Evolving Evolvability: How Can an Up-Modifier of Variability Spread?

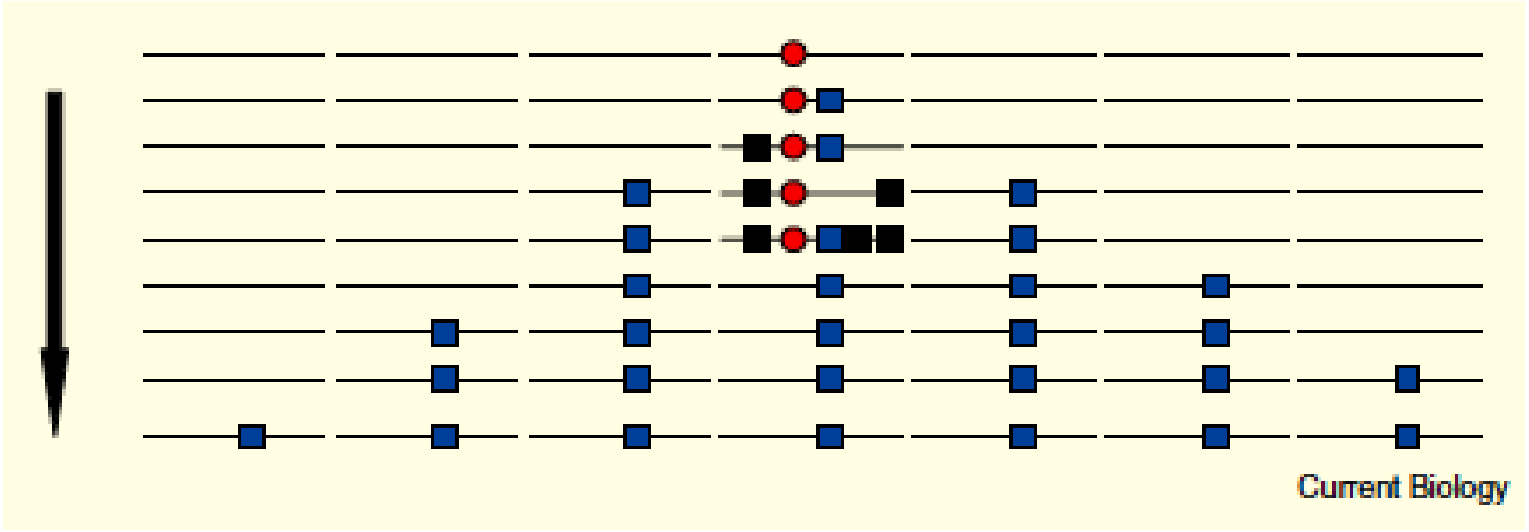


Figure 1. The population genetic problem with selection on evolvability.

A 'variability allele' (red circle) gives rise to a new beneficial allele (blue square) in the same genomic background. Because of recombination, the variability allele does not spread in the population along with the beneficial allele. Furthermore, because deleterious mutations (black squares) are far more common than beneficial ones, the variability allele occupies genomes that are more contaminated than average with deleterious mutations, and so it declines to very low frequency in the population.

Sniegowski and Murphy 2000

Mutation: the ultimate source of the variation that fuels evolution.

- Adenine (A), Guanine (G), Cytosine (C), and Thymine (T) make up the DNA alphabet. Their sequence in genomes encodes instructions for building and maintaining a new organism--and instructions for replicating DNA itself.

- To a first approximation, mutations may be thought of as typos or misprints that arise during genome replication. (Not quite the whole story, though.)

- The paradox of mutation is that random changes in the genome are far more likely to be harmful than beneficial, yet in the long run, evolutionary adaptation depends on the occurrence of mutation.

Mutation: the ultimate source of the variation that fuels evolution.

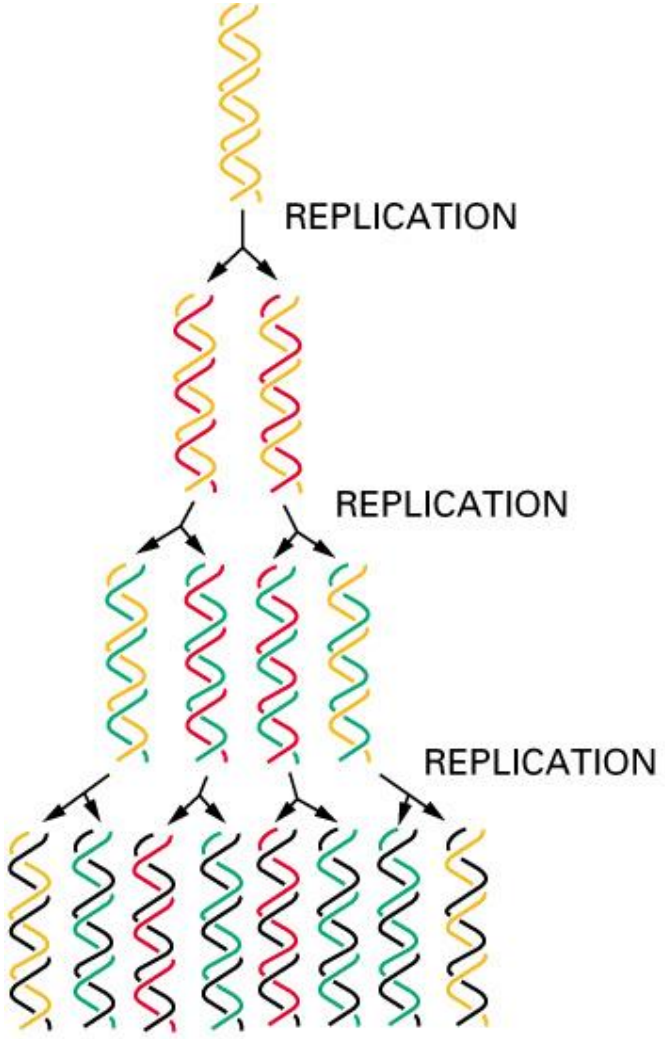
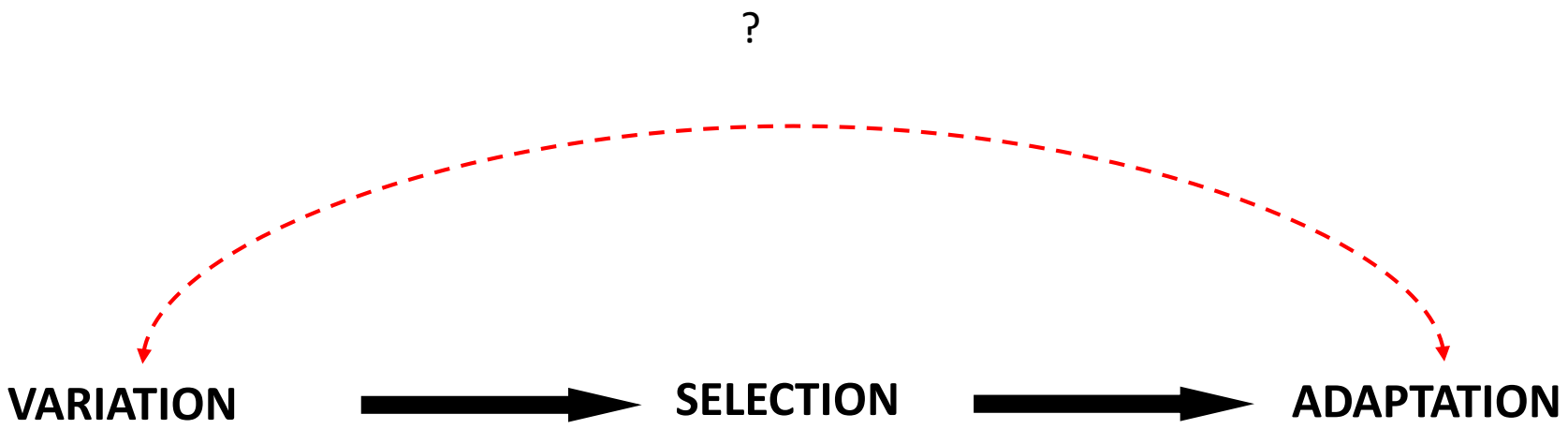


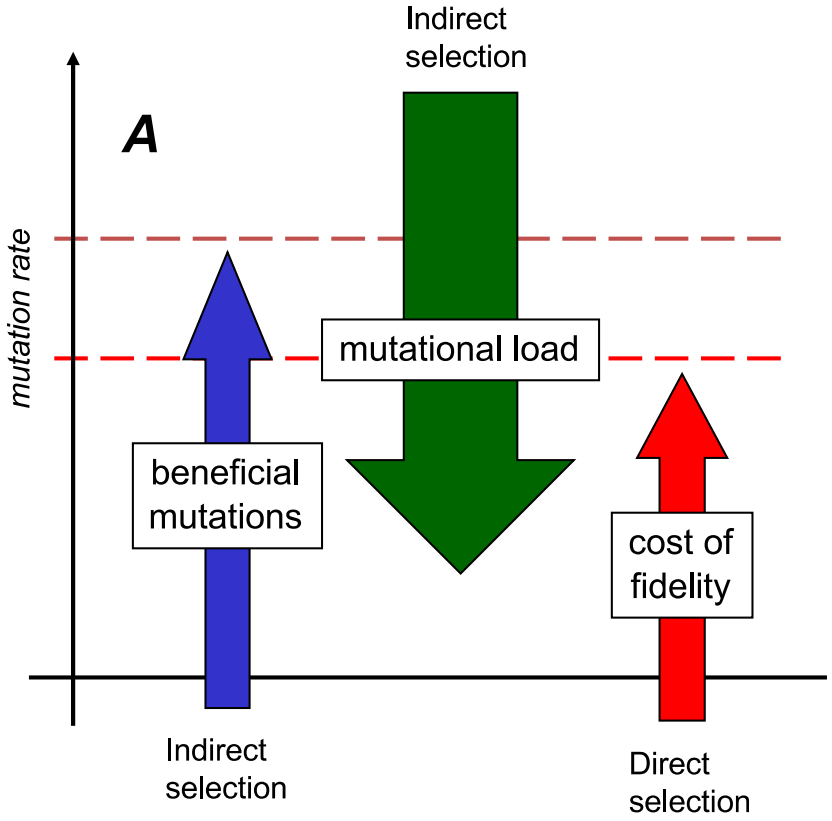
Figure 5–5. Molecular Biology of the Cell, 4th Edition.

