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

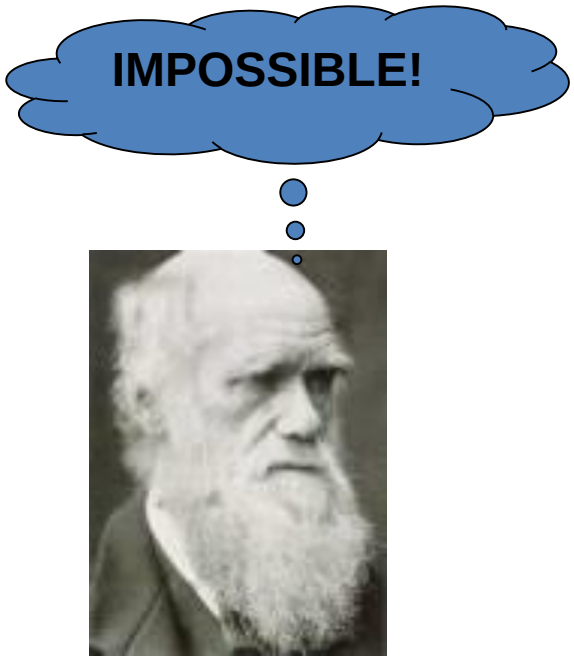
Complete genetic linkage can subvert natural selection

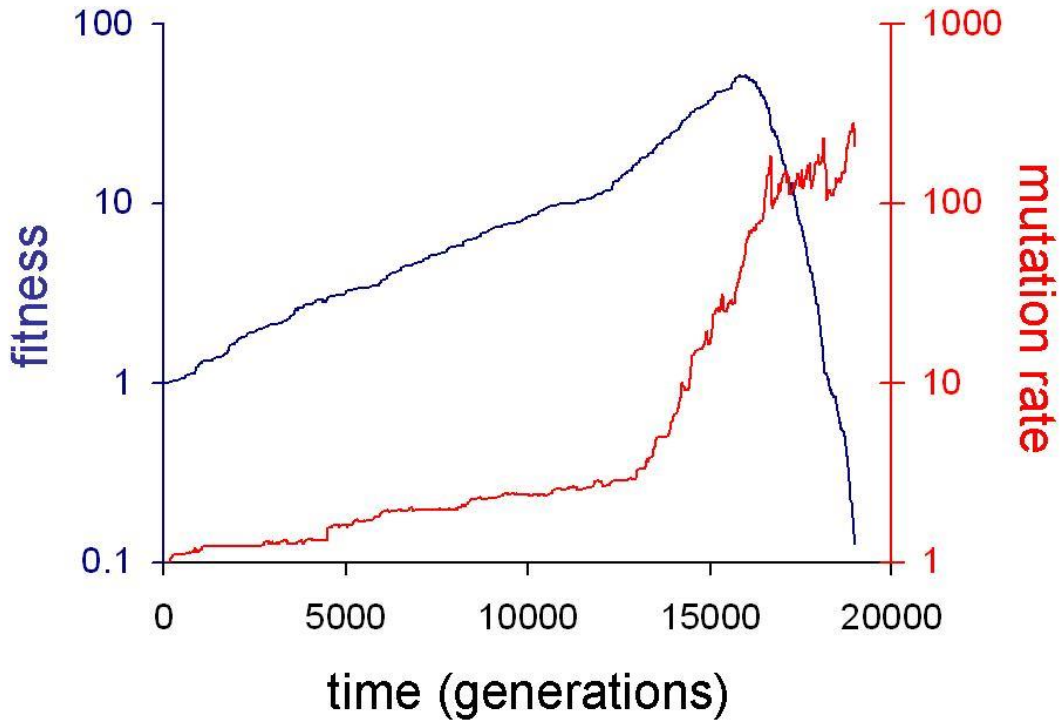
Philip J. Gerrish[†*§], Alexandre Colato[¶], Alan S. Perelson[†‖], and Paul D. Sniegowski[††]

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

Gerrish et al, 2007

A representative simulation result





Gerrish et al. 2007

A “mutation rate catastrophe hypothesis”: elements

1. The mutation rate in any organism--even viruses--is a polygenic trait. There are many potential mutator loci, and their effects can act cumulatively to produce extremely high mutation rates.

