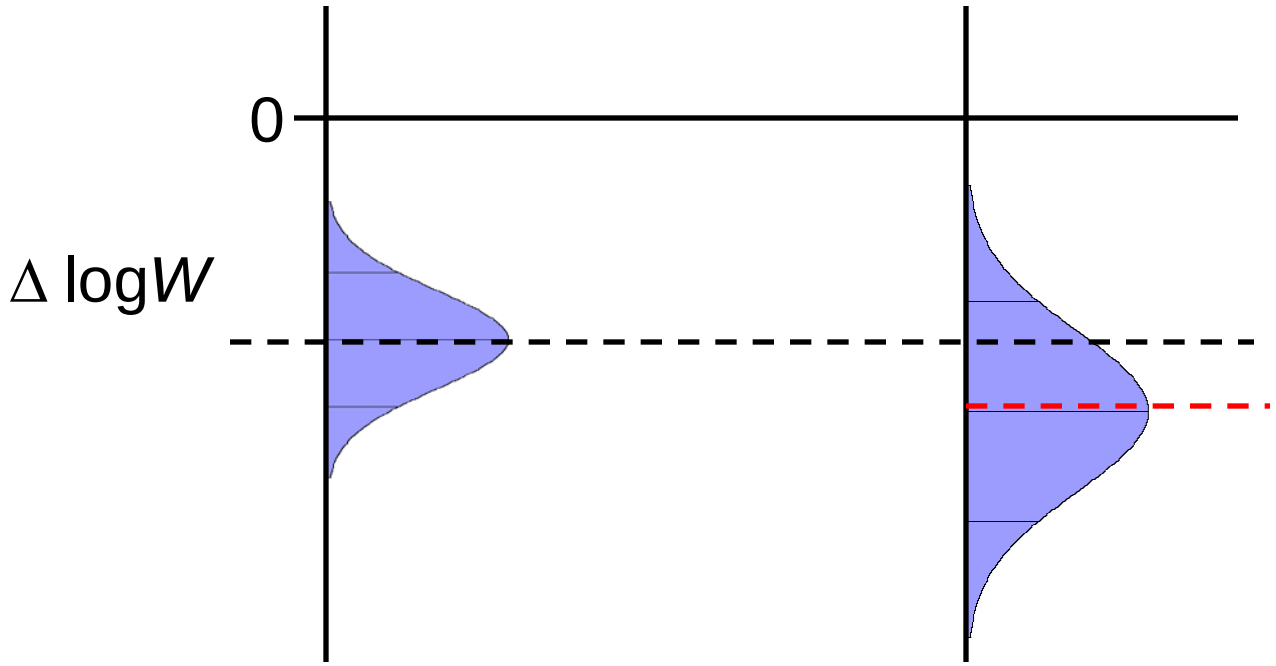
$\text{Mean}\ (\Delta \log W)_{\text{Clonal lineages}}$

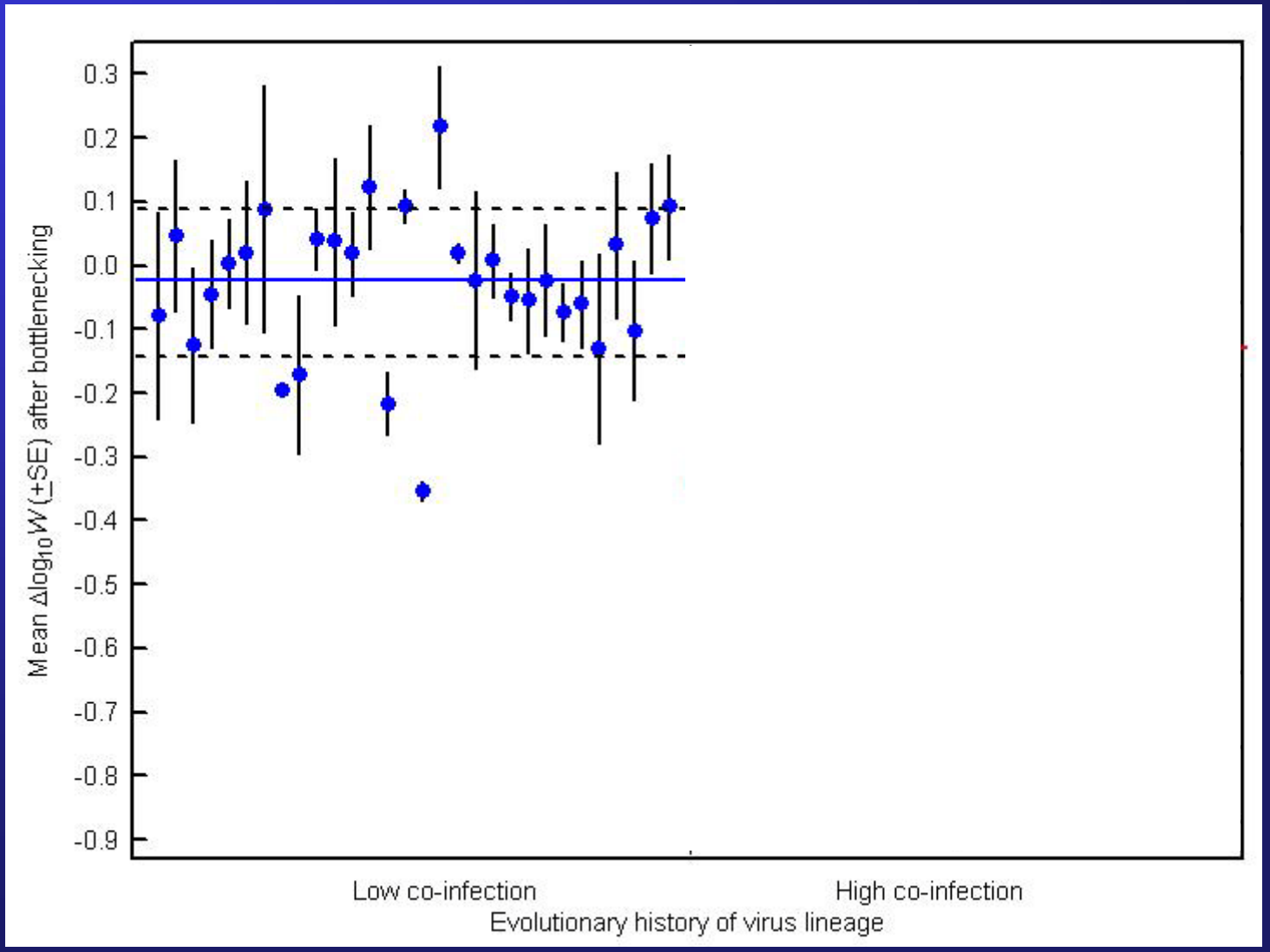


Predictions:

$$\text{Var}\,(\Delta \log W)_{\text{Clonal lineages}} < \text{Var}\,(\Delta \log W)_{\text{Co-infection lineages}}$$

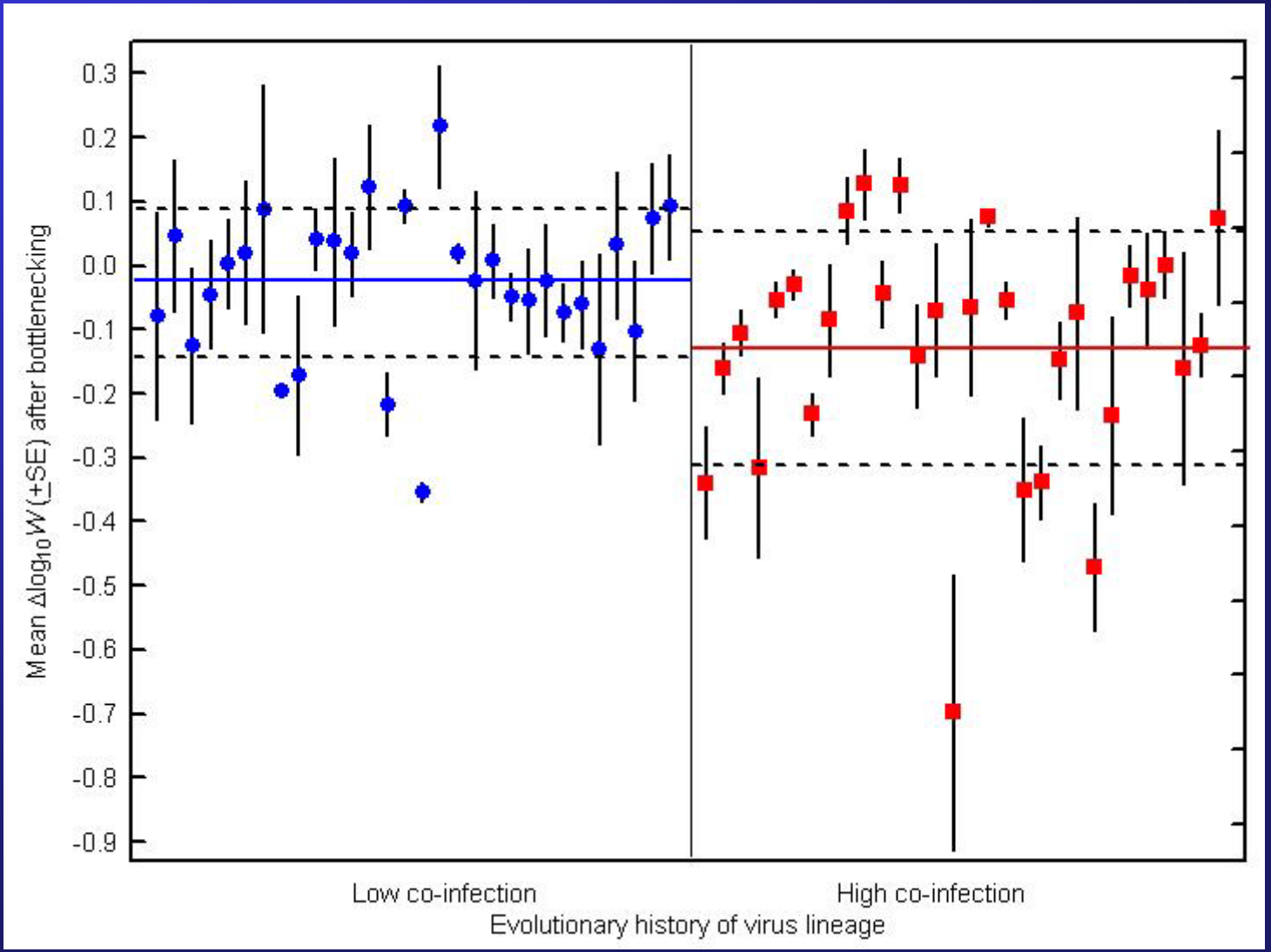
$$\text{Mean}\,(\Delta \log W)_{\text{Clonal lineages}} < \text{Mean}\,(\Delta \log W)_{\text{Co-infection lineages}}$$