Three main steps give rise to high fidelity DNA replication

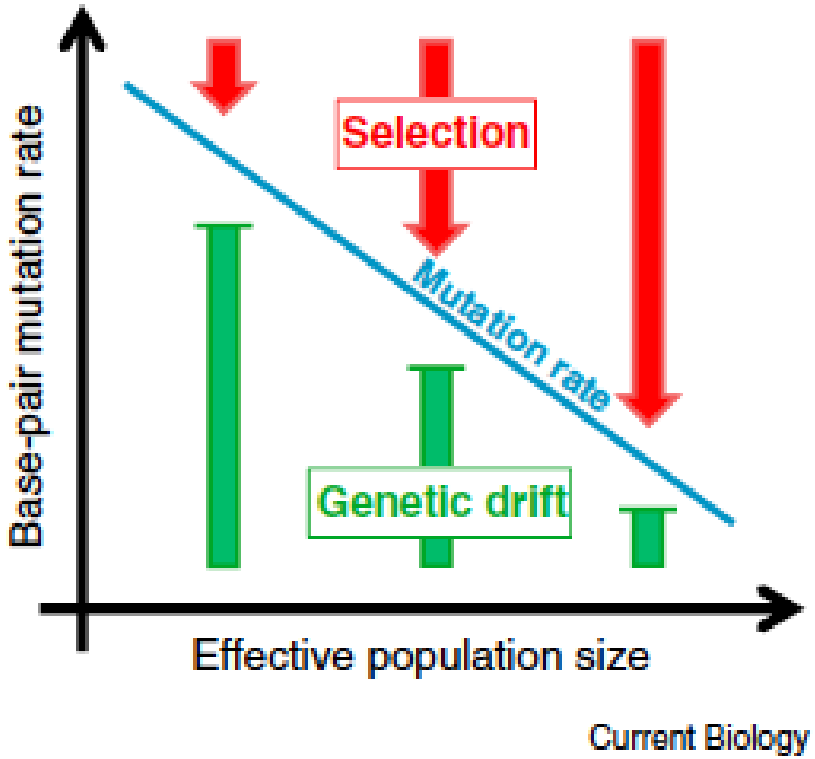
Replication or repair step	Cumulative error rate
5’-3’ polymerization	1×10^{-5}
3’-5’ exonucleolytic proofreading	1×10^{-7}
Postreplicative DNA repair	1×10^{-9}


?
VARIATION
SELECTION
ADAPTATION

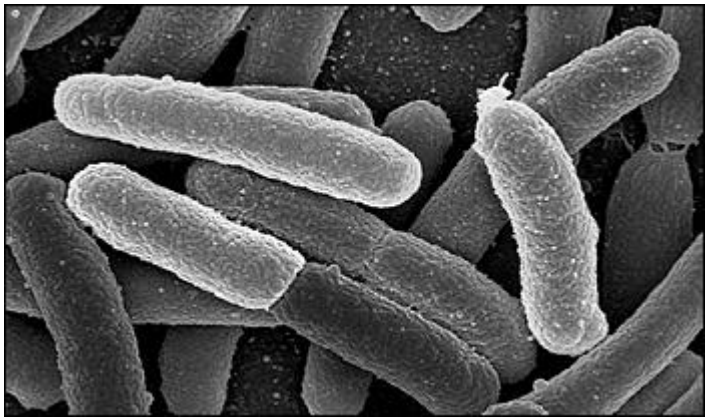
Mutation rate evolution



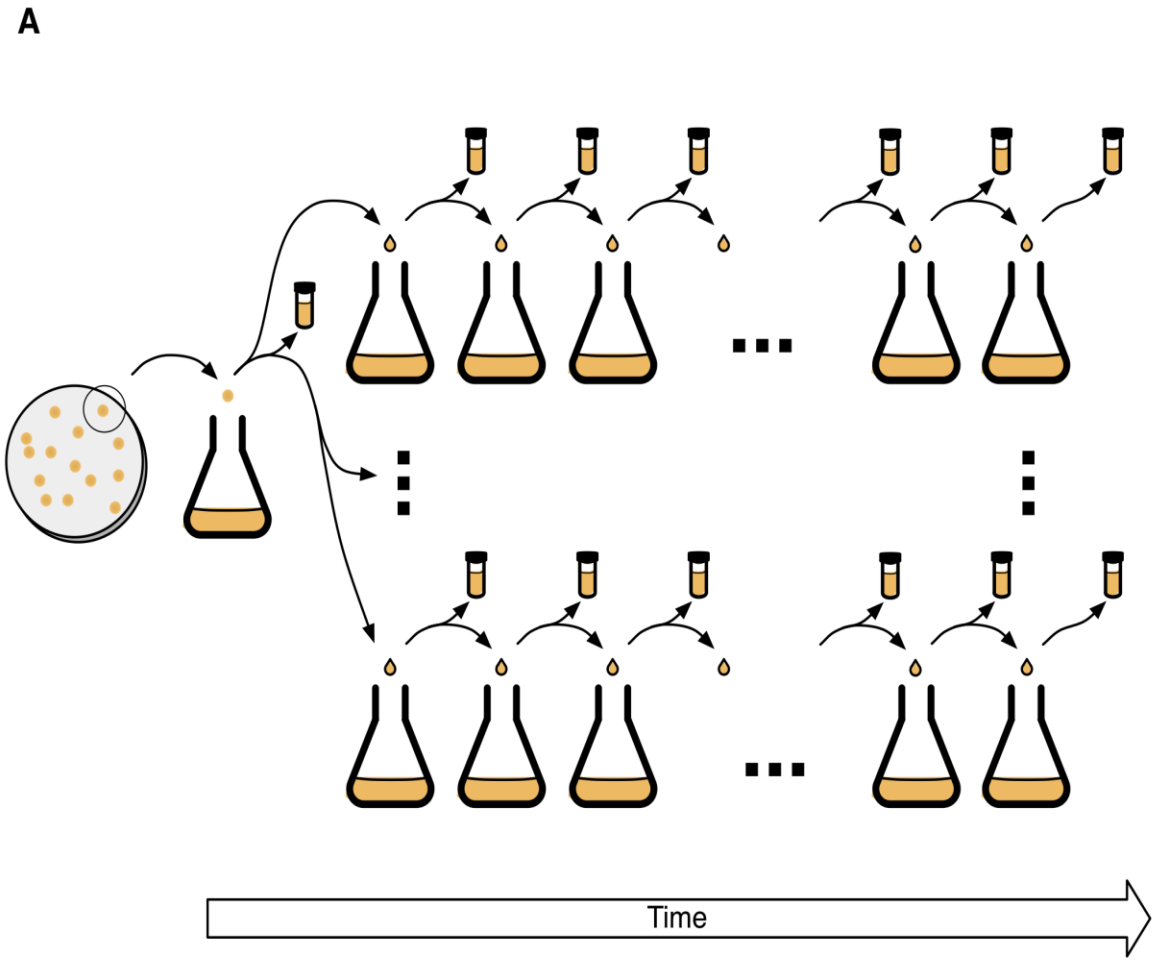
Sniegowski et al., 2000

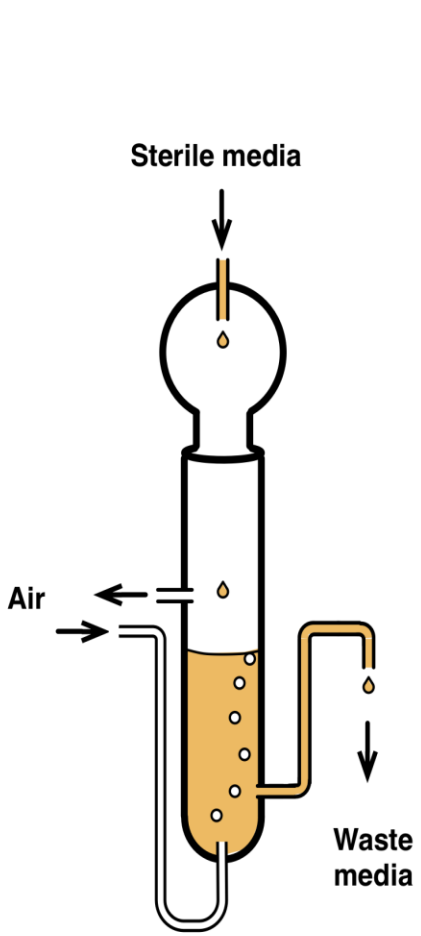


Raynes and Sniegowski, 2013; after Lynch, 2010



Experimental Microbial Evolution





Sprouffske et al., Current Biology 2012

Experimental Microbial Evolution: Some Strengths and Limitations

Strengths

- Experimental replication
- Short generation times
- Control of world in which evolution takes place
- (Phenomenology)[10]
- “Living fossil record”

Limitations

- Limited timespan
- Microbes are different from…
- Relative lack of ecological complexity
  - Spatial scales, dimensions
  - Coevolution
  - Changing abiotic environment

Experimental Microbial Evolution: What Can We Learn About?

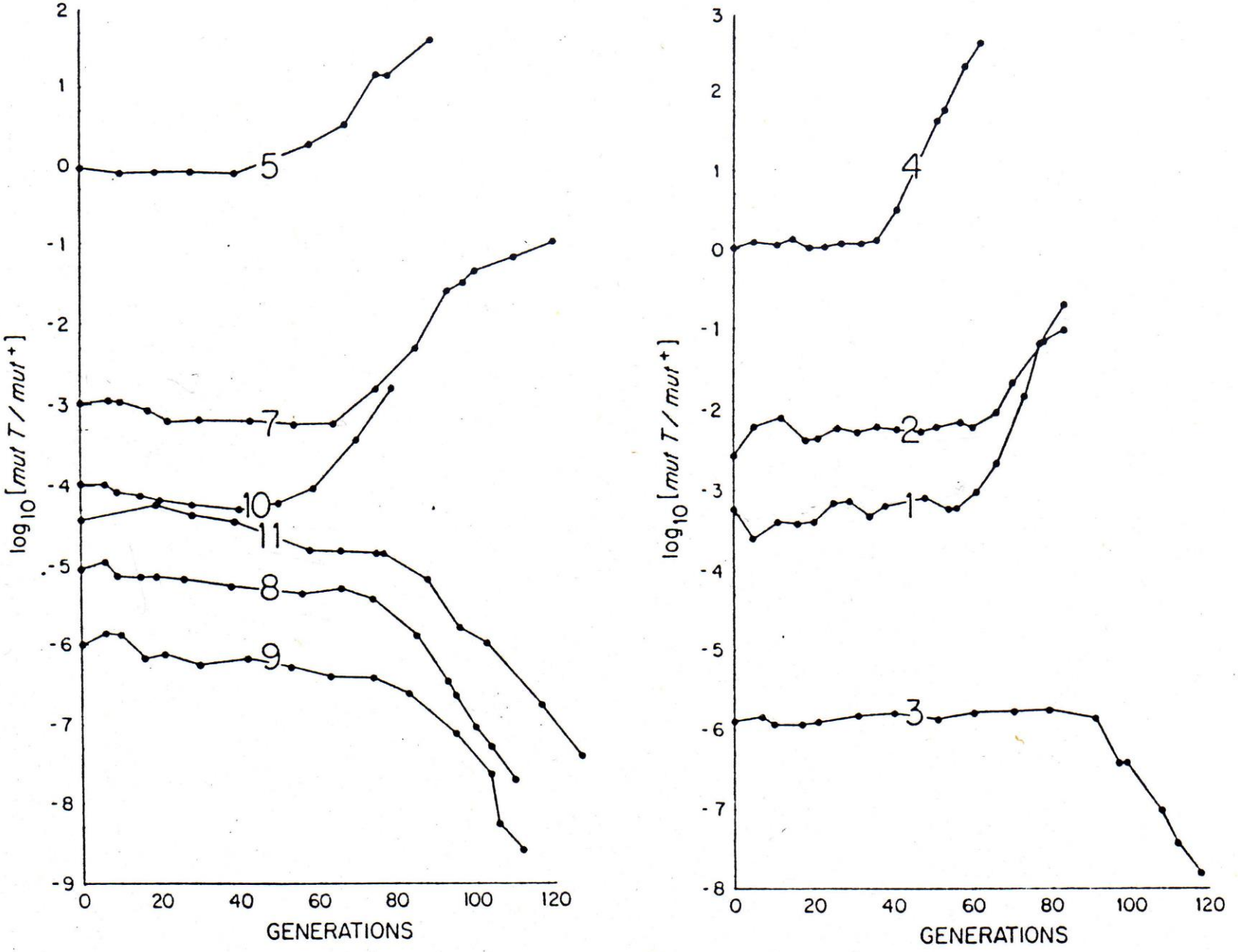
Evolutionary *process* (any world, in principle)

- Dynamics of genetic variants under selection, drift, mutation, recombination (or not), etc.
- Evolution of the genetic system itself: mutation rates, recombination rates, and their interaction (largely the subject of this talk)
- Roles of chance and history in evolution
- Nature of, and constraints on, major evolutionary transitions: unicellular→multicellular, etc.