2. Any *antimutator* allele that arises in a population is most likely to arise on the average loaded background and thus to have no immediate advantage over a mutator allele. Only its future advantage--a decreased accumulation of deleterious mutations over time--is likely to give an antimutator a selective edge. Although the equilibrium cost of a mutator allele in an asexual population is easily calculated and can be substantial, theory indicates that this equilibrium will be approached slowly if there are many mildly deleterious mutations.

3. In the meantime, additional mutator hitchhiking events may occur in association with beneficial mutations, driving the genomic mutation rate even higher.

4. Above a certain genomic mutation rate, the dispersive force of mutation on fitness can overwhelm natural selection and cause extinction.

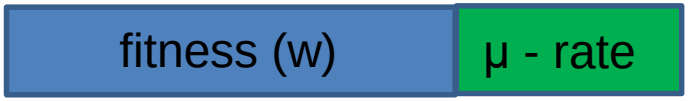
The required linkage

Loci that affect mutation rate: replication, repair, proofreading genes, etc…

Loci that affect fitness: use your imagination…

Simulations

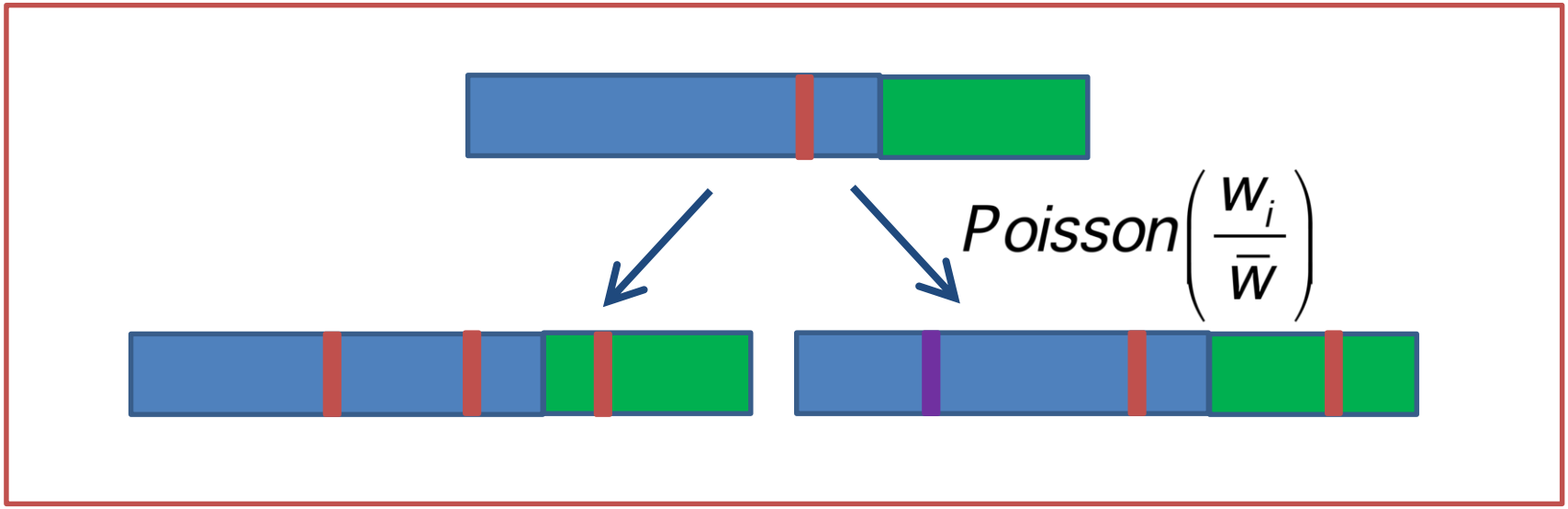
genome



↑ entropy: ↓ w, ↑ μ

↓ entropy: ↑ w, ↓ μ

Replication:



The mutation rate catastrophe occurs:

IF: $f_M > f_{AM}$

(mutator mutations more common than antimutator mutations)