Montville, Froissart et al. *PLoS Biology* 2005

Co-infecting viruses are less robust

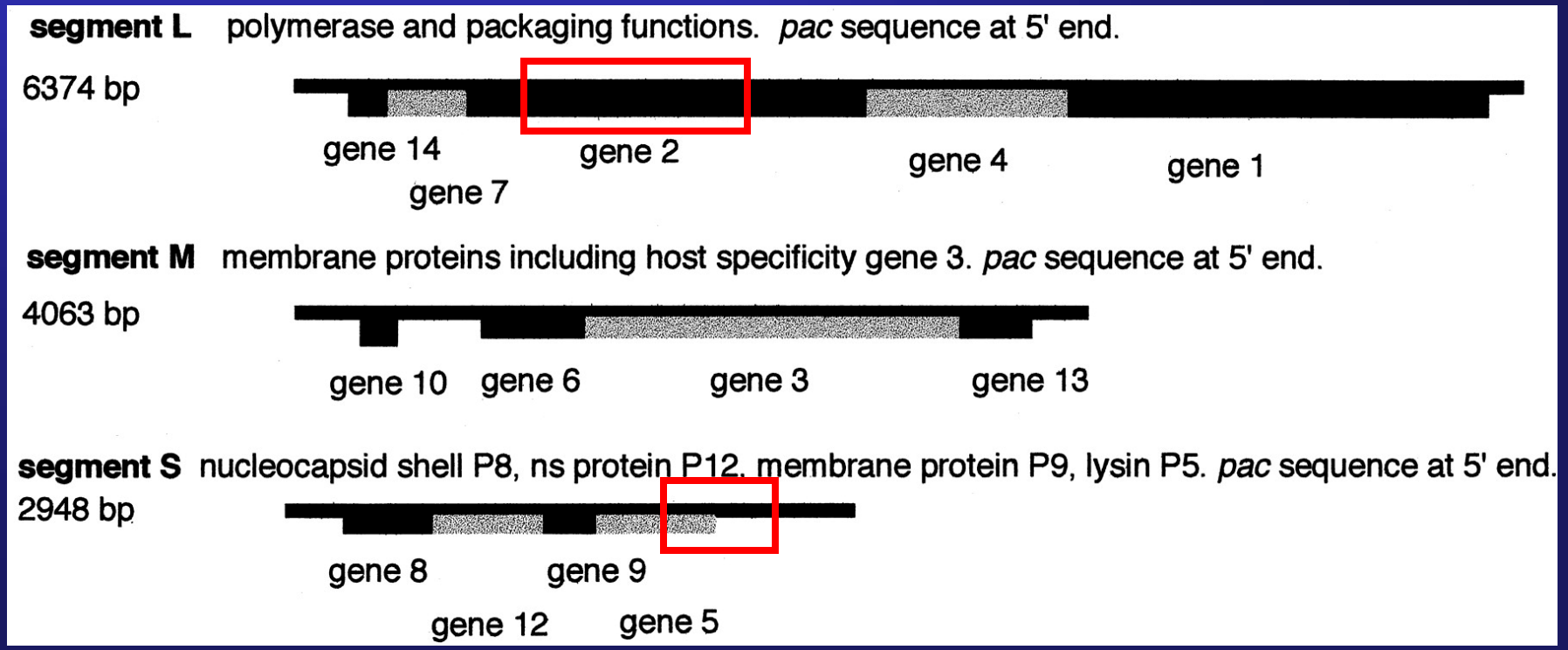


Montville, Froissart et al. *PLoS Biology* 2005

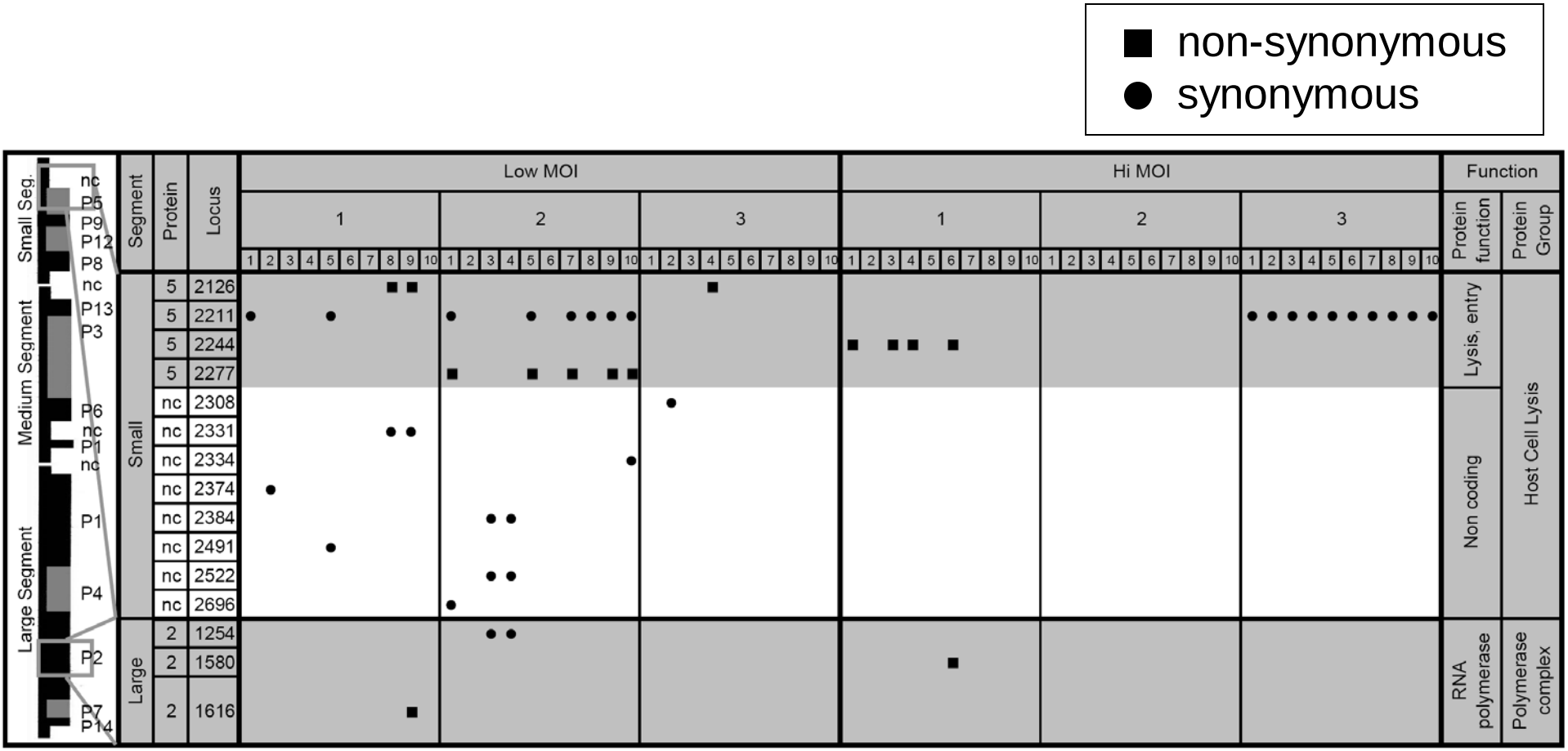
Molecular evidence?

Prediction:
Robust populations have more haplotypes & more substitutions/genotype

Analysis:
Sequenced regions of L and S segments for the 60 pre-bottleneck clones



Molecular evidence?



- more substitutions/genotype
- more inferred haplotypes

Duffy, Dennehy et al. (*unpubl*)

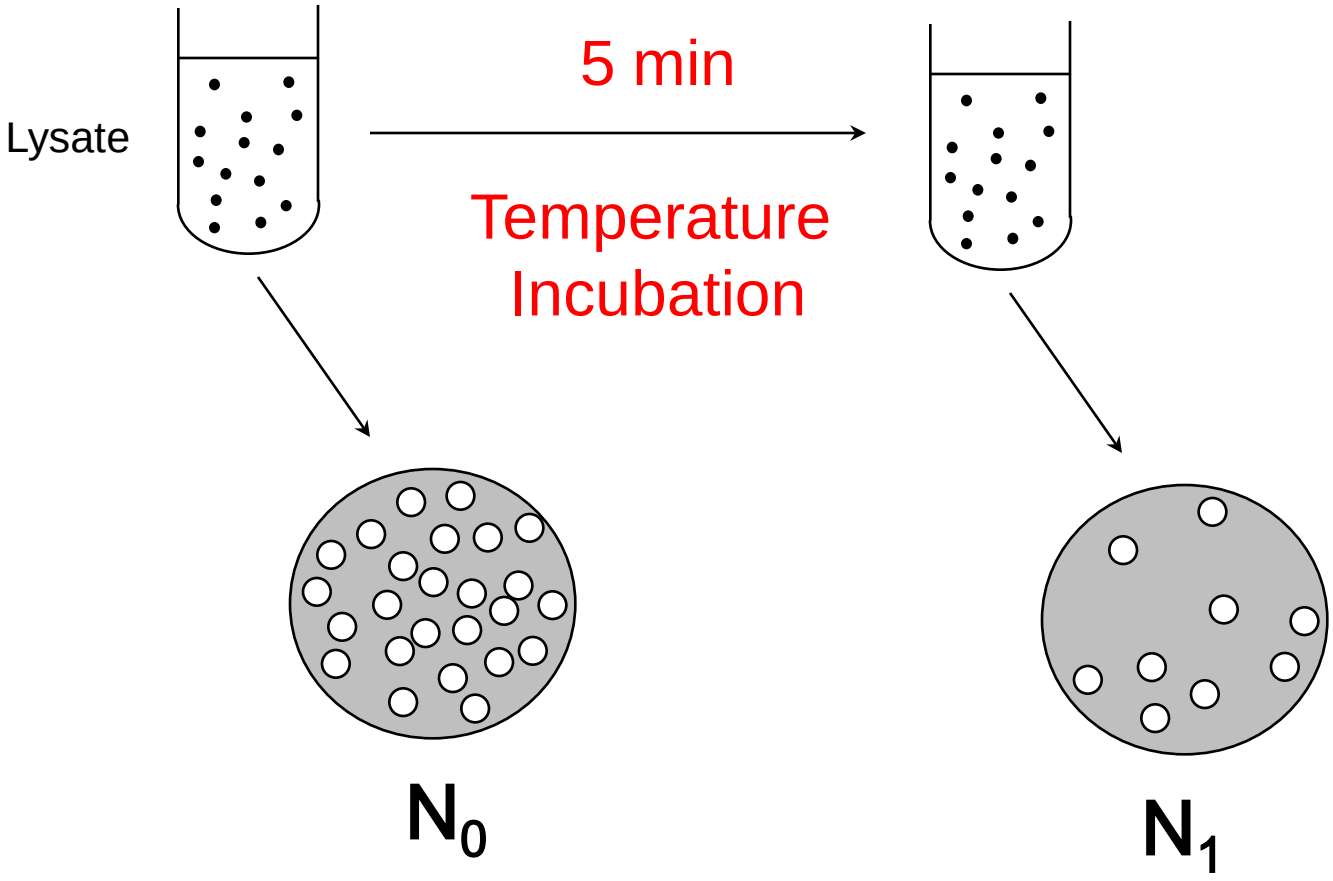
IS THERE A LINK BETWEEN ROBUSTNESS AND EVOLVABILITY?

Does robustness promote evolvability?