Evolved *mechanisms* (the particular world studied experimentally)

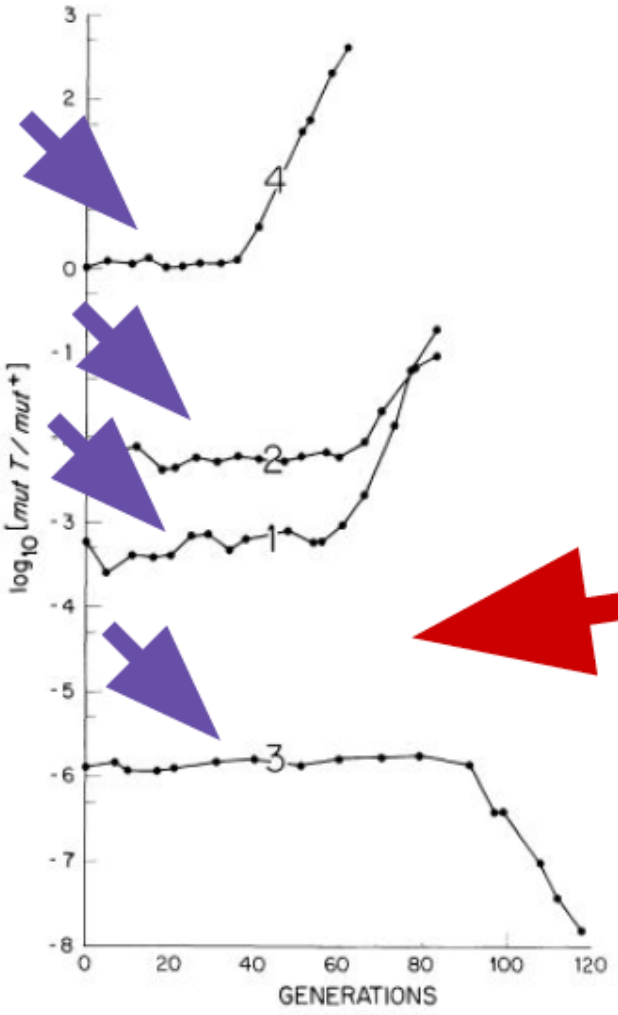
- Characterization, at phenotypic/genomic/proteomic levels, of evolutionary changes observed in specific organisms and specific environments

A Seminal Experiment on Mutation Rate Evolution



Source: Chao, L. and E.C. Cox. 1983. *Evolution* 37, 125-134

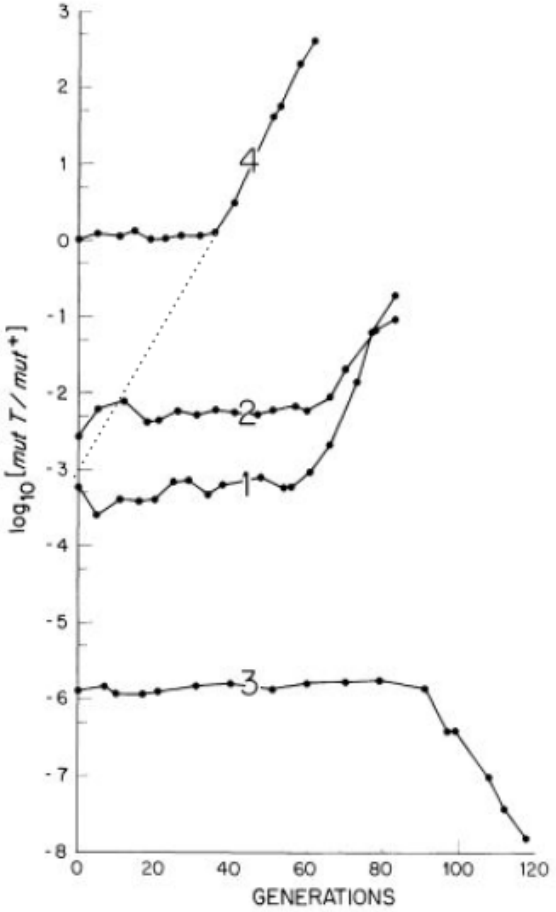
Chao & Cox 1983, two things to be explained



Lag

Threshold

Chao Cox results show a lag and then a straight line



Lag could be explained by:

- Waiting time till mutation
- Or, consequence of the fact that we're not tracking frequency of the particular mutator lineage that does shoot up--the true path of that lineage is as shown by the dotted line

Chao Cox results show a threshold of about 1e-04

- Above this starting frequency, the mutator fixes, and below it, it does not.
- mutT is elevates the point mutation rate by about 150-fold (Weilgoss 2012), yet the threshold is well below 1/150 -- why?
- Possibility 1: the particular spectrum of mutations caused by mutT (greatly elevates AT->CG transversions) means that with respect to some particular mutation(s), mutT is a much-more-than 150-fold mutator
- Possibility 2: multiple mutations were involved in the increase in mutator frequency

What is our *a priori* expectation for the threshold at which mutators should go on to fix, in competitions? (Ignoring deleterious mutations.)

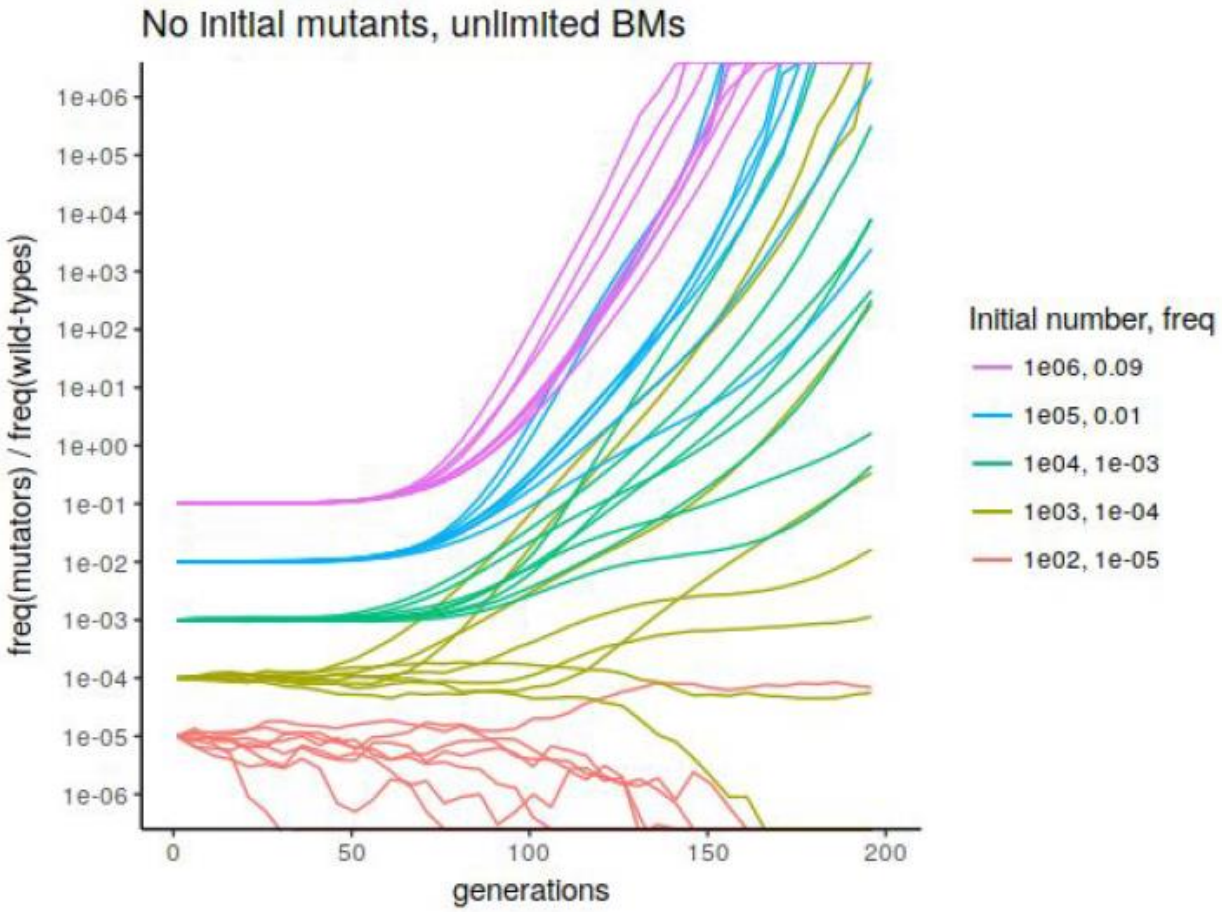
- If there's only one beneficial mutation to be had, the mutator lineage should win when $U_{mut} * N_{mut} > U_{wt} * N_{wt}$. So for a 100-fold mutator, the threshold frequency should be 0.01 (that is, U_{wt} / U_{mut})
- Suppose there are three available BMs. Triple mutants are generated at a rate of U^3. So the threshold frequency in this case is $(U_{wt} / U_{mut})^3$, or for a 100-fold mutator 1e-06. If there's two available BMs, the threshold frequency would be 1e-04.
- Multiple mutants are probably not in fact generated at once (mutation rates are too low/populations too small). But the threshold should be in between, since a lineage can enter a positive feedback loop where it expands enough to have a good chance of getting a next mutation (Tenaillon et al 1999).

Chao Cox parameters

- N: 1e10
- U_b: supposing that it's something like knockout of a gene, as high as 1e-07 for the nonmutators.
  - This would mean the nonmutators are producing 1000 beneficial mutants per generation
  - The mutators, at the threshold frequency of 1e-04, would be producing about 10 beneficial mutants per generation.
  - Since the mutator and nonmutator populations were grown up separately, at time 0 the mutator population likely has a much higher relative frequency of already-existing mutants than does the wild-type population.

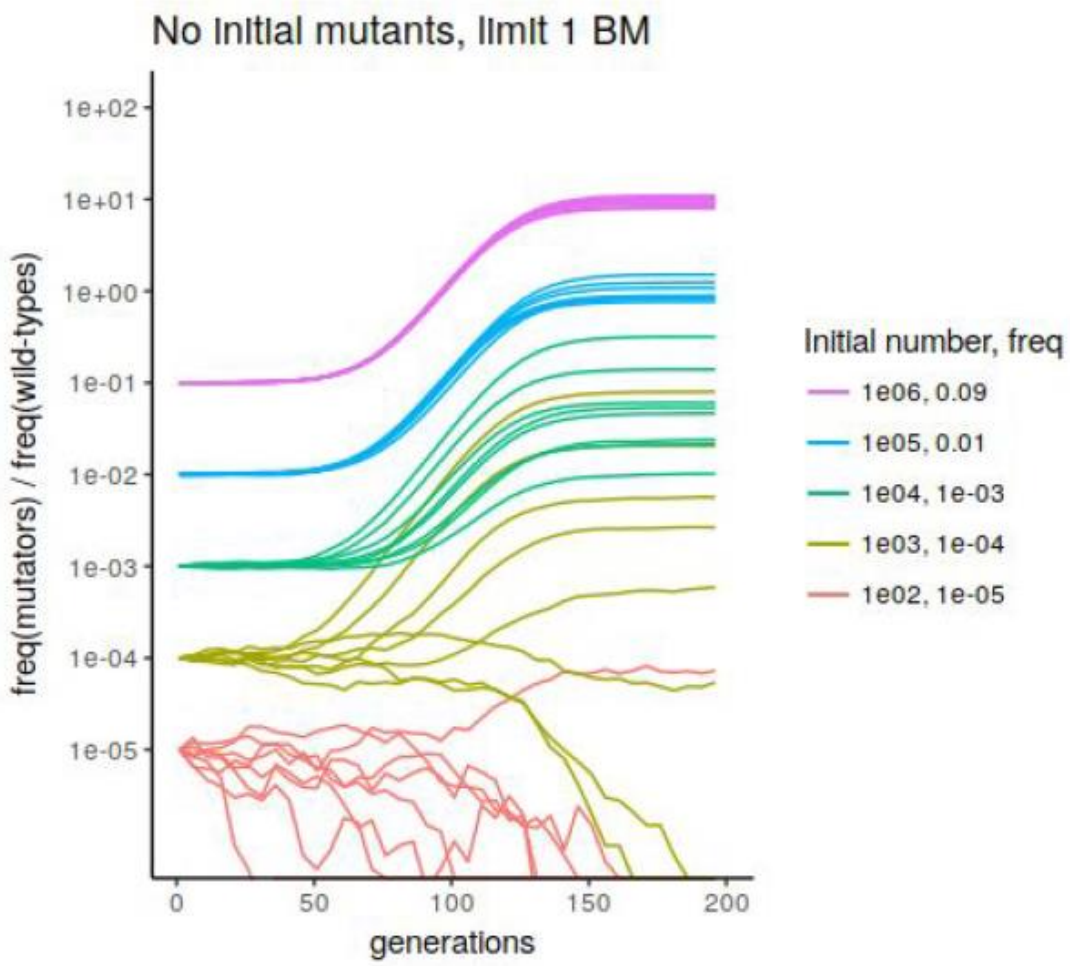
Simulation parameters:

- N: 1e07 (lessened to make it run-able in reasonable time).
- U_b: 1e-06 (raised because pop was lessened).
- Mutator fold increase: 100

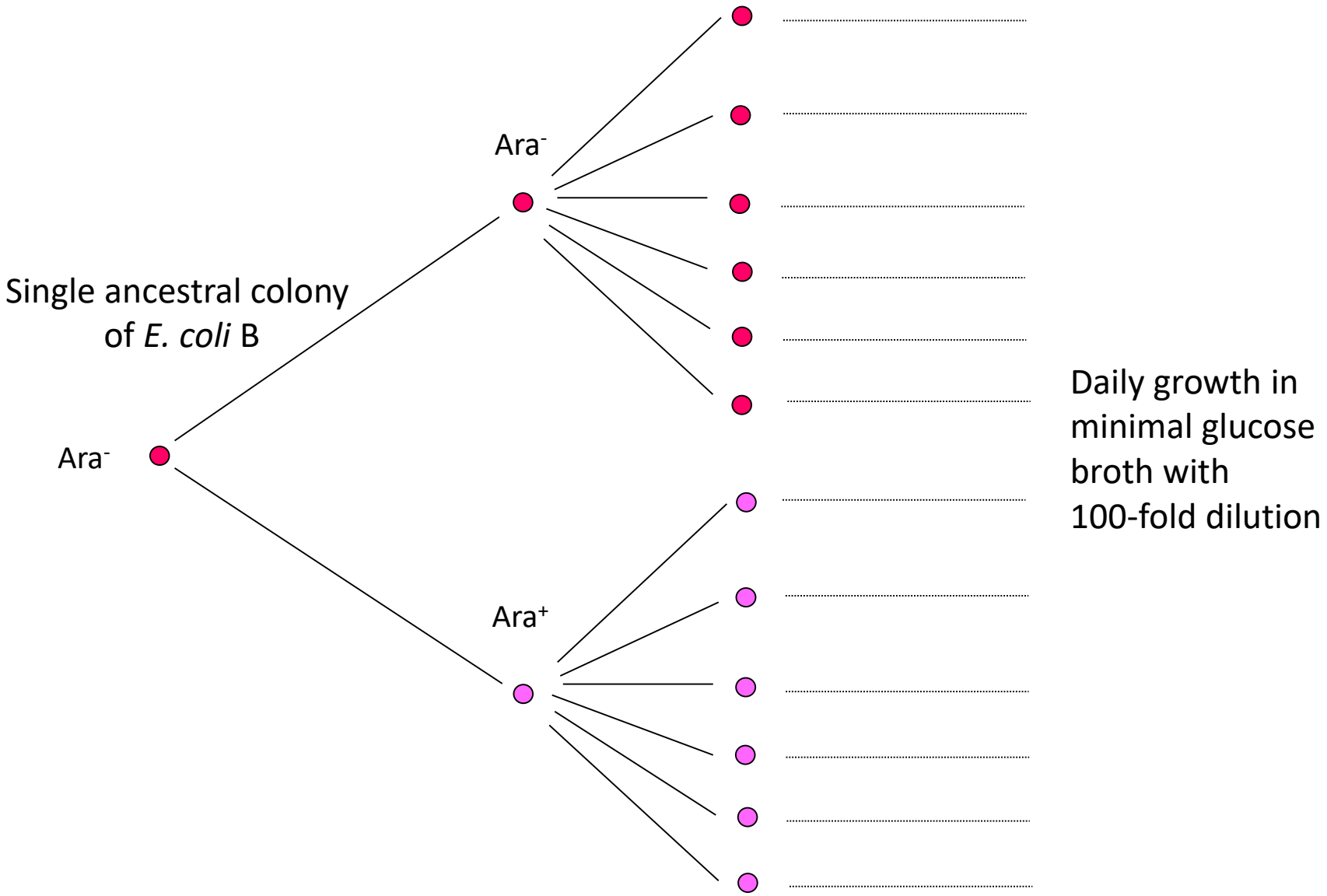


A lot of stochasticity but a threshold somewhere around 1e-04. Lag observed.

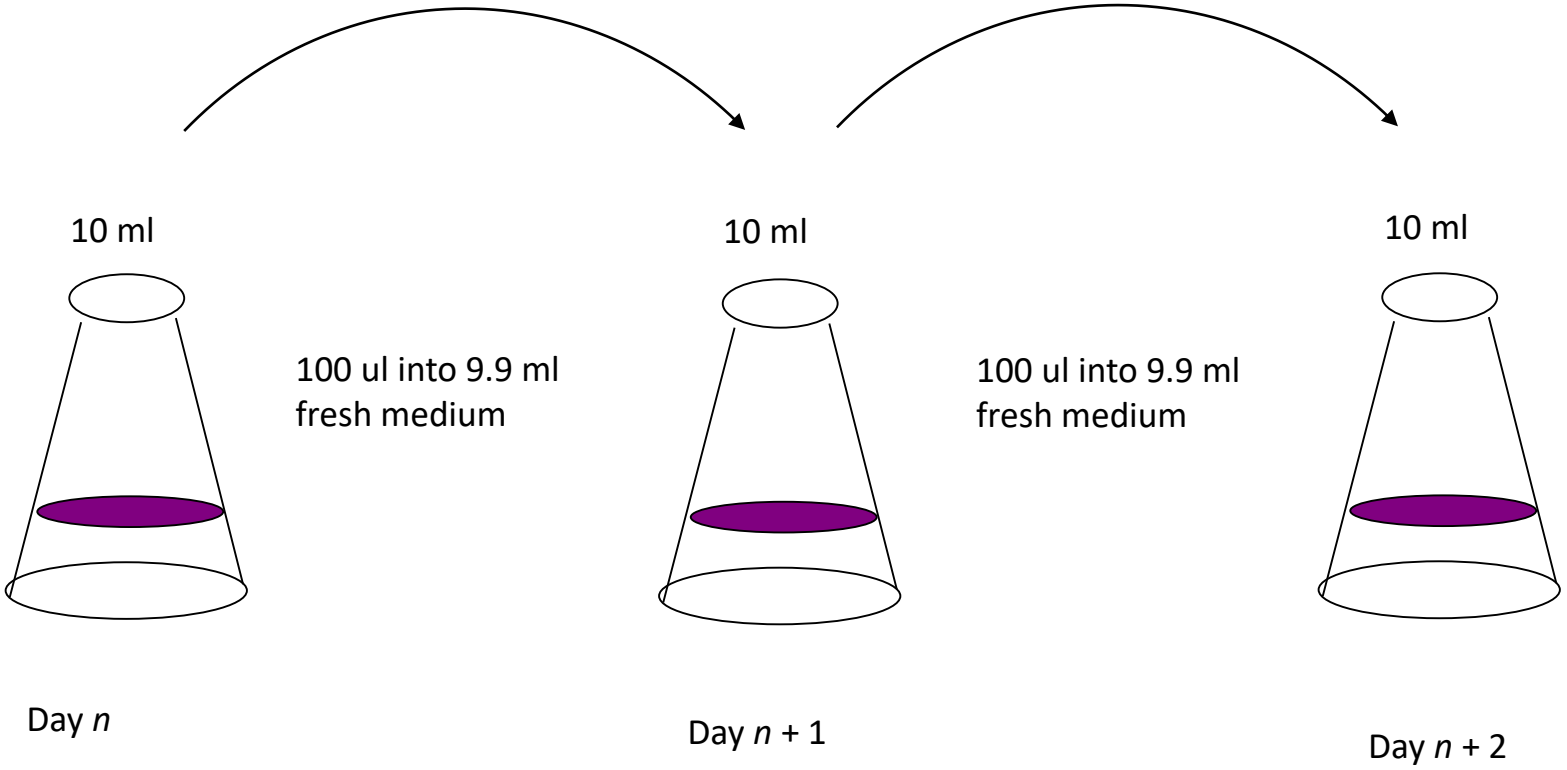
Limit 1 (for everyone)



Lenski's Long Term Evolution Experiment in *E. coli*: An Iconic Study in Experimental Evolution