IFF: $f_D > f_B > 0$

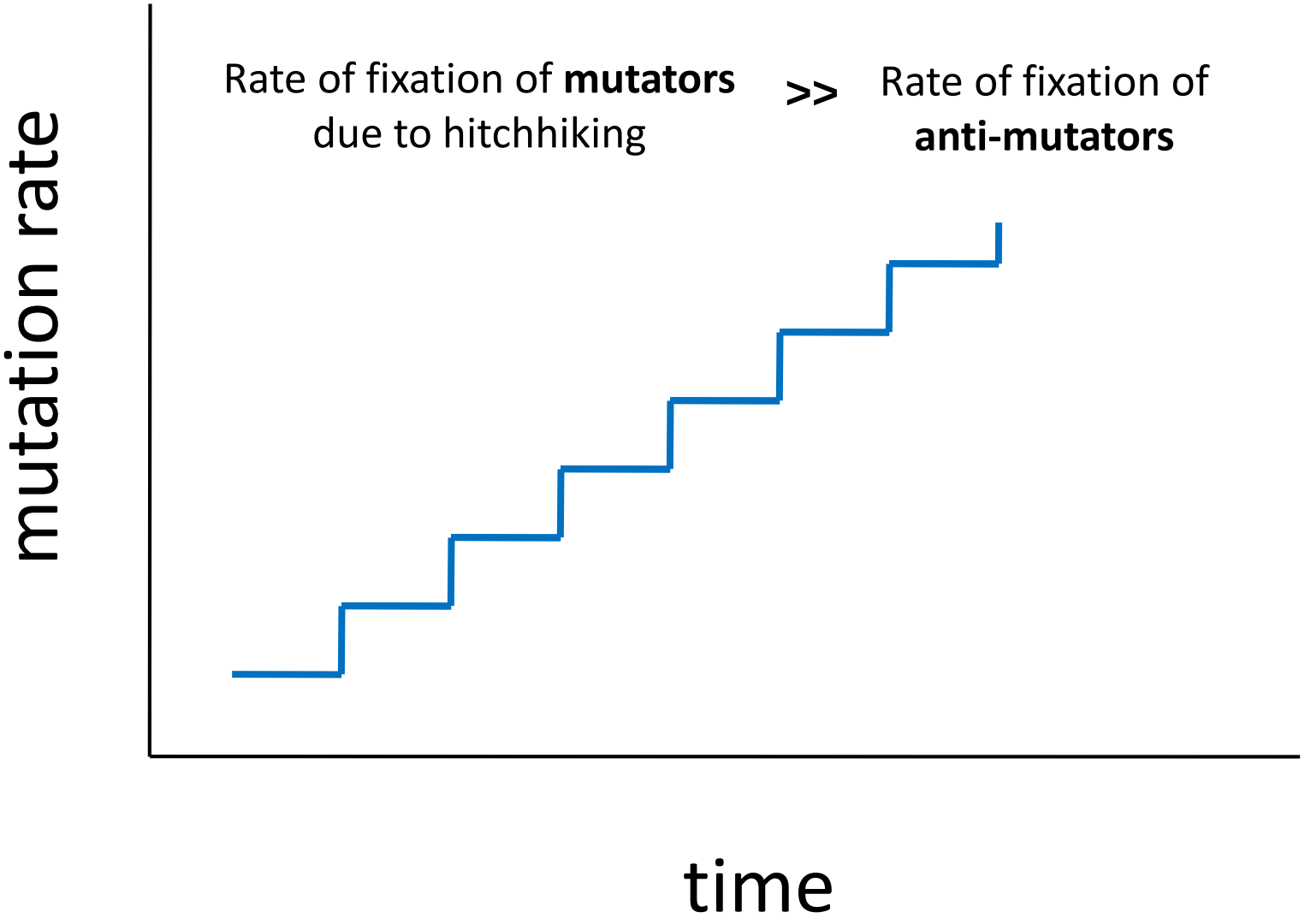
(deleterious mutations more common than beneficial mutations)

Mutation-rate ratchet

Evolutionary asymmetry:

Rate of fixation of **mutators** due to hitchhiking **>** Rate of fixation of **anti-mutators**

Mutation-rate ratchet?