NO: Robustness reduces phenotypic variation, thereby impeding selection

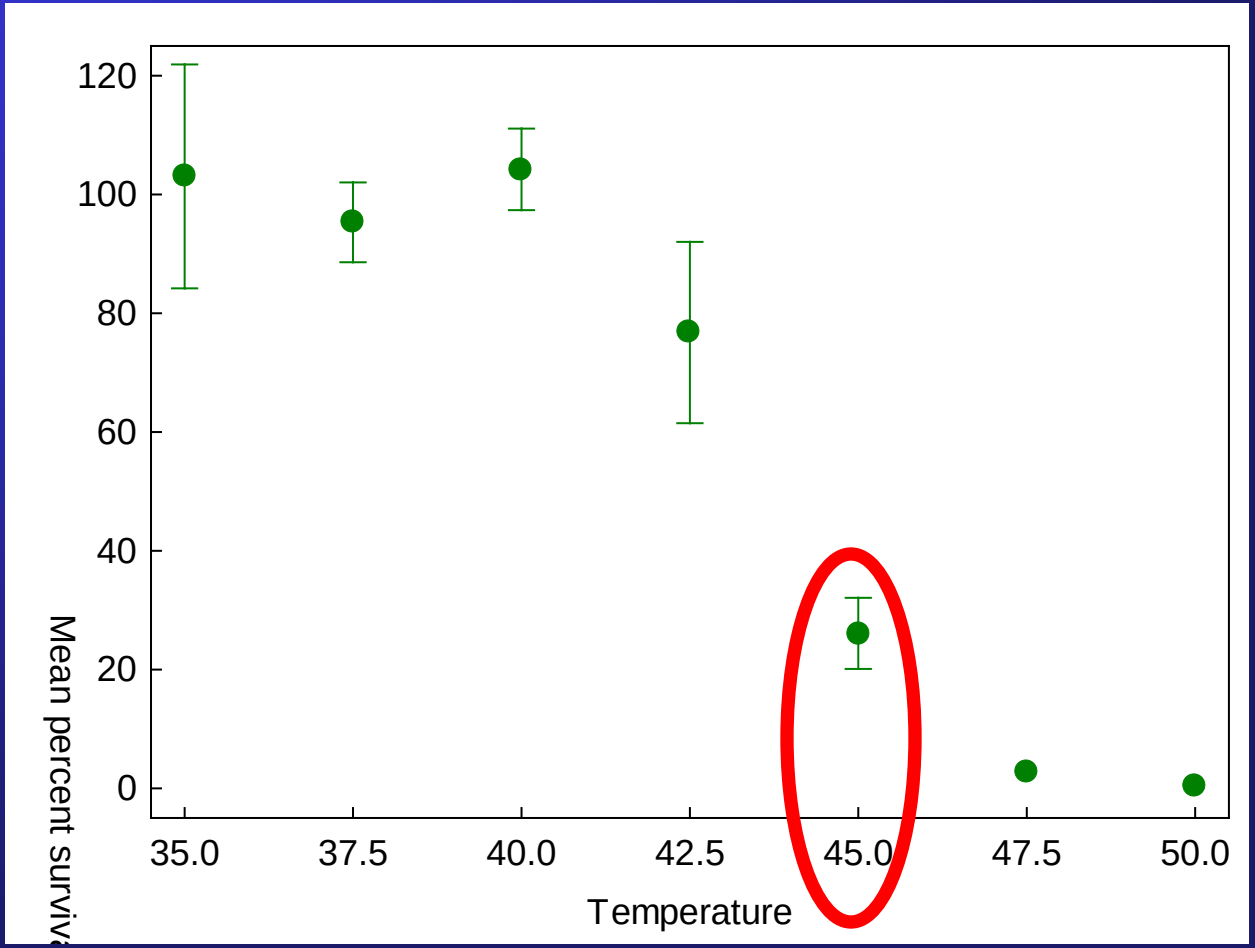
YES: Robustness allows protein folding/stability despite mutation, facilitating protein innovation

Temperature Survival Assay



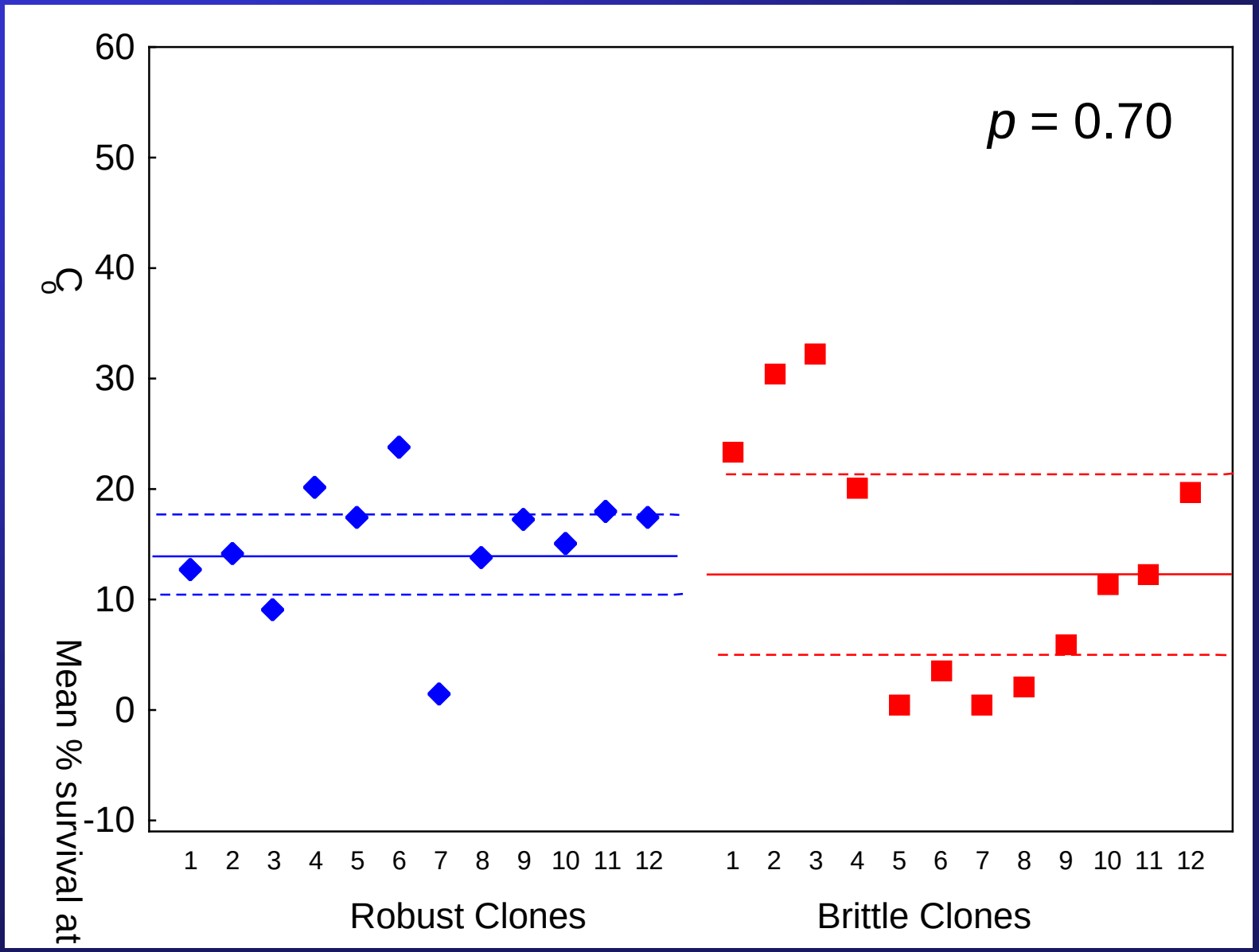
$$\% \text{ Survivors} = (N_1/N_0) * 100$$