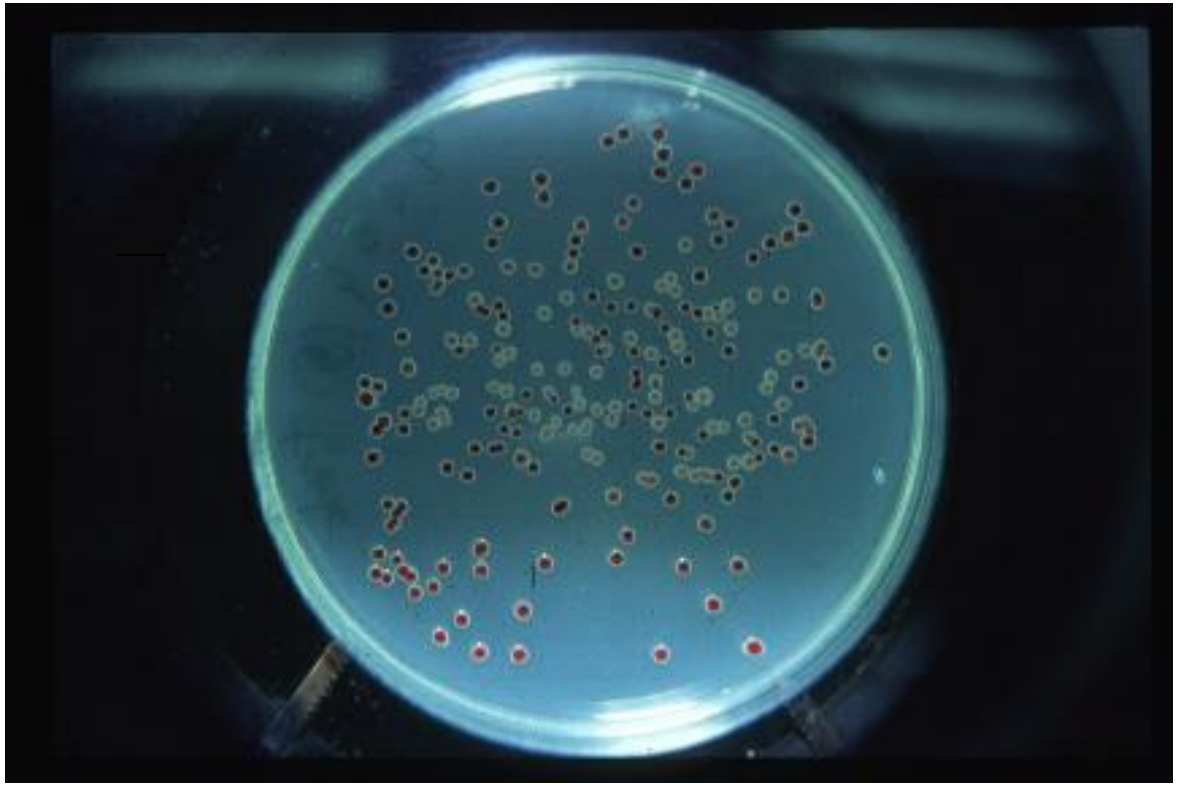


Daily transfer regime



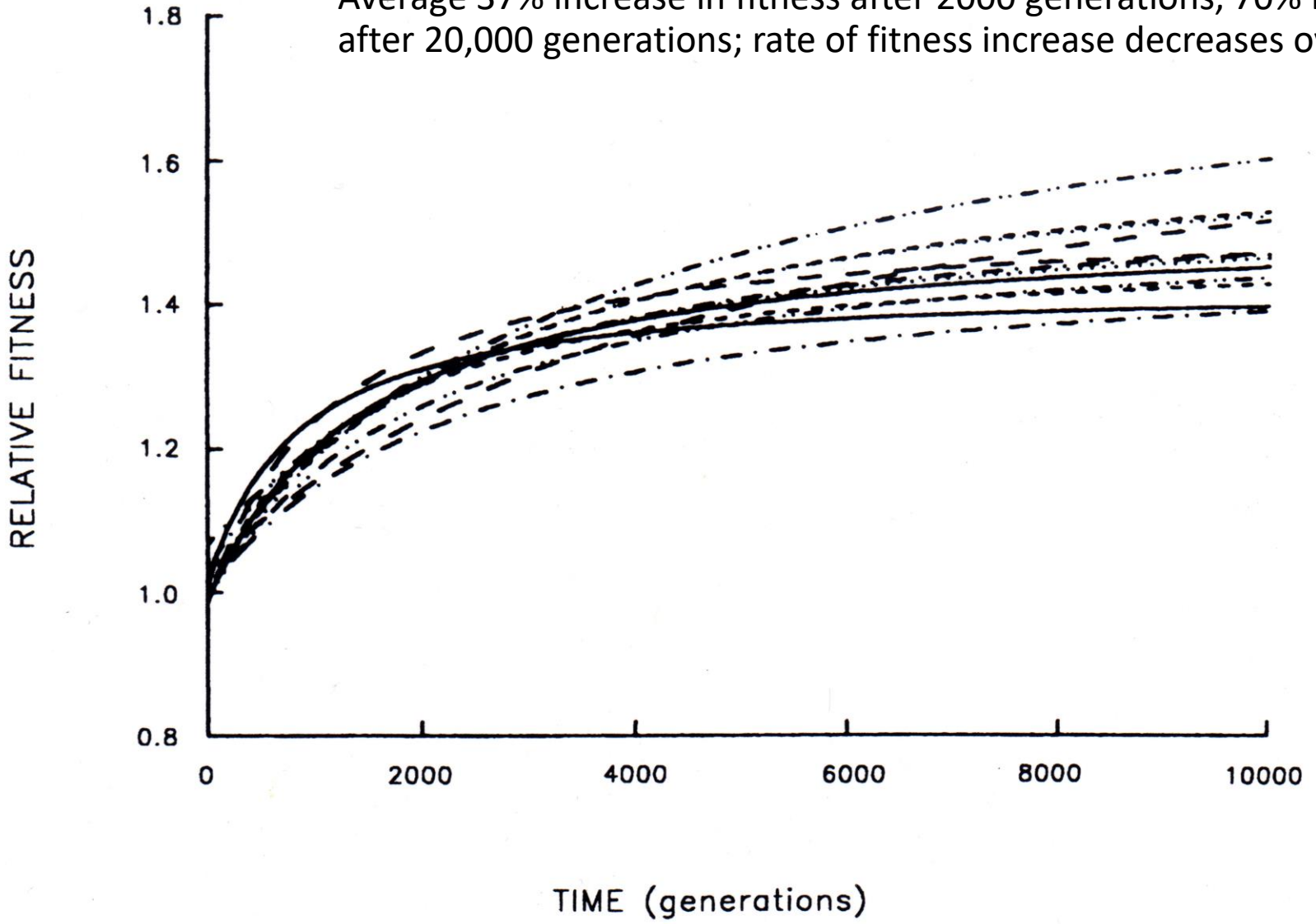
Fitness

- Classical definition: survival x reproduction
- In Lenski World, define fitness based on growth of evolved strains when competed against ancestor carrying opposite arabinose marker



Evolution Experiment

Average 37% increase in fitness after 2000 generations, 70% increase after 20,000 generations; rate of fitness increase decreases over time.



Source: Lenski, R.E. and M. Travisano. 1994. *PNAS* 91, 6808-6814.

How do we measure the genomic mutation rate?

- *Fluctuation assays* are a common method for measuring microbial mutation rates.
- These assays have roots in the classic Luria-Delbrück experiment, which demonstrated a fundamental biological concept.

The Luria-Delbrück experiment, 1943

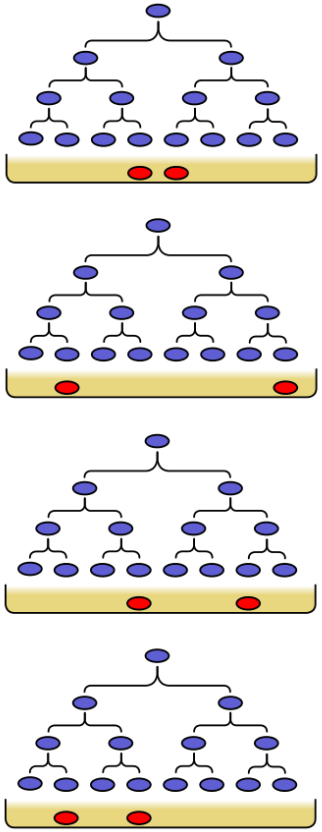
The question: Are bacterial mutations *random* (spontaneous), or do the mutations arise *in response* to selection?

When Luria & Delbrück first began investigating this question, they grew cultures of *E. coli* and exposed them to phages (which normally infect and kill *E. coli*) to observe patterns of resistance.

To their annoyance, the numbers of survivors varied a lot between experimental replicates.

Why?

If it were true that mutations arise in response to selection…

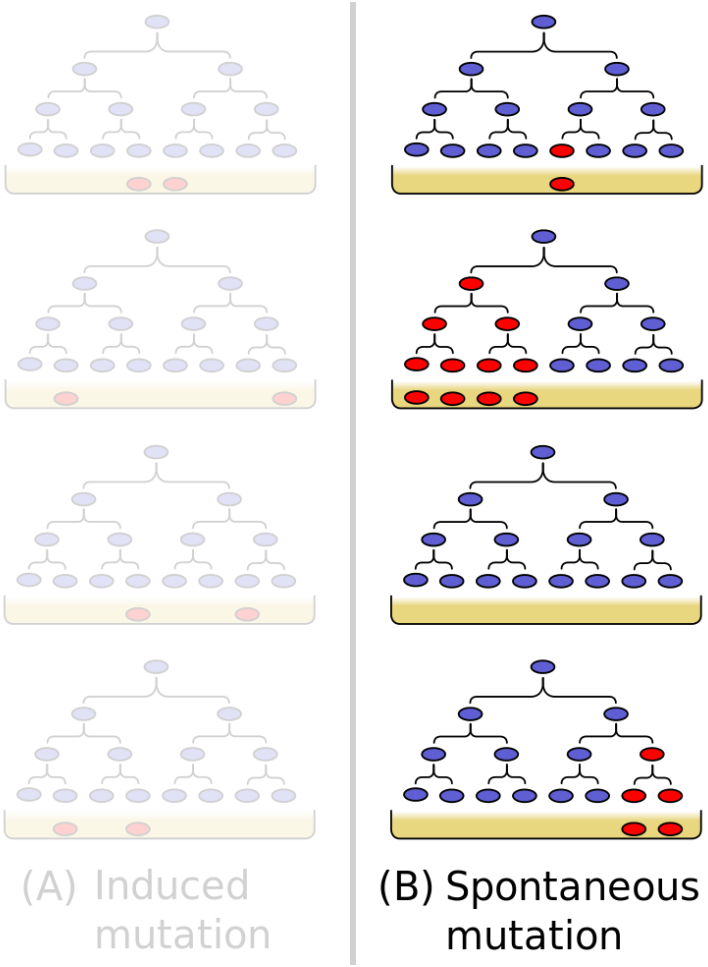


(A) Induced mutation

Similar (not necessarily equal) numbers of surviving bacteria across replicates.

Image credit: Madeleine Ball (modified by MME)

In reality, Luria & Delbrück saw this:

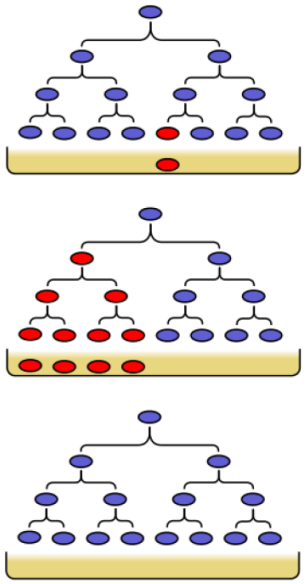


The numbers of surviving bacteria greatly vary across replicates.

Image credit: Madeleine Ball (modified by MME)

Interpreting Fluctuation Test Data

(i.e. the # of colonies growing on the selective plates)



During this culture's incubation, a mutation occurred in the final generation of growth—a result more likely at a low mutation rate than a high one..

In this culture, a mutation occurred early in growth—a result more likely at a high mutation rate.

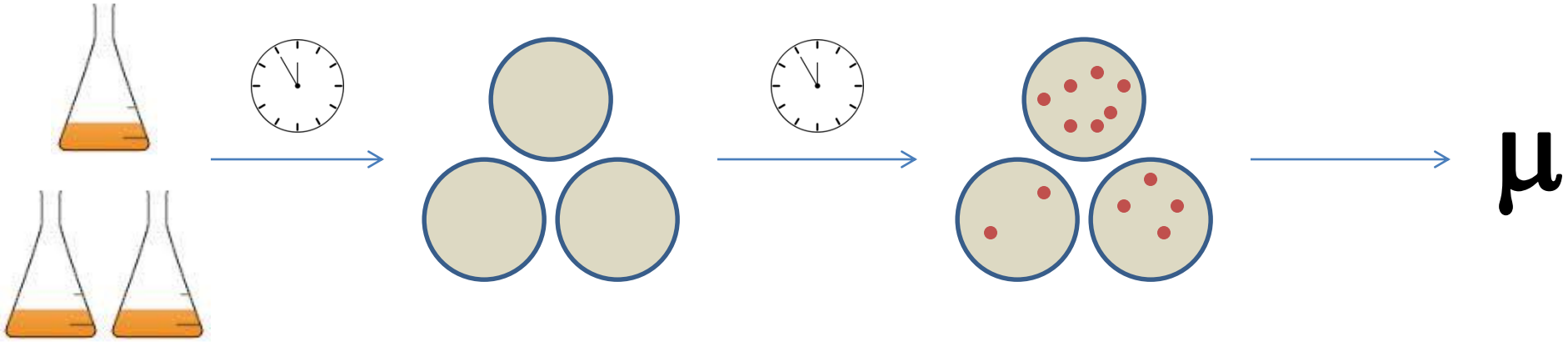
In this culture, no mutations occurred during the course of the incubation. (The mutation rate is unlikely to be high.)

Selection (via the selective plate) acts upon the mutations that were randomly generated during the culture growth in the flask of permissive medium.

The # of colonies growing on the selective plates correlates with the mutation rate.

Image credit: Madeleine Ball (modified by MME)

Measuring mutation rate (μ): the fluctuation test (FT) in the contemporary lab



1) Inoculate 3 replicate flasks of permissive medium with a tiny # of clonal cells.