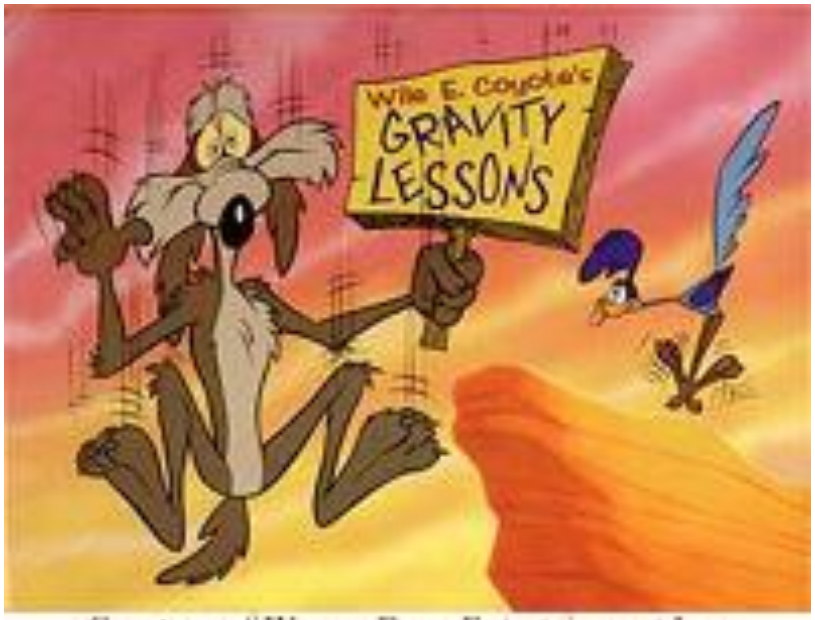


Why it works (at least in simulations)

How could natural selection, a process that operates through the avoidance of extinction, be involved in driving a population extinct?

Natural selection is short-sighted

- Mutational load takes time to accumulate.
- Natural selection does not anticipate the realization of excess mutational load.



Courtesy of Warner Bros. Entertainment Inc.

Ways in which an elevated genomic mutation rate can threaten population viability in asexuals

1. Muller's ratchet is accelerated (stochastic).

2. Mutational meltdowns are accelerated (stochastic).

3. The lethal mutation rate is exceeded (deterministic).

4. "Error threshold" for fitness and/or mutation rate is exceeded (deterministic).

5. Rate at which net effect of mutation degrades fitness exceeds rate at which natural selection operates to increase/maintain it. (Related to 4.)

Muller's Ratchet: A cost of asexuality

"…an asexual population incorporates a kind of ratchet mechanism, such that it can never get to contain, in any of its lines, a load of mutations smaller than that existing in its at present least-loaded lines."