Reaction norm for wild type



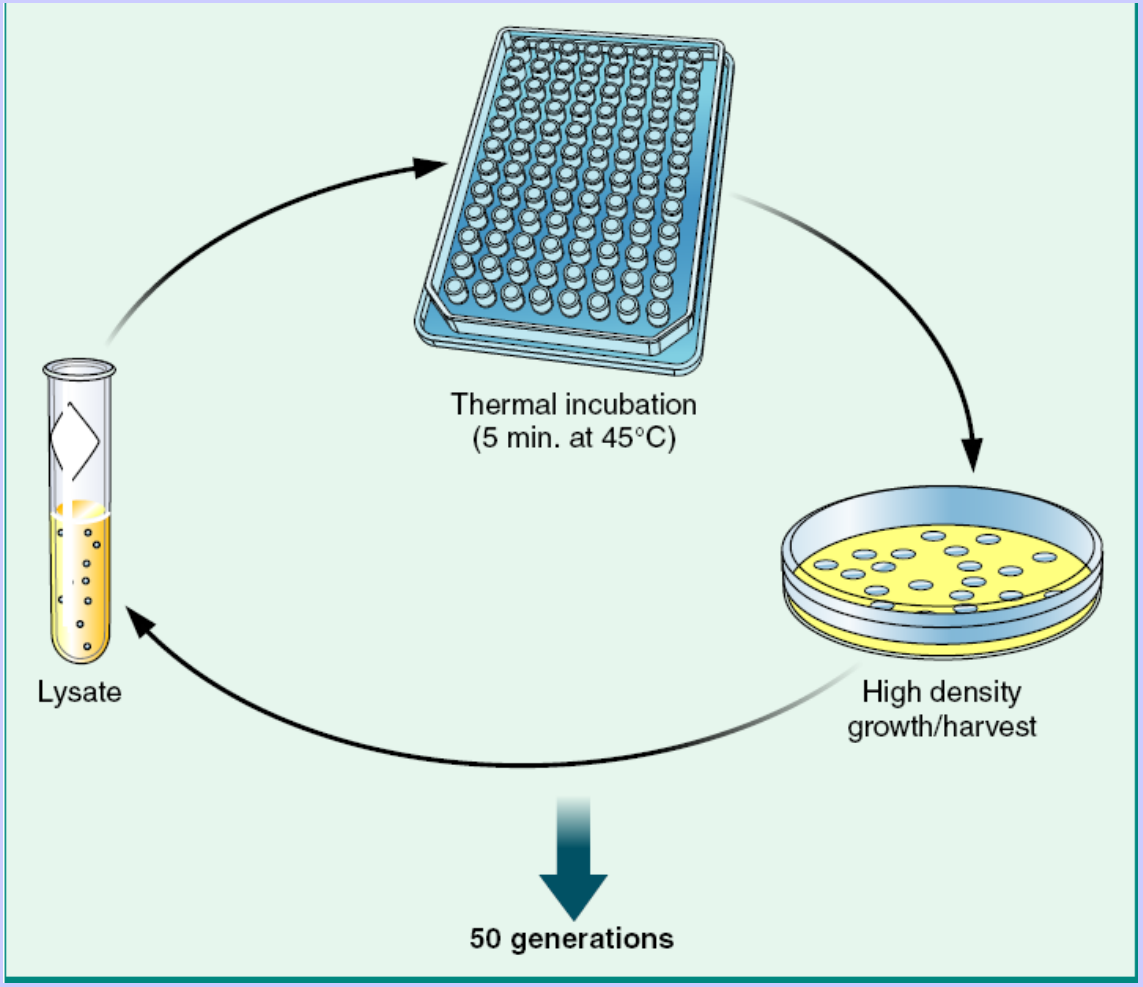
McBride et al. *BMC Evol Biol* 2008

Robust and brittle clones survive equally at 45ºC

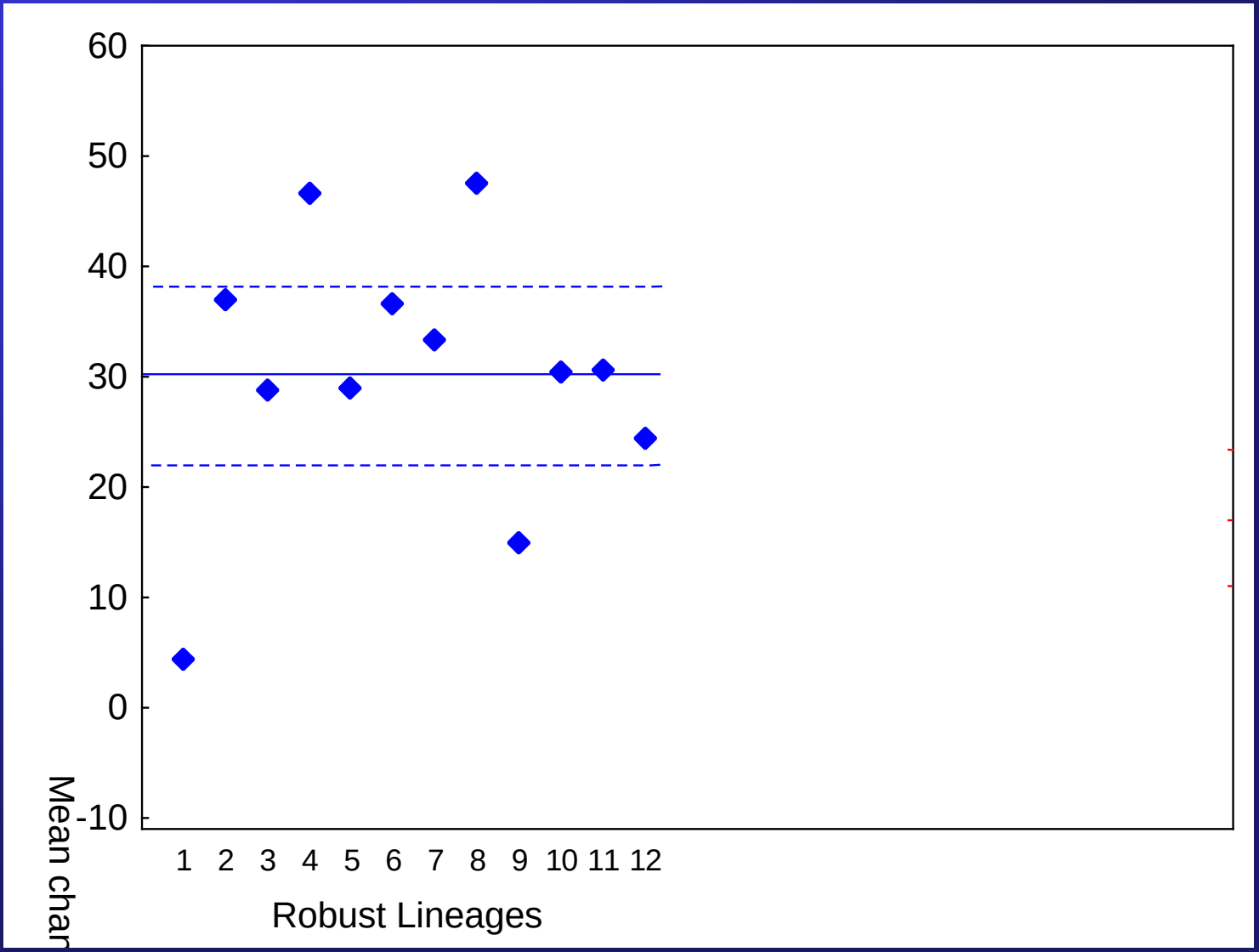


McBride et al. *BMC Evol Biol* 2008

Do robust clones show greater evolvability?

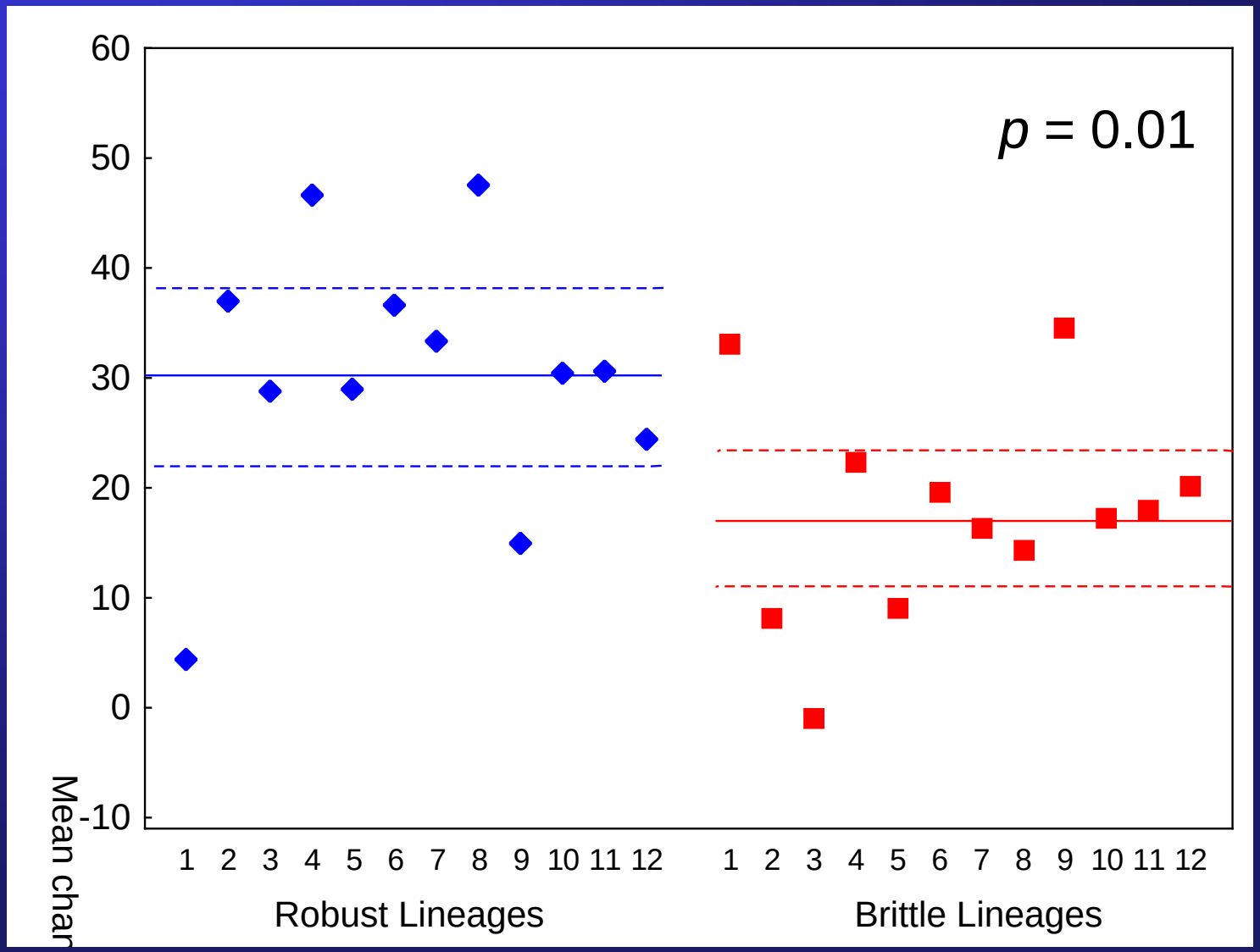


$$\overline{\Delta\%S} = \overline{\%S}_{\text{Evolved}} - \overline{\%S}_{\text{Ancestral}}$$