2) Grow cultures to a given size.

The cells may acquire new mutations.

3) Plate each flask on a selective plate. (example: an antibiotic plate)

4) Incubate the selective plates.

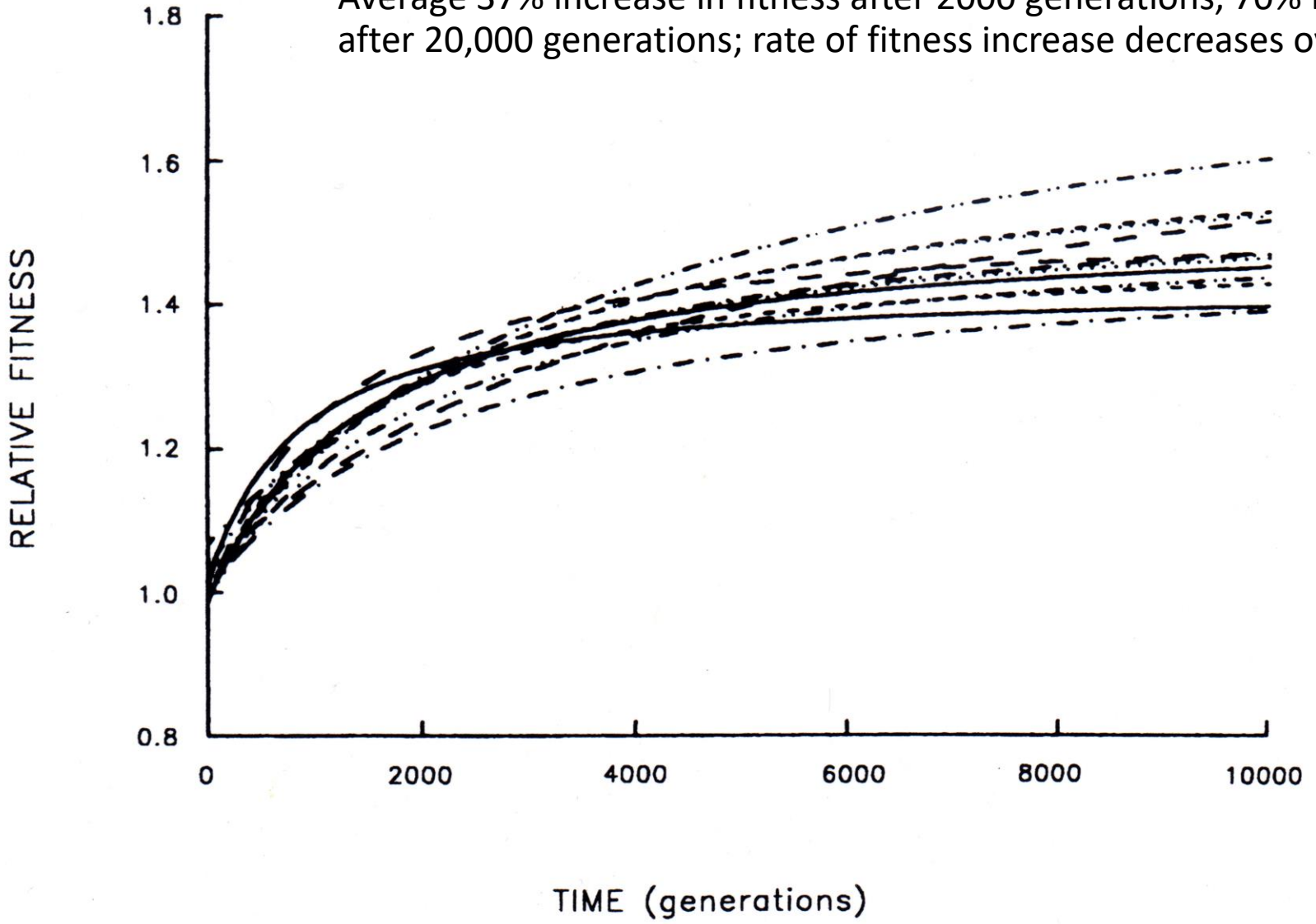
5) Count the colonies.

The # of colonies are correlated with the clone's mutation rate.

6) Calculate a mutation rate estimate from the distribution of the numbers of mutants per culture and the (common) culture size.

Evolution Experiment

Average 37% increase in fitness after 2000 generations, 70% increase after 20,000 generations; rate of fitness increase decreases over time.



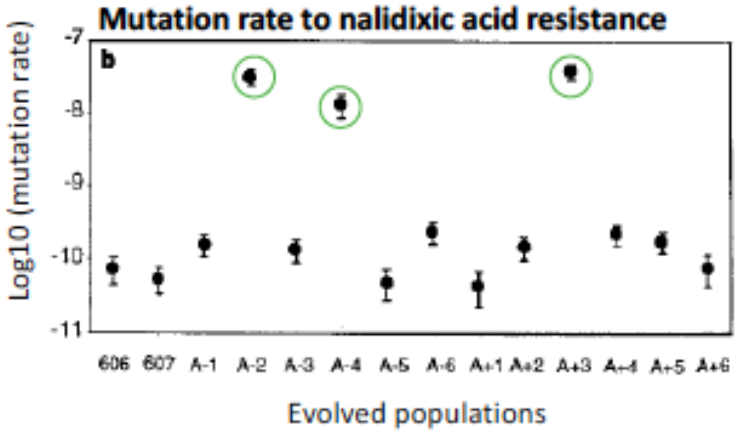
Source: Lenski, R.E. and M. Travisano. 1994. *PNAS* 91, 6808-6814.

High mutation rates evolved

Evolution of high mutation rates in experimental populations of *E. coli*

Paul D. Sniegowski*, Philip J. Gerrish† & Richard E. Lenski†

* *Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA*
† *Center for Microbial Ecology, Michigan State University, East Lansing, Michigan 48824, USA*

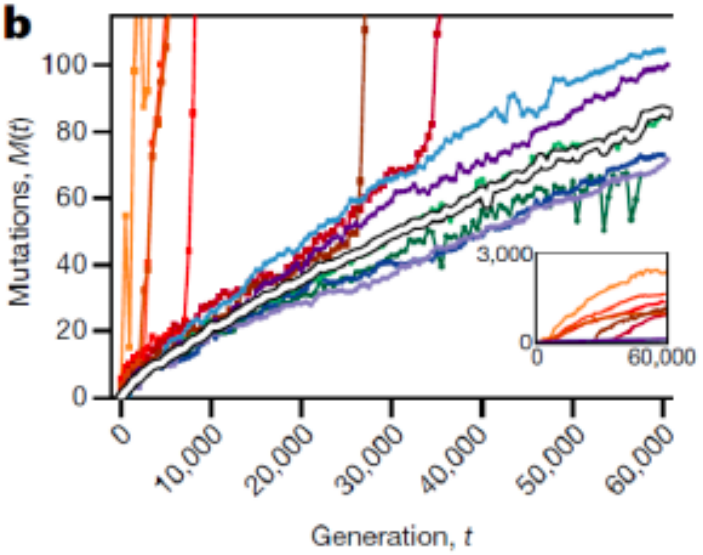


ARTICLE

doi:10.1038/nature24287

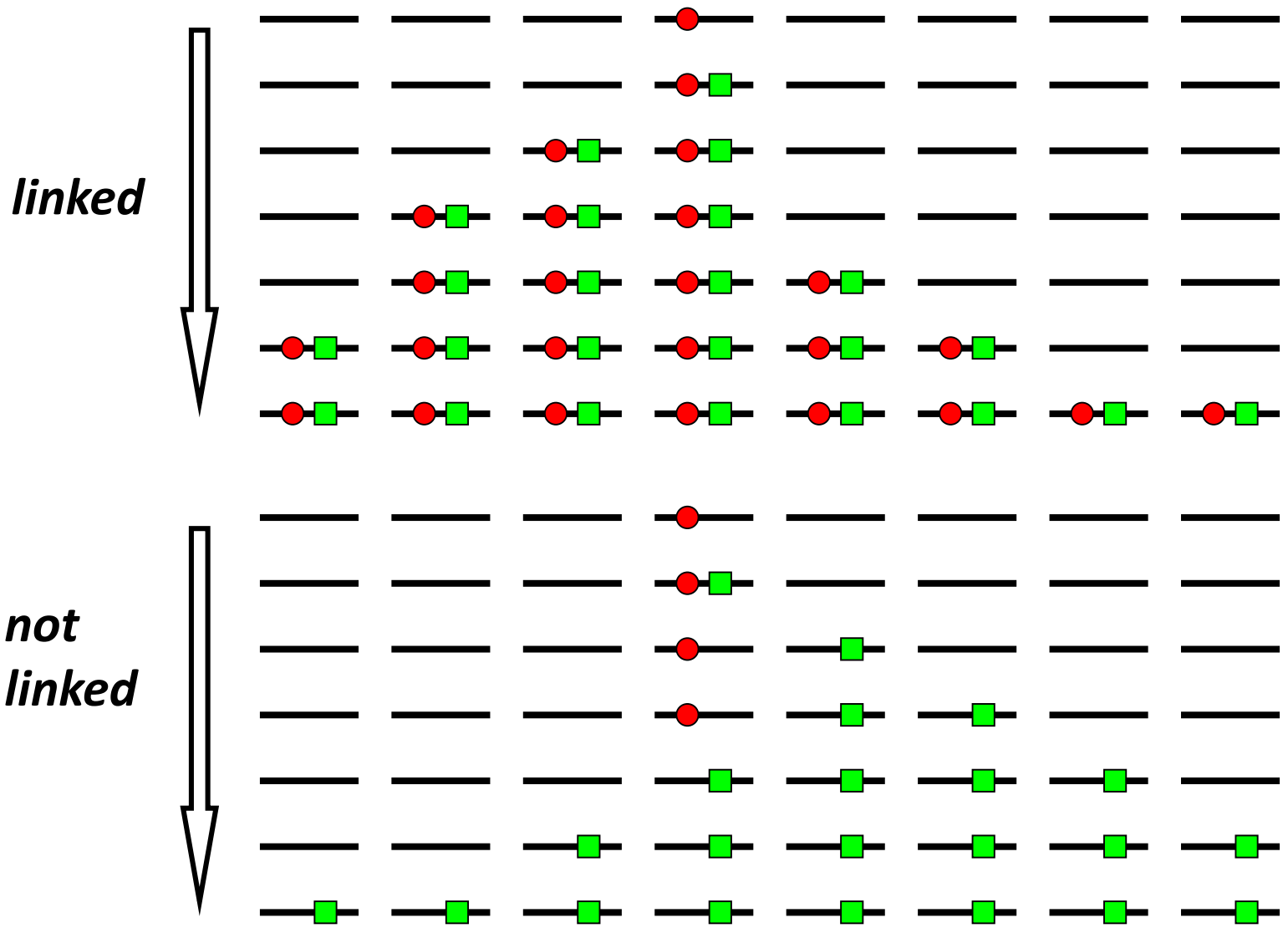
The dynamics of molecular evolution over 60,000 generations

Benjamin H. Good, Michael J. McDonald, Jeffrey E. Barrick, Richard E. Lenski & Michael M. Desai



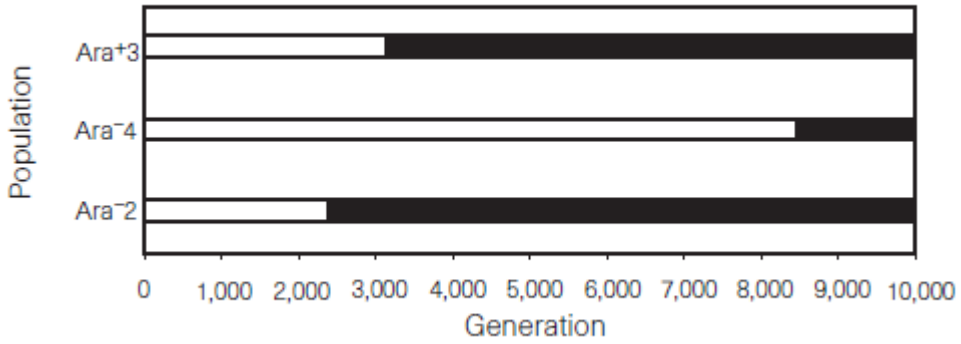
- Evolution of high mutation rates (100 fold) in three of the 12 Lenski populations by spontaneous mutation at 2500, 3000 and 8500 generations
- Several other instances of this later in history of system, too.
- Hitchhiking, genetic drift or direct selection?