Muller 1964

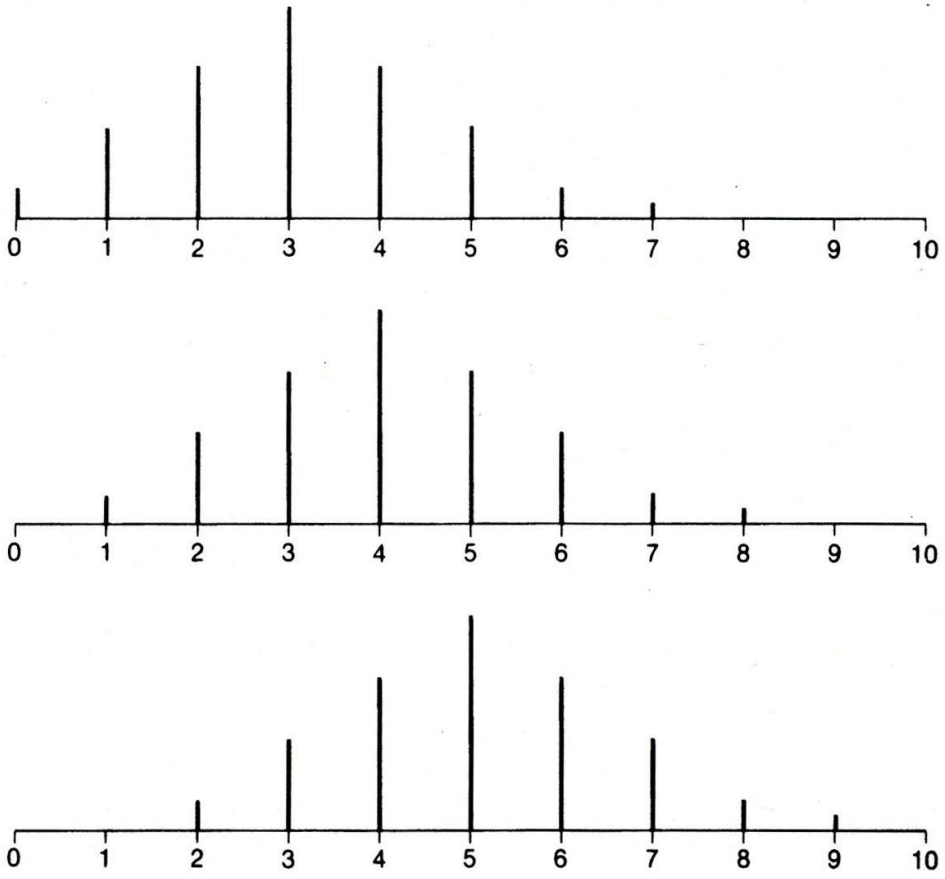


Figure source: Maynard Smith, J. *Evolutionary Genetics*, 2nd ed. Oxford U. Press

Note: Muller's ratchet and mutational meltdowns can be slowed and even stopped by large population size and/or beneficial mutations.

The idea of “lethal mutagenesis” in asexuals (papers by Bull, Wilke et al.; process was actually modeled before them by Fontanari, Colato and Howard 2003)

Assume:

-mutations per genome are Poisson-distributed
-all mutations deleterious or neutral
-deleterious mutations arise at genomic rate U_d
-population infinite

Then mean fitness at equilibrium is the Poisson fraction of mutation-free genotypes:

$$\overline{w} = e^{-U_d}$$

Let *b* represent the number of offspring that would reproduce in the absence of mutation.

Then extinction in the largest population is assured if equilibrium absolute fitness is less than one:

$$b e^{-U_d} < 1$$

Lethal mutagenesis, contd.

Consider a simple model for a bacterial population that in the absence of mutation would be growing exponentially by binary fission (and ignoring absolute generation time). For this situation, $b = 2$ and extinction is assured if

$U_d > 0.69$.

Under more realistic circumstances, a lower genomic rate can cause extinction.

Notes:

--The process is deterministic and does not depend on population size.

--Approach to equilibrium fitness may take many generations and depends on distribution of effects of deleterious mutations. (Equilibrium occurs in 1 generation if all mutations are lethal…) This point about the approach to equilibrium fitness has already been made.

--Beneficial mutations are not considered in the model but can slow or even prevent extinction, as shown by later experimental work of Bull and collaborators.

The idea of the “error threshold”

This concept comes from the quasispecies theory of Eigen and Schuster, which considers the evolution of polynucleotide sequences of infinite length in an infinite population under mutation and selection.

For a simple fitness landscape with a single peak, adaptation of the population (by which is meant localization around the peak in sequence space) is impossible if $u > 1/L$, where u is per site mutation rate and L is sequence length. Beyond this threshold the population undergoes a transition toward a state in which all sequences become equally likely.

Notes: The significance of the error threshold for extinction is controversial. Its original formulation is for an infinite population, so extinction cannot occur, strictly speaking. (There is no demography.)

Another alternative: Dynamical definition of mutation rate at which fitness declines