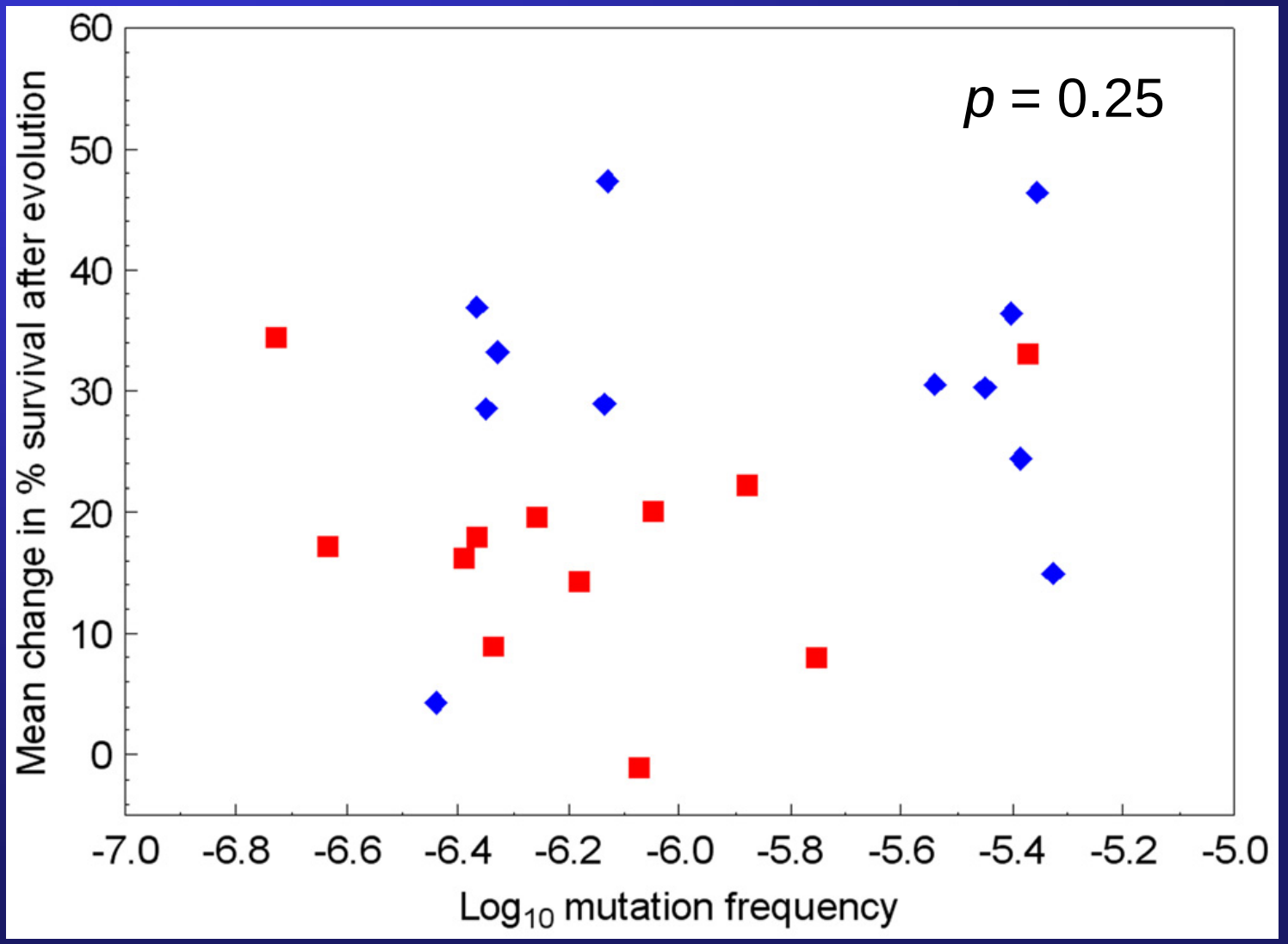
McBride et al. *BMC Evol Biol* 2008

Lineages founded by robust viruses are more evolvable



McBride et al. *BMC Evol Biol* 2008

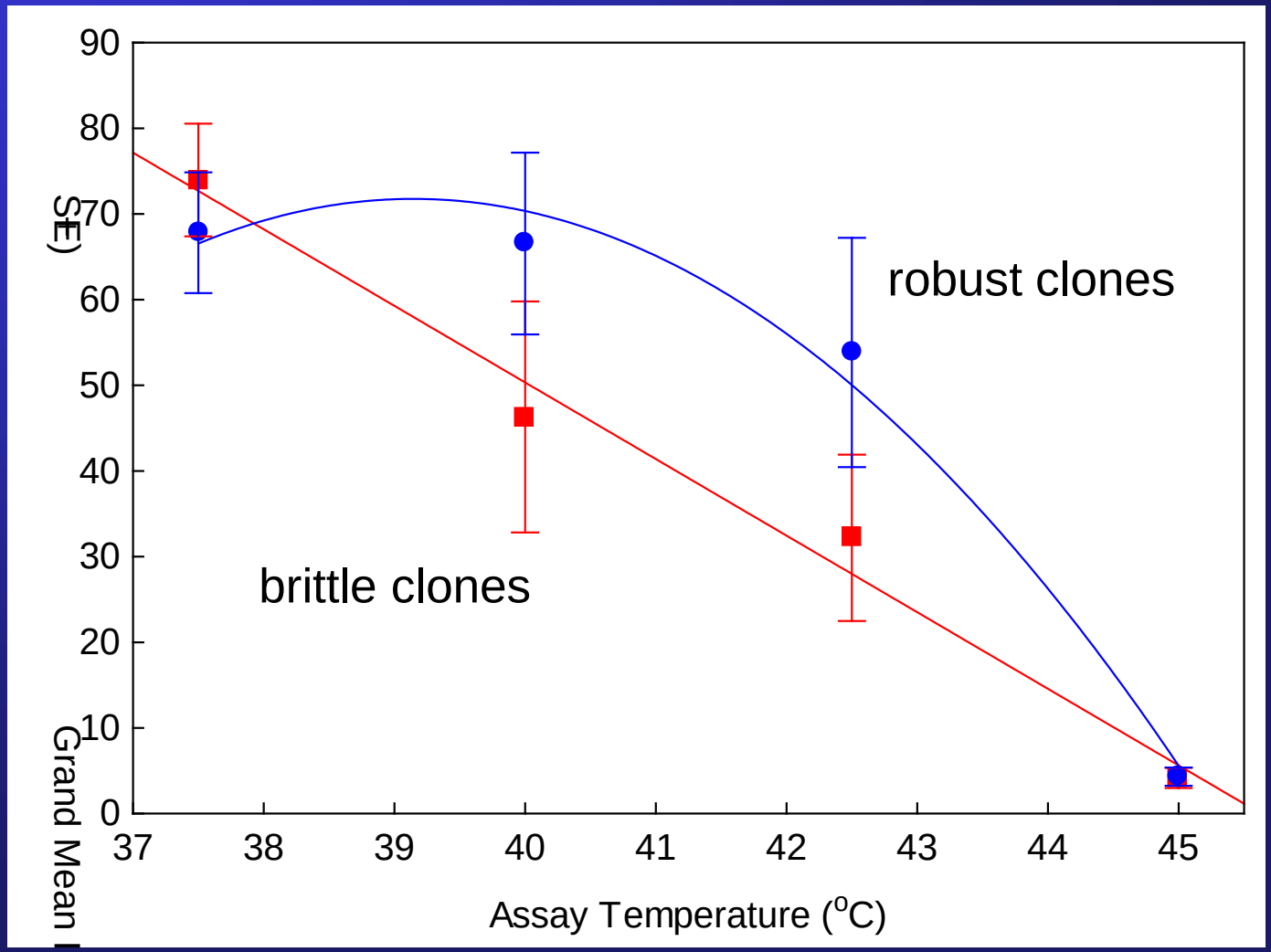
Mutability does not explain differences in evolvability



McBride et al. *BMC Evol Biol* 2008

Mechanism for genetic robustness?

Protein stability/thermotolerance



Ogbunugafor et al. 2009

Summary 1

- Co-infection (complementation) weakens selection to maintain robustness
- Robust viruses more evolvable under heat shock
- Evolution itself has capacity to evolve

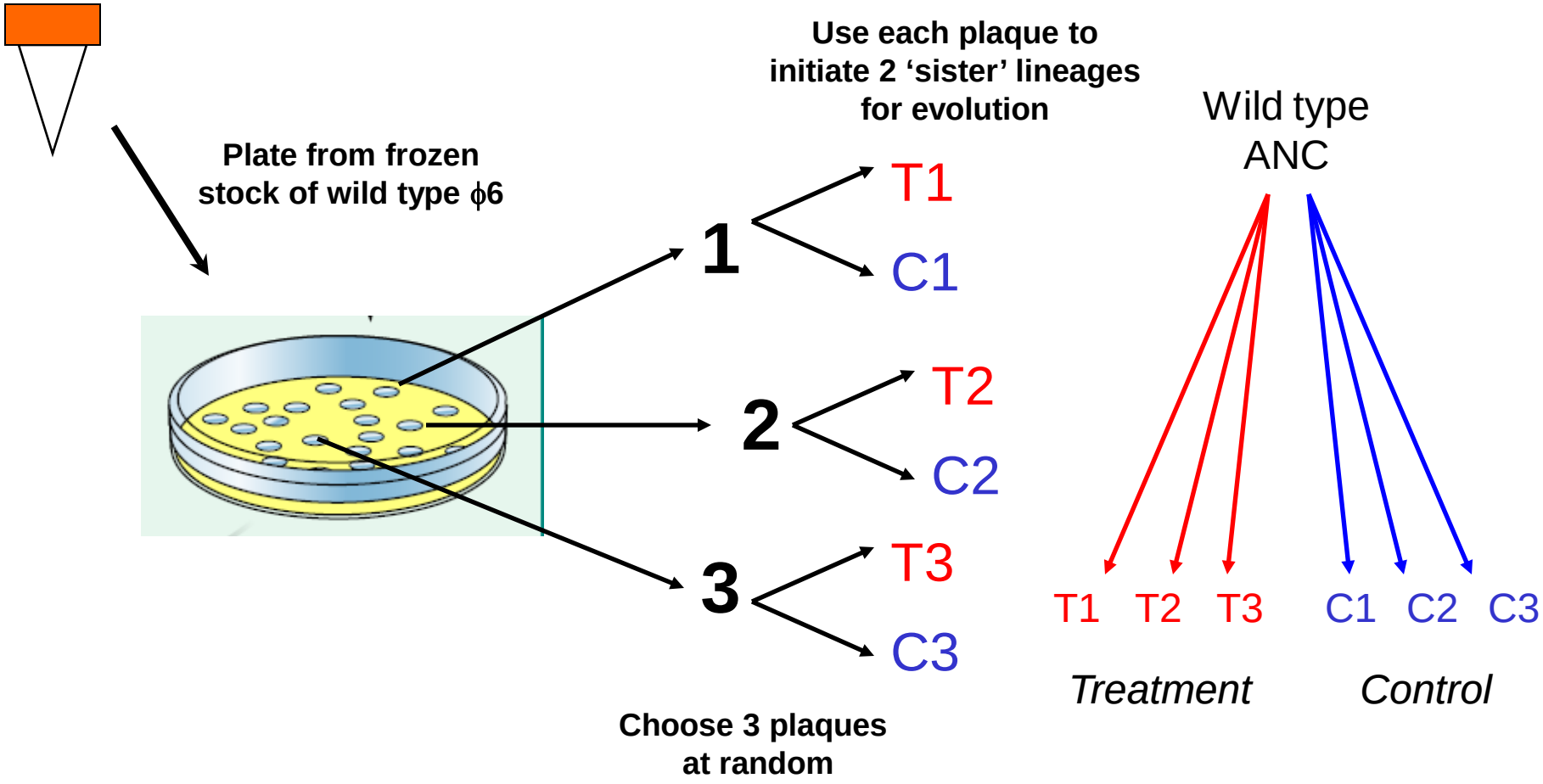
CAN ROBUSTNESS BE SELECTED?

How can robustness be selected in phage ϕ6?

- Evolved changes in robustness led to differential evolvability of thermotolerance
- Robustness and thermotolerance seem to be correlated
- Thus, selection for thermotolerance should yield robustness i.e., *Bidirectional Selection* should be possible

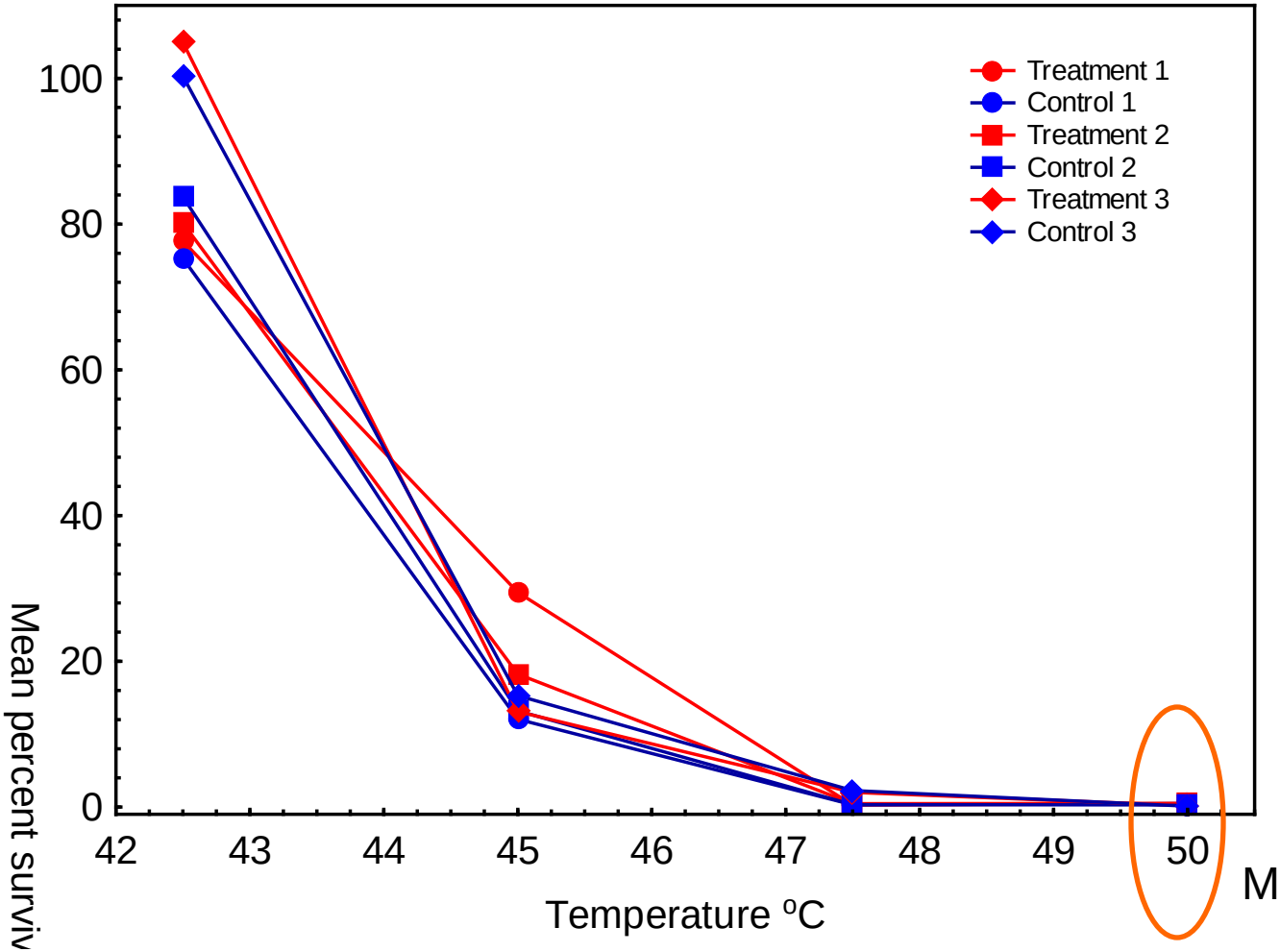
HYPOTHESIS: *Robustness should evolve as a by-product of evolved thermotolerance*