Linkage and “mutator” hitchhiking

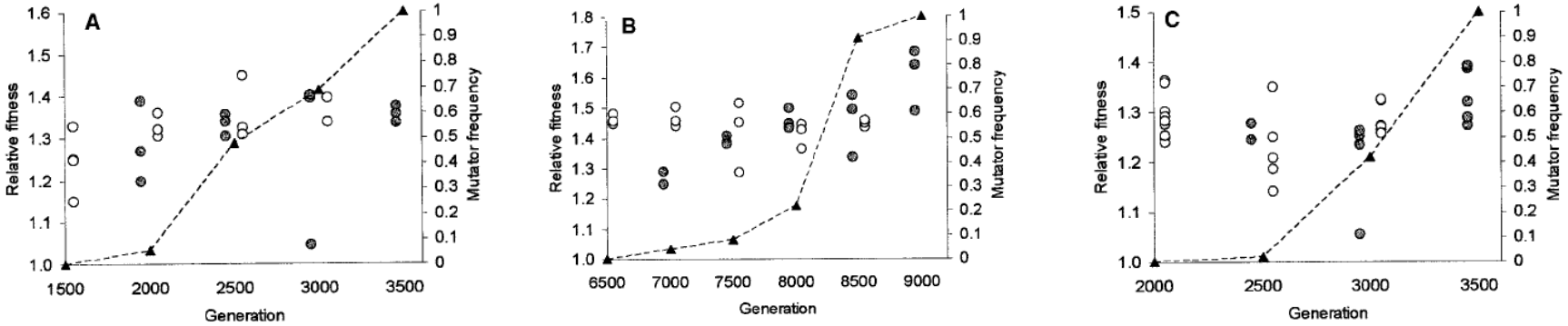


Sniegowski et al., 2000

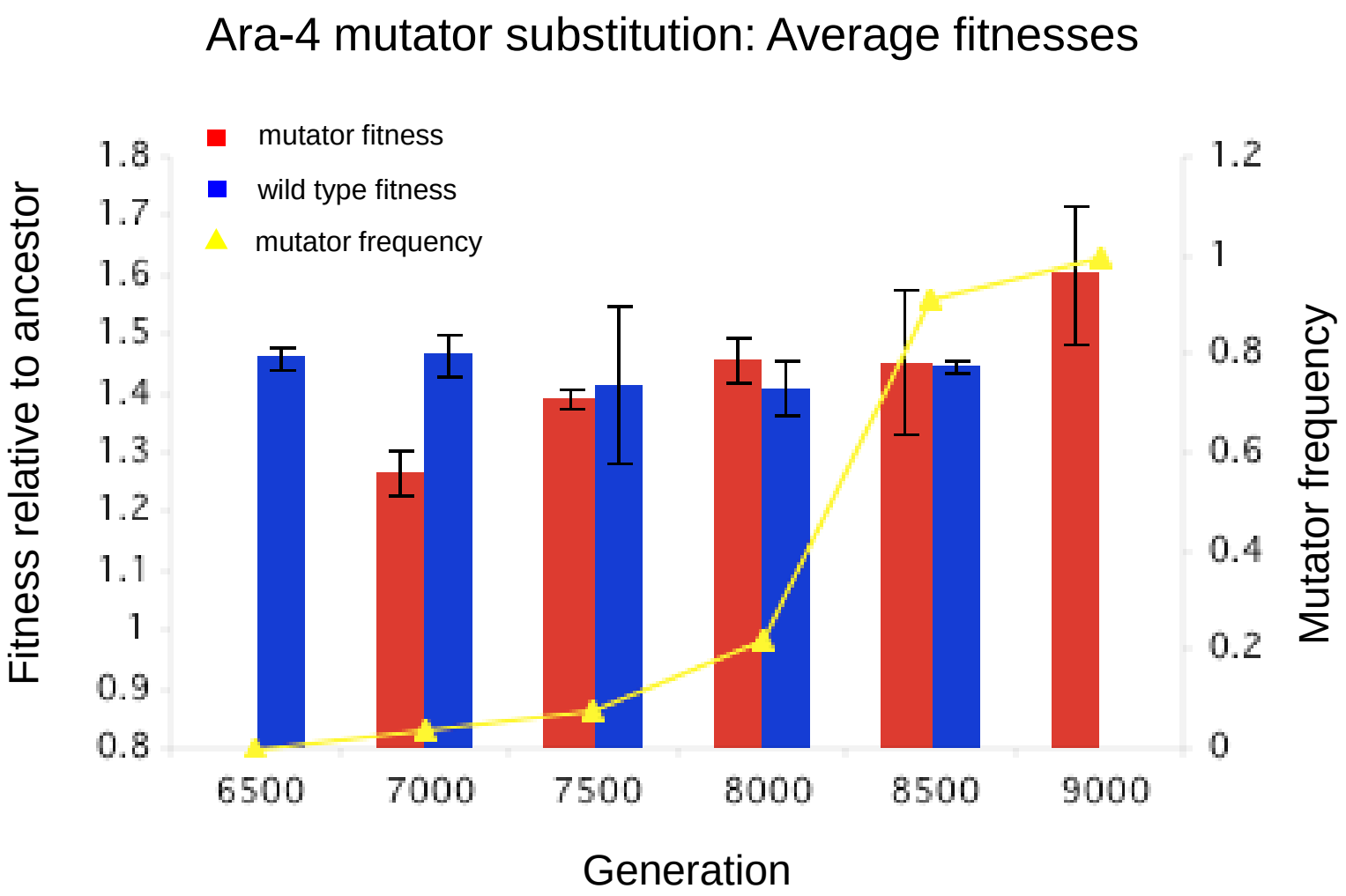
Hitchhiking of spontaneously arising mutators



Sniegowski *et al*, 1997



Shaver *et al*, 2000


Ara-4 mutator substitution: Average fitnesses
mutator fitness
wild type fitness
mutator frequency
Fitness relative to ancestor
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
1.2
1
0.8
0.6
0.4
0.2
0
Mutator frequency
6500
7000
7500
8000
8500
9000
Generation

Mutator sequence analysis

Population	Mutator mutation	Effect	Additional coding changes at 20,000 generations
Ara^{+}3	insertion of G after position 521	frameshift in 1st third of protein	I->V at a.a. 694, assuming original reading frame
Ara^{-}2	insertion of two a.a. repeat (LA) after a.a. position 68	*see below*	G->E at a.a. 32 M->T at a.a. 135 V->A at a.a. 274
Ara^{-}4	deletion of two a.a. repeat (LA) after a.a. position 68	*see below*	G->D at a.a. 281 A->V at a.a. 606

Note: An LALALA repeat makes up the end of the B α-helix of MutL. Immediately following the repeat is a loop which leads to the C α-helix. This helix-loop-helix structure is thought to form the lid of the ATP binding site for MutL, implying that the mutations altering the repeat number alter the properties of the ATP binding pocket.

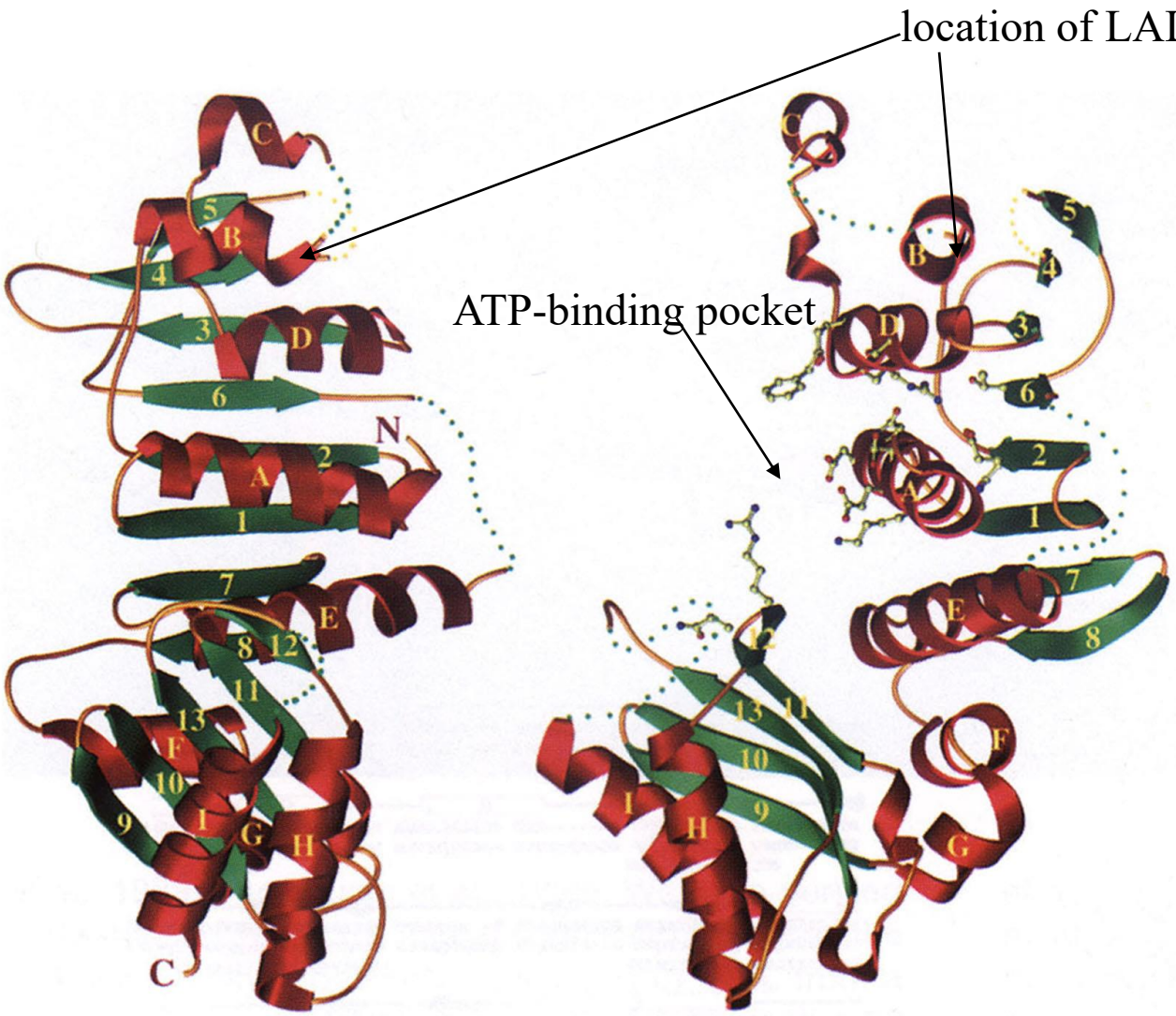
Repeat alterations in *mutL*

CTGGCG CTGGCG CTGGCG

Ancestor 68 KKDELALALARHATS

Ara-2 68 KKDELALALALARHATS

Ara-4 68 KKDELALARHATS



Source: Ban, C. and W. Yang. 1998. *Cell* 95, 541-552.