$$U\bar{s} > \sigma_x^2$$

$\bar{s}$ = *mean deleterious effect of mutations generally*

$\bar{s} = f_D m_D - f_B m_B$

U = *genomic mutation rate*

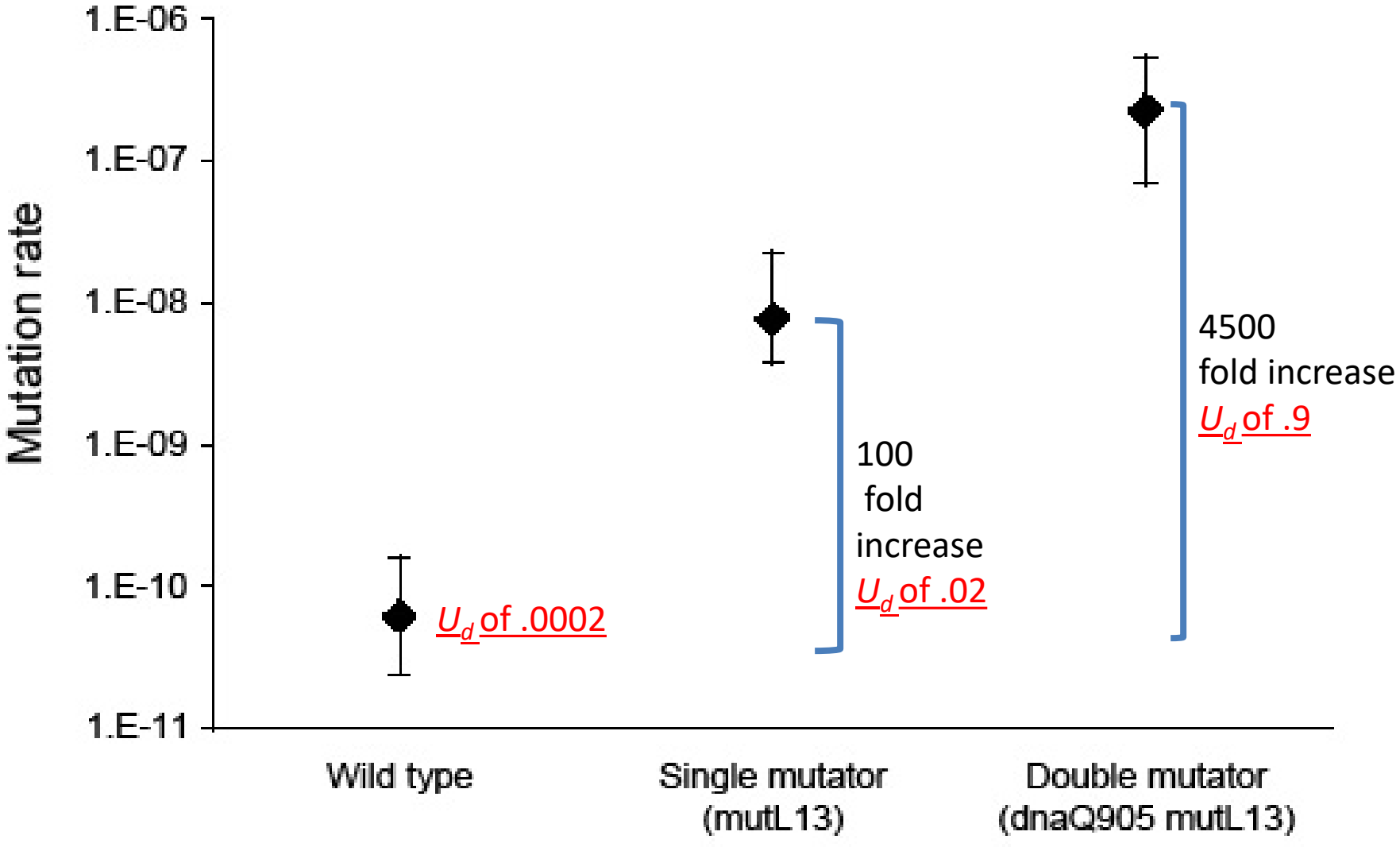
σ_x^2 = *fitness variance*

Gerrish, Colato, Sniegowski 2013

How to test the mutation rate ratchet idea experimentally?