Isolation of ancestor clones



McBride and Turner (unpublished)

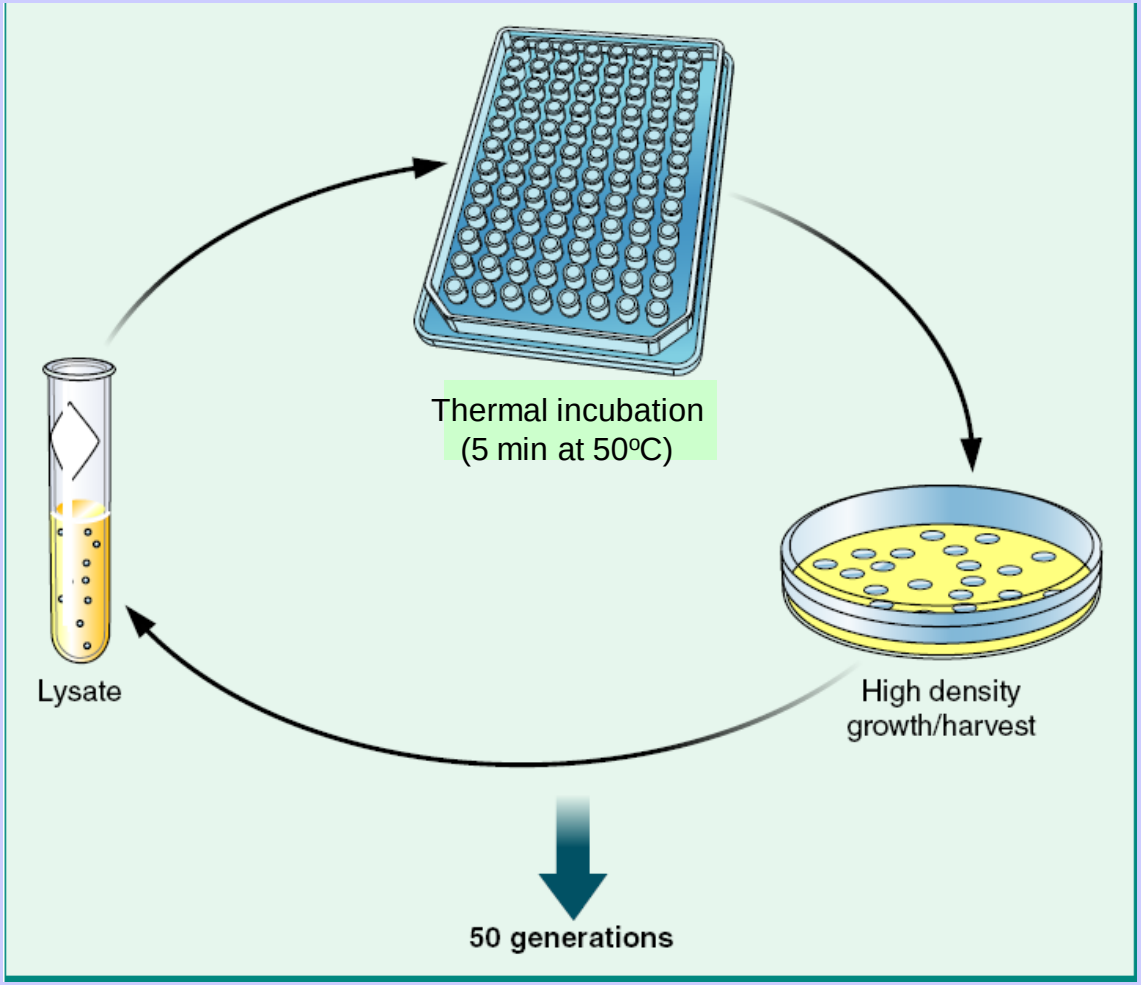
Pre-evolution reaction norm

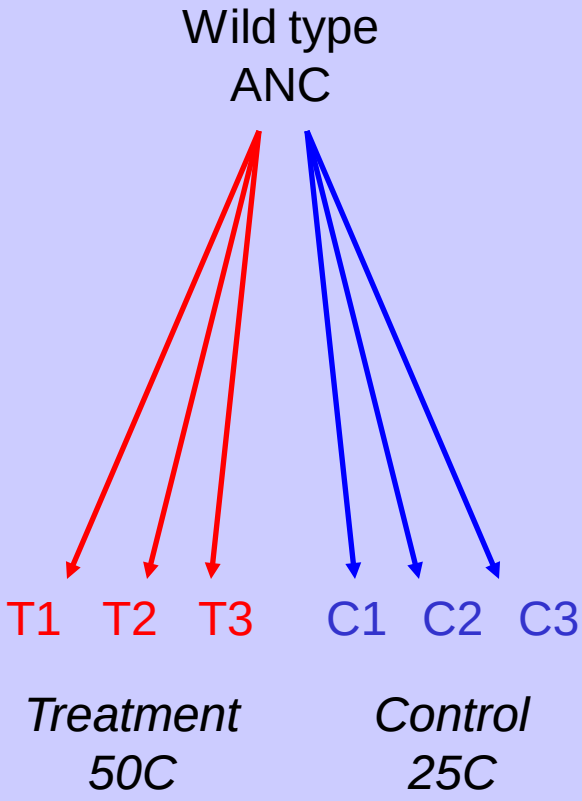


Mean %S ≈ 0.05

McBride and Turner (unpublished)

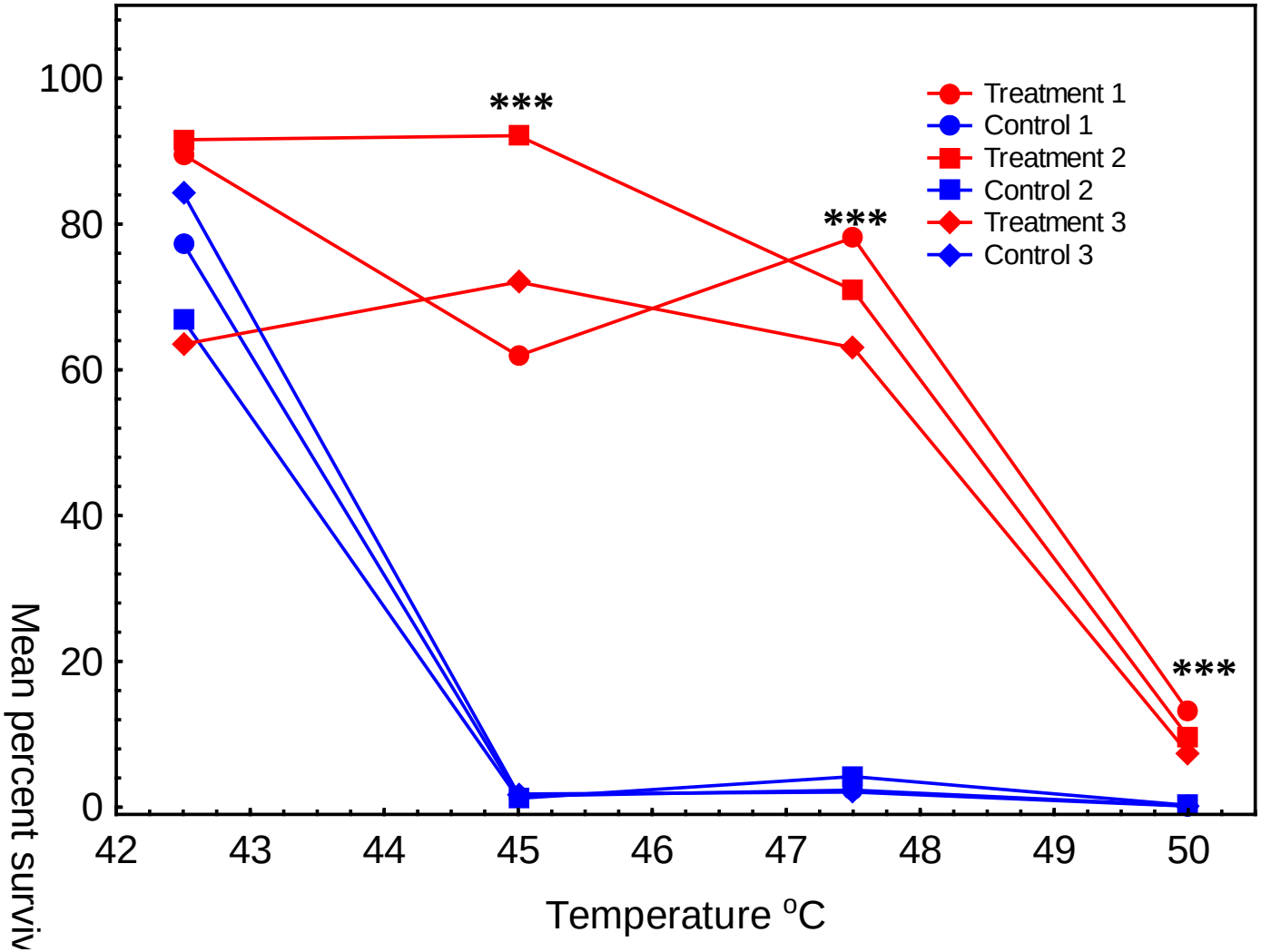
Experimental design





McBride and Turner (unpublished)

Post-evolution reaction norm



McBride and Turner (unpublished)

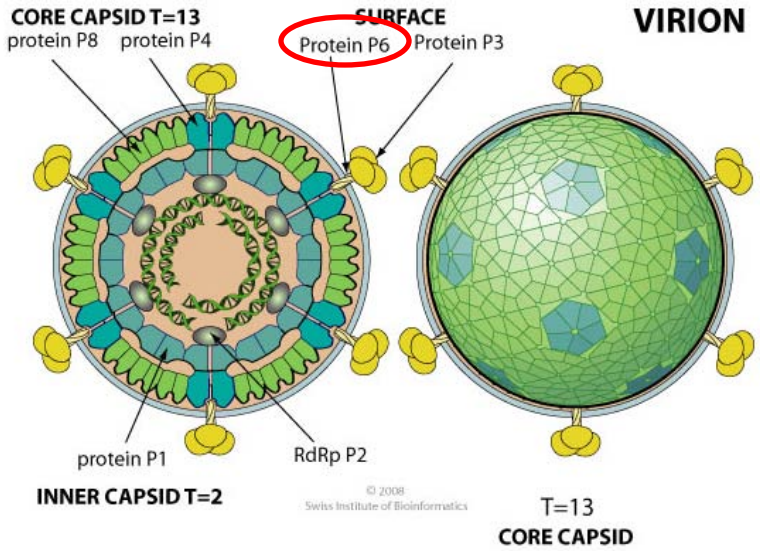
Testing for the bidirectional response

- Treatment populations survive heat shock better than controls

- Are they also more robust against mutations?