The CTG GCG repeat array is not well conserved

	210							233
E. coli B	GAG	CTG	GCG	CTG	GCG	CTG	GCG	CGT
E. coli O157:H7	...	...	...	...	...	...	...	...
E. coli K-12	...	...	...	...	...	...	..T	...
S. typhimurium	...	...	...	...	...	...	..C	...
S. enterica	...	...	...	...	...	...	..C	...
S. flexneri	...	...	...	...	...	...	..T	...
Y. pestis	..T	T..	..A	...	...	T..	..C	..C
P. aeruginosa	..C	...	C..	...	..C	...	..T	..C
B. subtilis	..T	TGC	AA.	.GA	..T	T.C	CG.	..C
N. meningitidis	..C	A.C	.AA	..C	...	..C	CAC	..C

Mutators can hitchhike in natural populations

The frequency of mutators in populations of *Escherichia coli*

Lars Boe [a], Morten Danielsen [a], Steen Knudsen [b,*], Jesper Breum Petersen [a], Jakob Maymann [a], Peter Ruhdal Jensen [a]

High Frequency of Hypermutable *Pseudomonas aeruginosa* in Cystic Fibrosis Lung Infection

Antonio Oliver, Rafael Cantón, Pilar Campo, Fernando Baquero,* Jesús Blázquez*

Hypermutable *Haemophilus influenzae* with mutations in *mutS* are found in cystic fibrosis sputum

Michael E. Watson Jr,[1,2] Jane L. Burns[3] and Arnold L. Smith[1]

Highly Variable Mutation Rates in Commensal and Pathogenic Escherichia coli

Ivan Matic, *et al.*
Science **277**, 1833 (1997);

High Mutation Frequencies Among *Escherichia coli* and *Salmonella* Pathogens

J. Eugene LeClerc, Baoguang Li, William L. Payne, Thomas A. Cebula*

High Rate of Macrolide Resistance in *Staphylococcus aureus* Strains from Patients with Cystic Fibrosis Reveals High Proportions of Hypermutable Strains

Anne-Laure Prunier,[1] Brigitte Malbruny,[1] Muriel Laurans,[2] Jacques Brouard,[2] Jean-François Duhamel,[2] and Roland Leclercq[1]

Consequences of mutator hitchhiking in asexual populations

The approach to mutation–selection balance in an infinite asexual population, and the evolution of mutation rates

Toby Johnson

The fitness cost of an increased mutation rate does not apply immediately

Johnson, 1999

The evolution of genomic mutation rate is upwardly biased

The Evolution of Mutation Rate in Finite Asexual Populations

Jean-Baptiste André[1] and Bernard Godelle[2]

Andre and Godelle, 2006

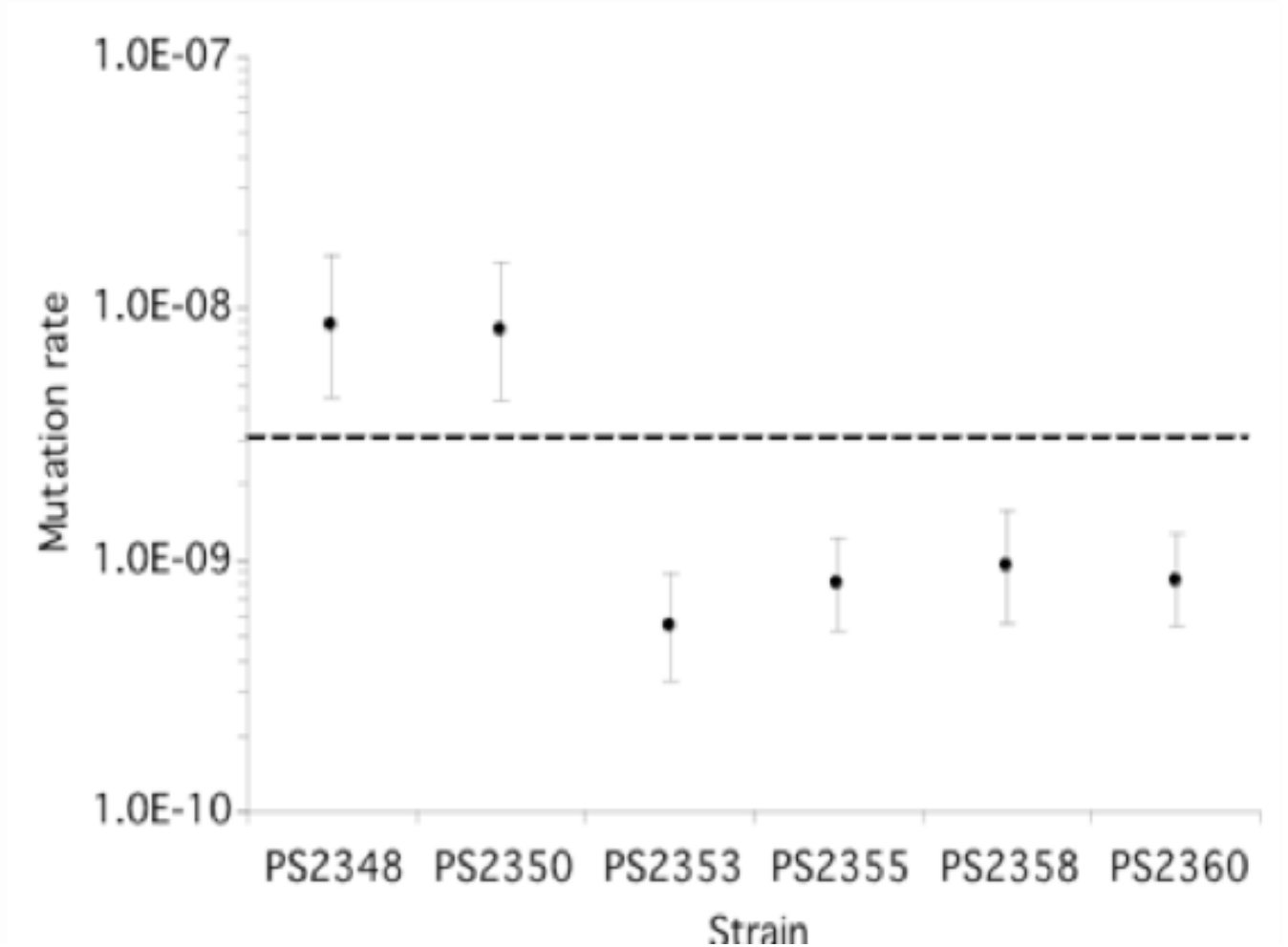
Complete genetic linkage can subvert natural selection

Philip J. Gerrish[†*§], Alexandre Colato[¶], Alan S. Perelson[†‖], and Paul D. Sniegowski[††]

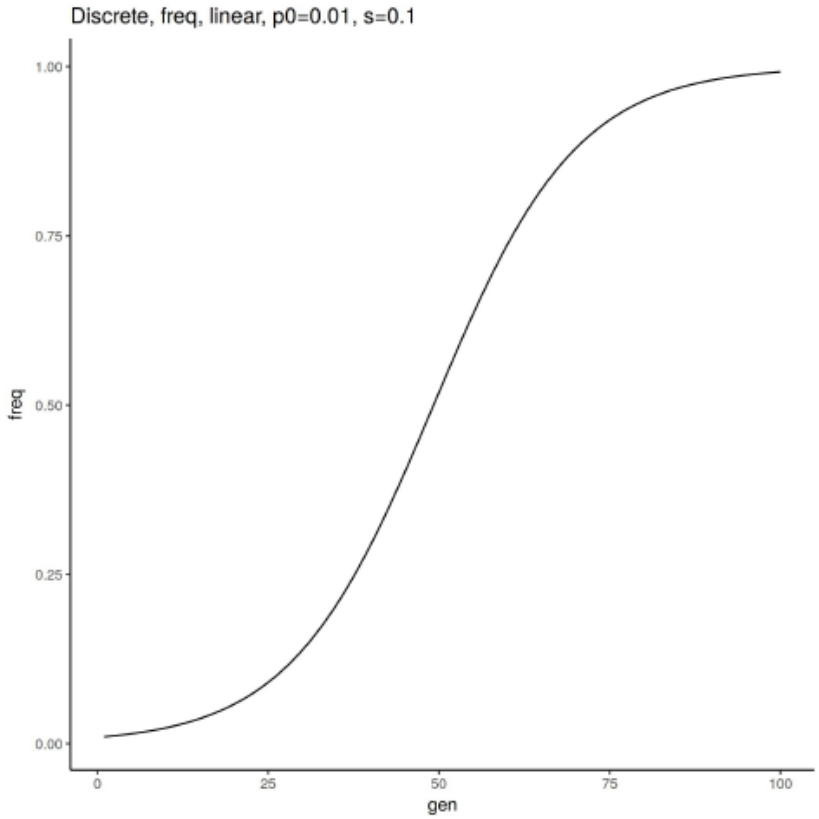
The mutation rate may evolve upward to a level that threatens population viability

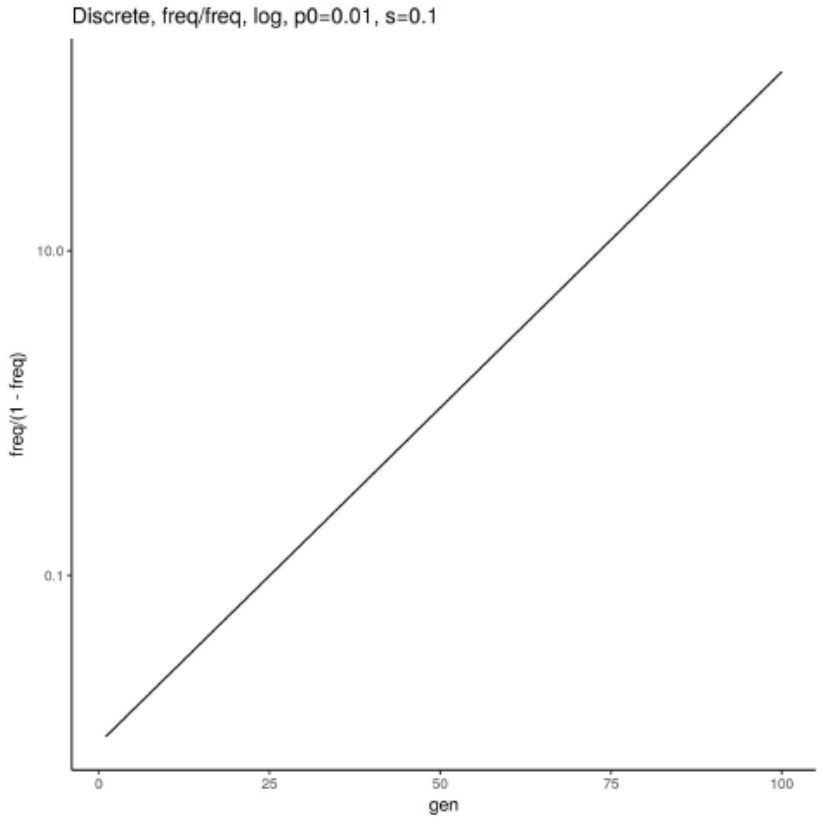
Gerrish et al, 2007

Allele replacement experiments confirm *mutL* repeat mutations as cause of mutator phenotype in Ara-2 and Ara-4 populations



Aside: deterministic selection looks like a straight line on a log of freq ratio plot.





- Mutators are able to fix starting well lower than a threshold of U_{wt} / U_{mut} because (at these parameters) they get multiple beneficial mutations.
- They need these multiple beneficial mutations to fix. With only one BM, they cannot.
- With just one BM, a mutator + 1BM lineage will increase roughly 10- to 100-fold in frequency. This means that starting at 10^-4, mutators may be bumped up to 10^-2, the point at which they have an equal chance of getting the 2nd BM.
- To say it another way: there are initially 10,000 times more wild-types than mutants. But there are soon only 100 times as many wt+1BM lineages as their are mut+1BM lineages.
    - This is in part because the population is large enough that many lineages can climb dramatically in frequency without affecting each other, at the beginning.