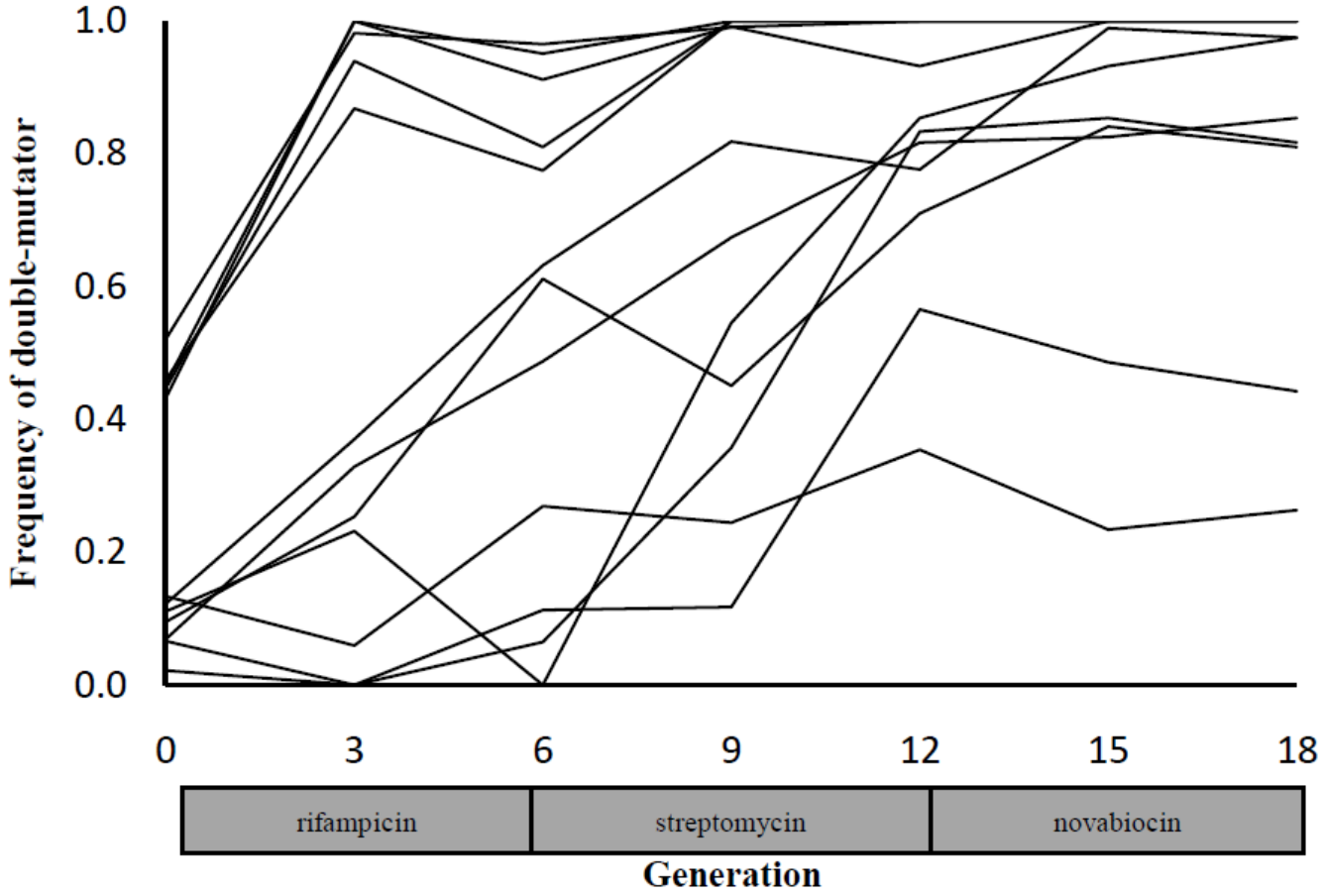
- Can’t wait for whole process: too long.
- However, mutator hitchhiking *is* predictable in short run (recall Chao and Cox experiment).
- Some other approaches: “divide and conquer”
  - Create strains with one and two mutator defects. (Done.)
  - Test for hitchhiking of double mutator over single, á la Chao and Cox; this is one click of the mutation rate ratchet.
  - Test for *spontaneous* evolution of higher-order mutators.
  - Look for evidence that very high mutation rates can lead to reductions in fitness (”dis-adaptation”) or even extinction.

Engineered “Single-” and “Double-mutator *E. coli*



Estimates of U_d are based on Kibota and Lynch’s (1996) mutation accumulation experiments in minimal media