Testing for the bidirectional response

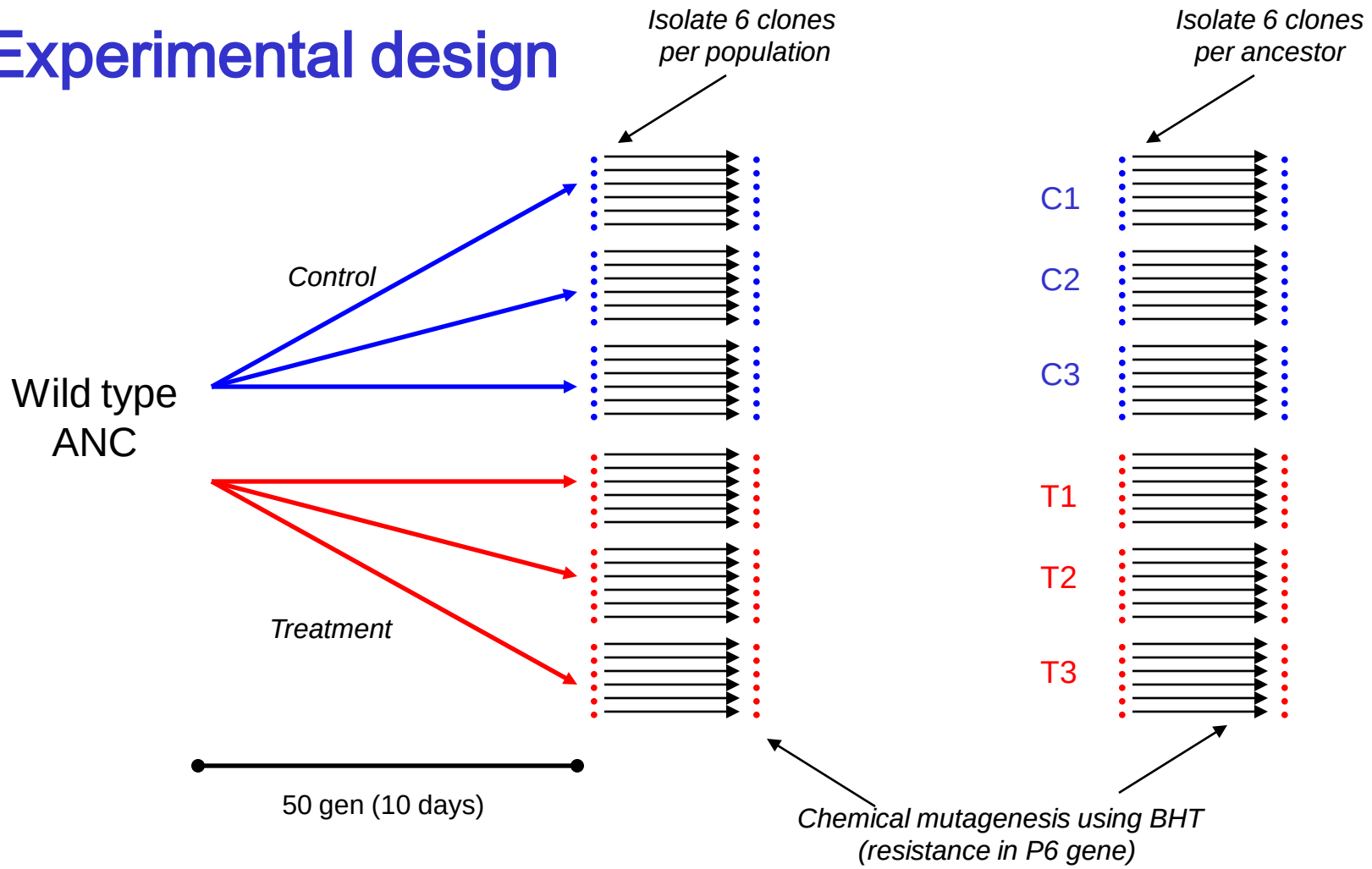
Experimental design



McBride and Turner (unpublished)

Testing for the bidirectional response

Experimental design

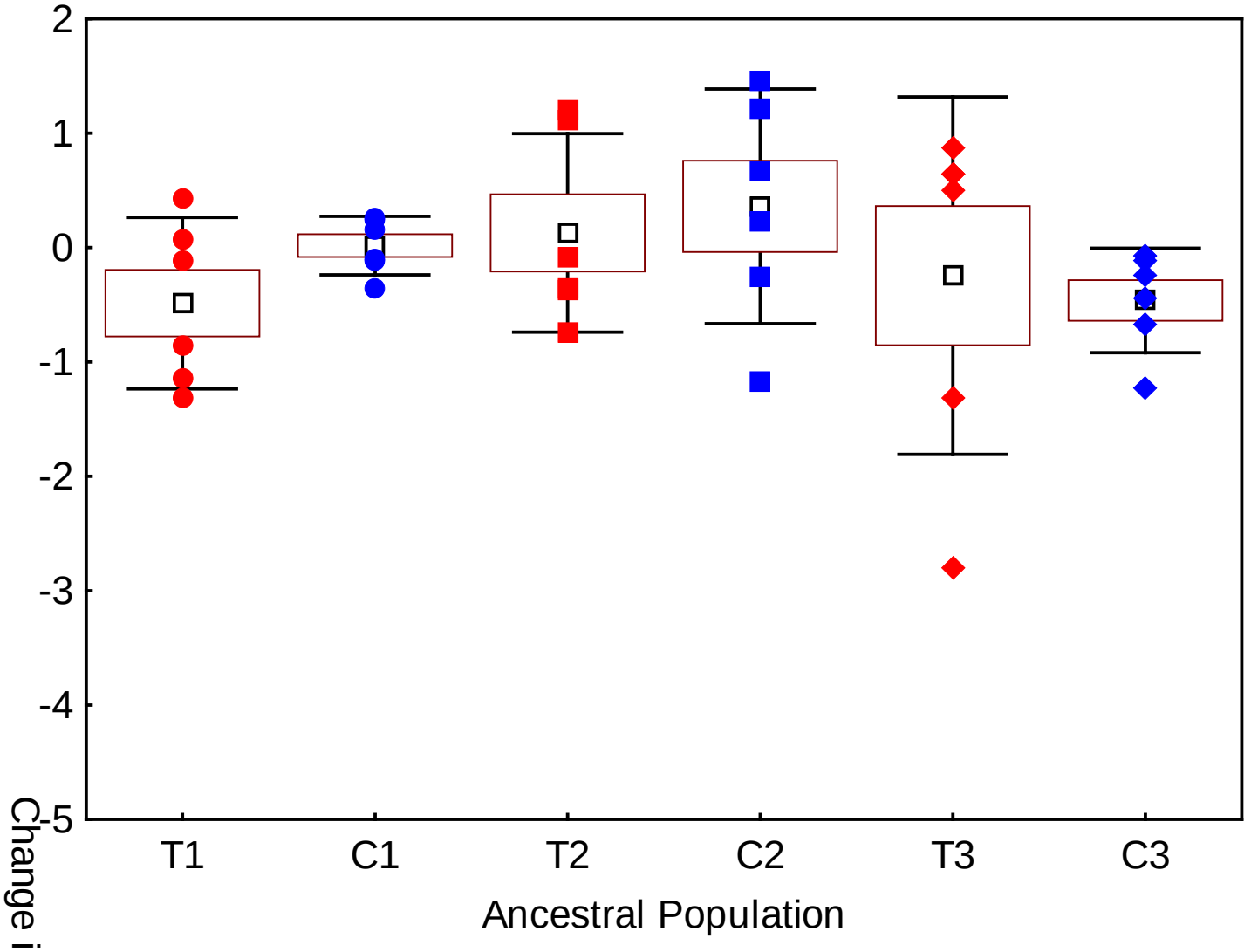


McBride and Turner (unpublished)

Measure fitness (*W*) on *P. phaseolicola* for each set of ancestor clones before and after mutagenesis

$$\Delta \log W = \log W_{\text{post-BHT}} - \log W_{\text{pre-BHT}}$$

Pre-evolution robustness



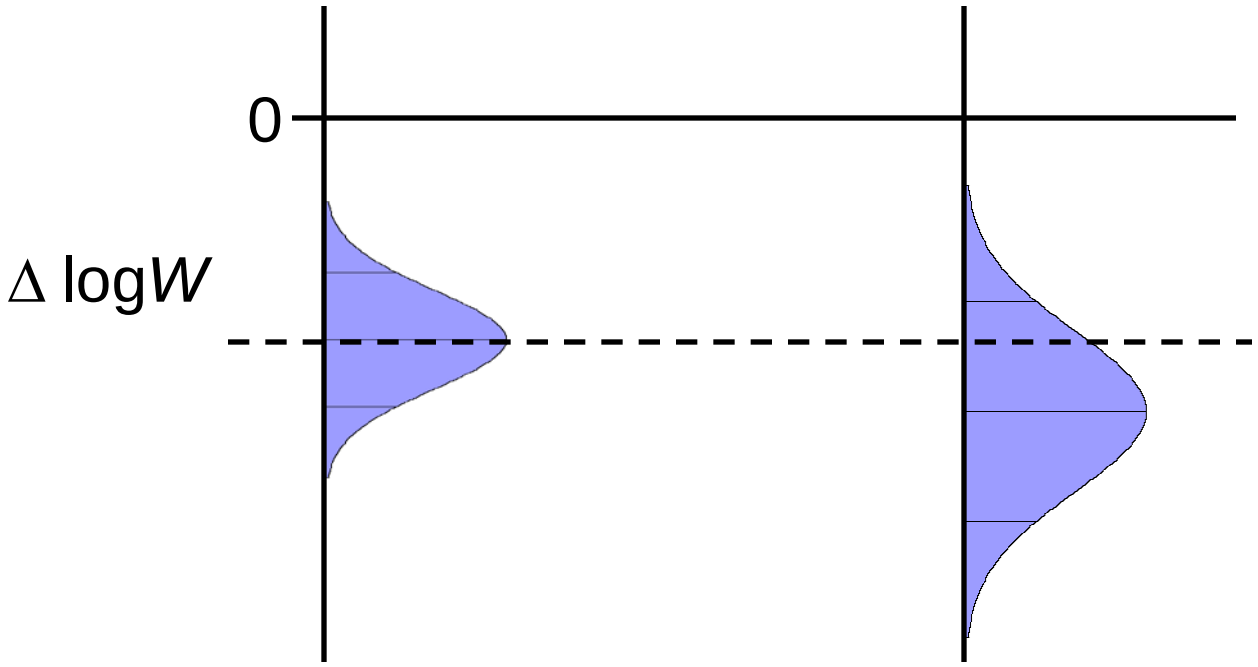
McBride and Turner (unpublished)

Measure fitness (*W*) on *P. phaseolicola* for each ancestor and evolved lineage before and after mutagenesis

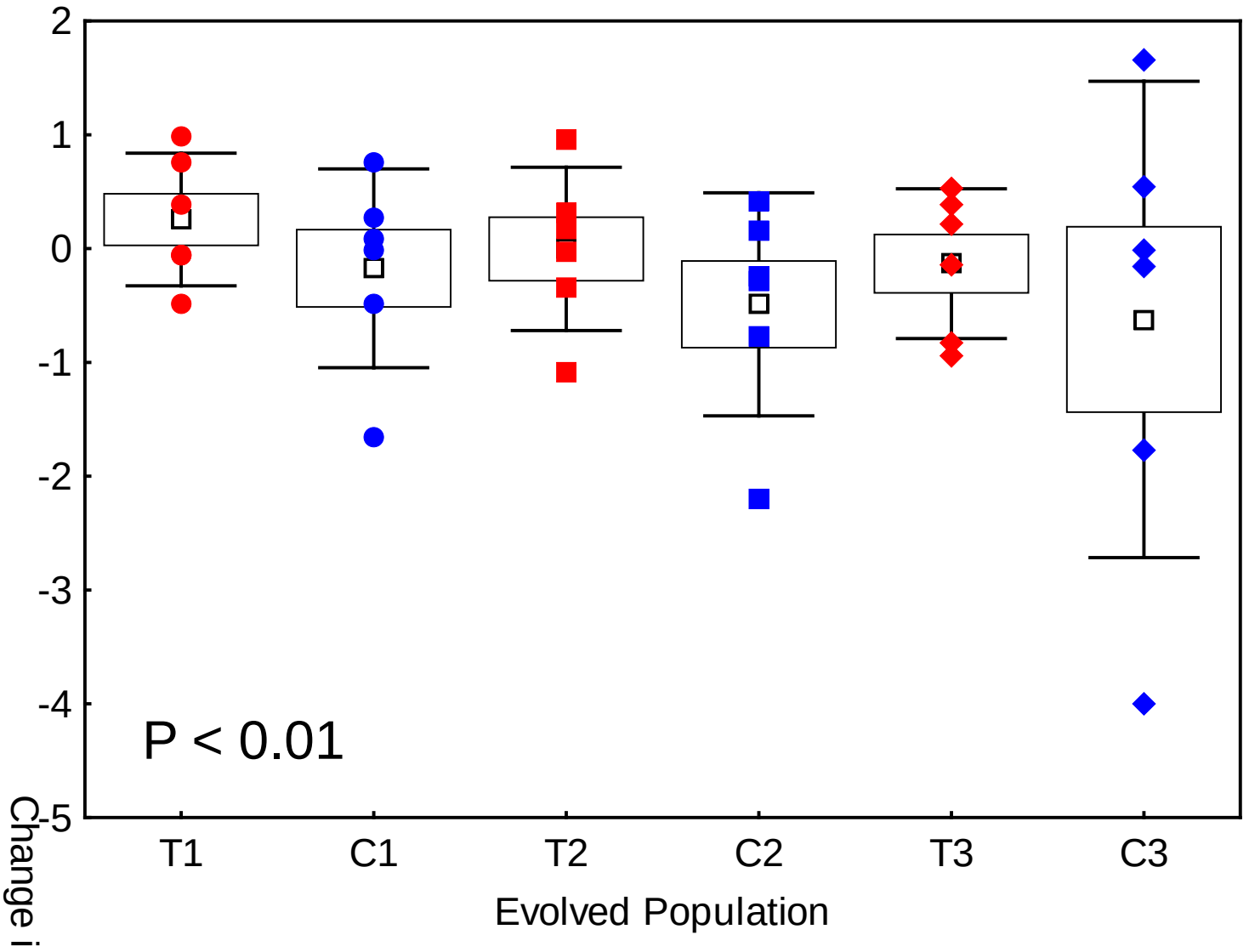
$$\Delta \log W = \log W_{\text{post-BHT}} - \log W_{\text{pre-BHT}}$$

Prediction following evolution:

$$\text{Var}\,(\Delta \log W)_{\text{Treatment lineages}} < \text{Var}\,(\Delta \log W)_{\text{Control lineages}}$$



Post-evolution robustness



- Evolved thermotolerance fosters robustness
- Mutation accumulation assays underway

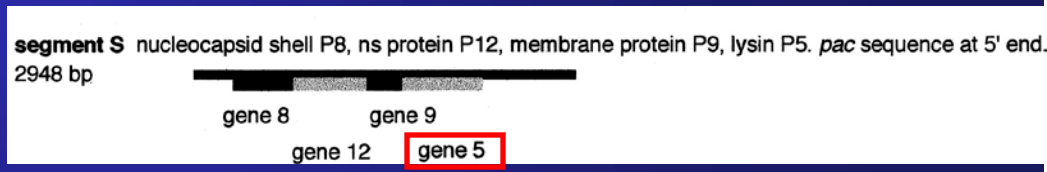
McBride and Turner (unpublished)

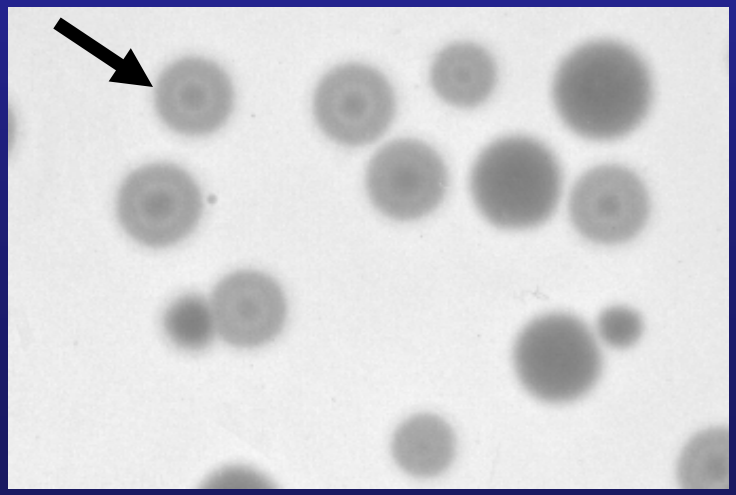
What is the mechanism?

3 thermotolerance evolution studies

- Robust vs. Brittle clones evolved at 45°C
  (McBride et al. 2008)
- Robust vs. Brittle populations evolved at 45°C
  (Goldhill and Turner, unpubl.)
- Wild type clones evolved at 50°C
  (McBride and Turner, unpubl.)

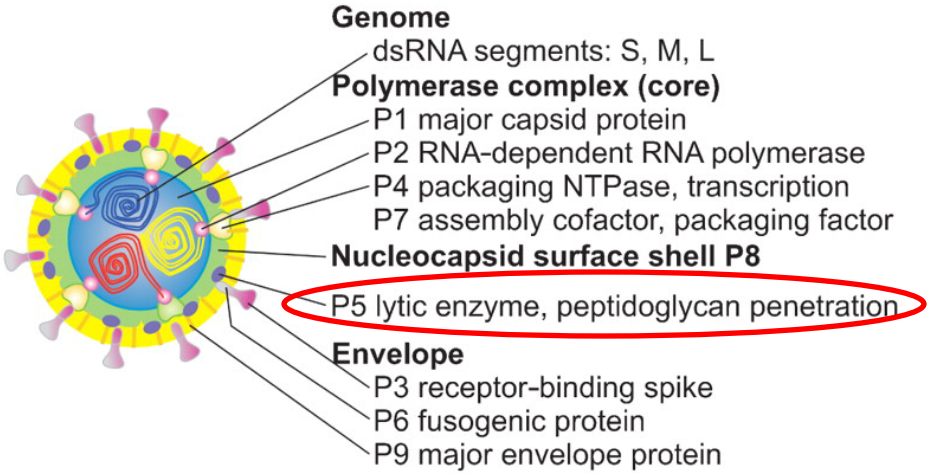
S segment:
P5 lysin gene mutation
G2238U transversion V→F

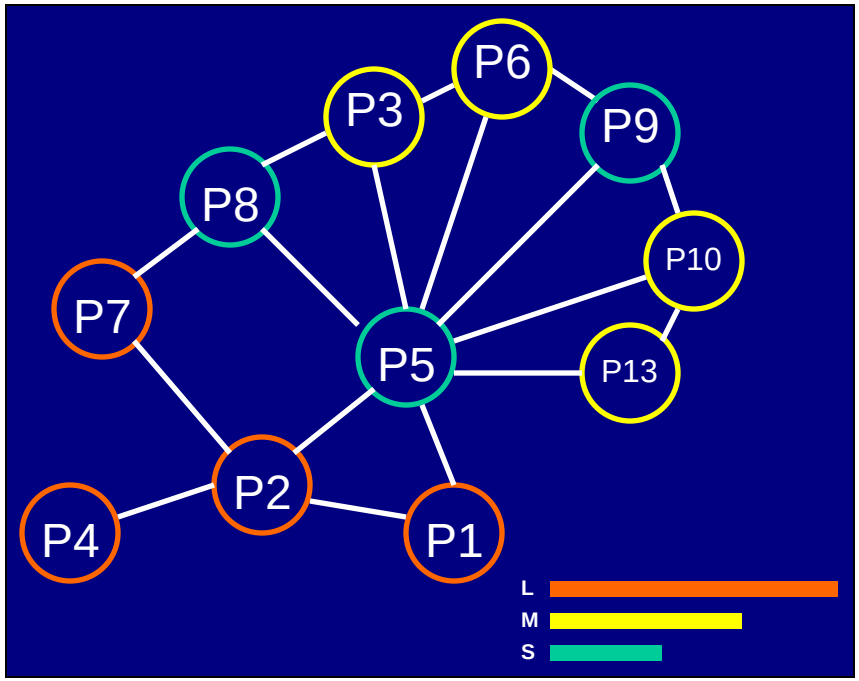


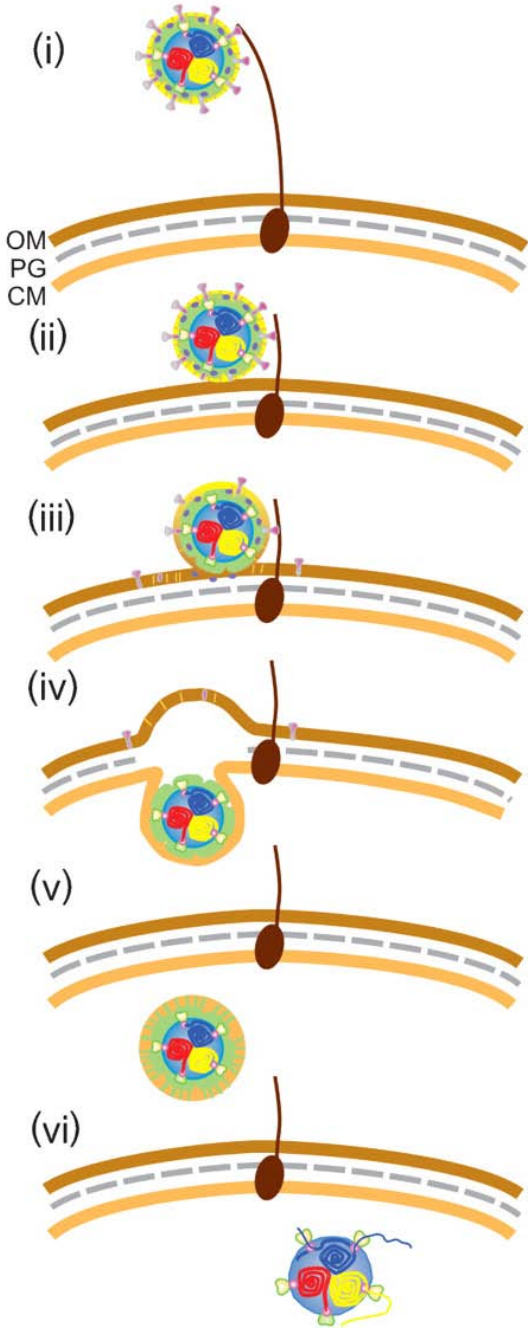


Bulls-eye plaque at 25°C
Mildly deleterious allele

phi6 P5 gene: robustness locus?







Cvirkaite-Krupovic et al 2010 *J Gen Virol*

Summary 2

- Adaptation to extreme heat shock broadens the thermal niche
- Selection for thermotolerance causes indirect selection for robustness (bidirectional response)
- The lysin gene may be underlying mechanism (global robustness regulator)

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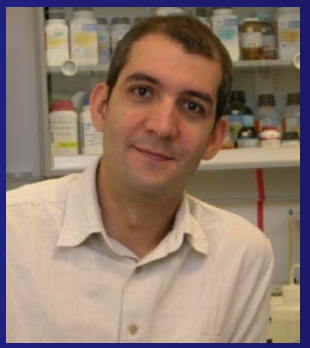

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