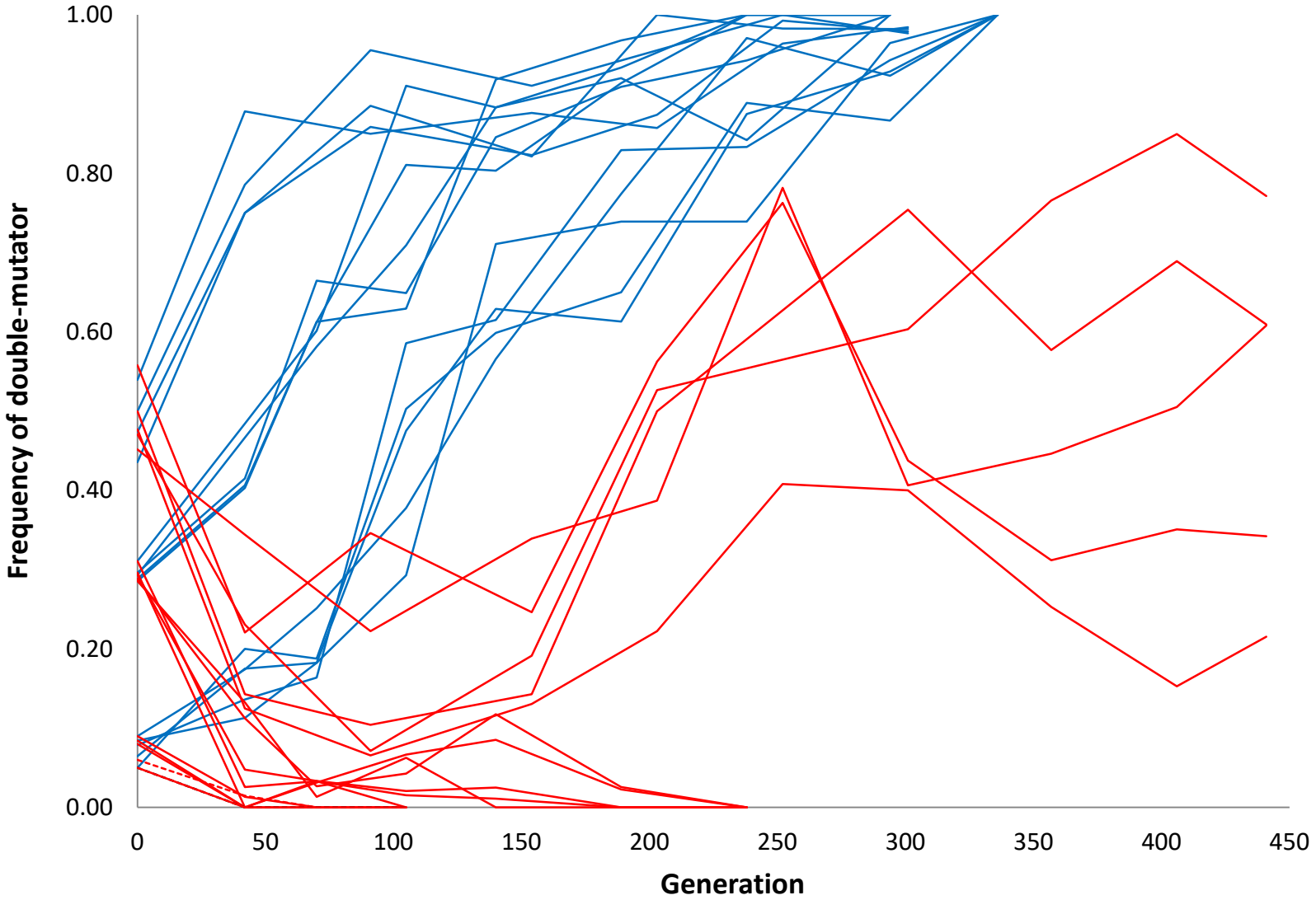
Single/Double-mutator competitions under lethal selection



Grey boxes indicate periods of antibiotic exposure. The number of generations reported in the figure is a minimal estimate because of sharp reductions in population size and subsequent regrowth upon exposure to antibiotics.

Gentile et al., 2011

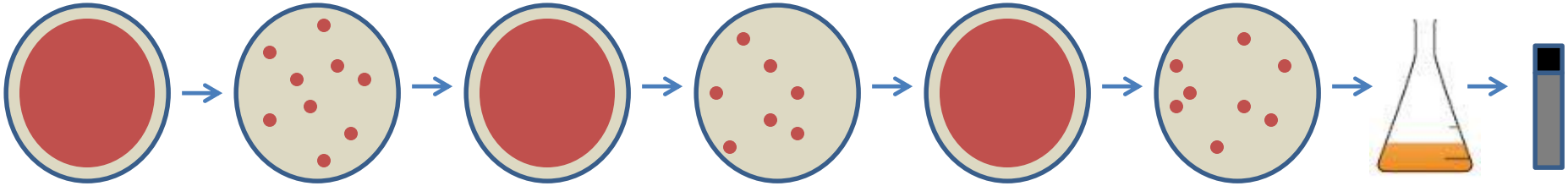
Single/Double-mutator competitions



: populations propagated in low glucose medium : populations propagated in high glucose medium. Ten populations in low glucose medium in which the double-mutator began at low frequency (~0.03) and was lost within 70 generations are not shown.

Gentile et al., 2011

Testing for spontaneous evolution of higher-order mutator genotypes. II: Effect of repeated lethal selection on a mutator population.



1) Grow a mutator ancestor clone, with a mutation in *mutL*, on plain LB.

2) Replica-plate to **fosfomycin**. Incubate O/N. Colonies will grow.

3) Respread colonies with saline. Incubate O/N. A lawn will grow.

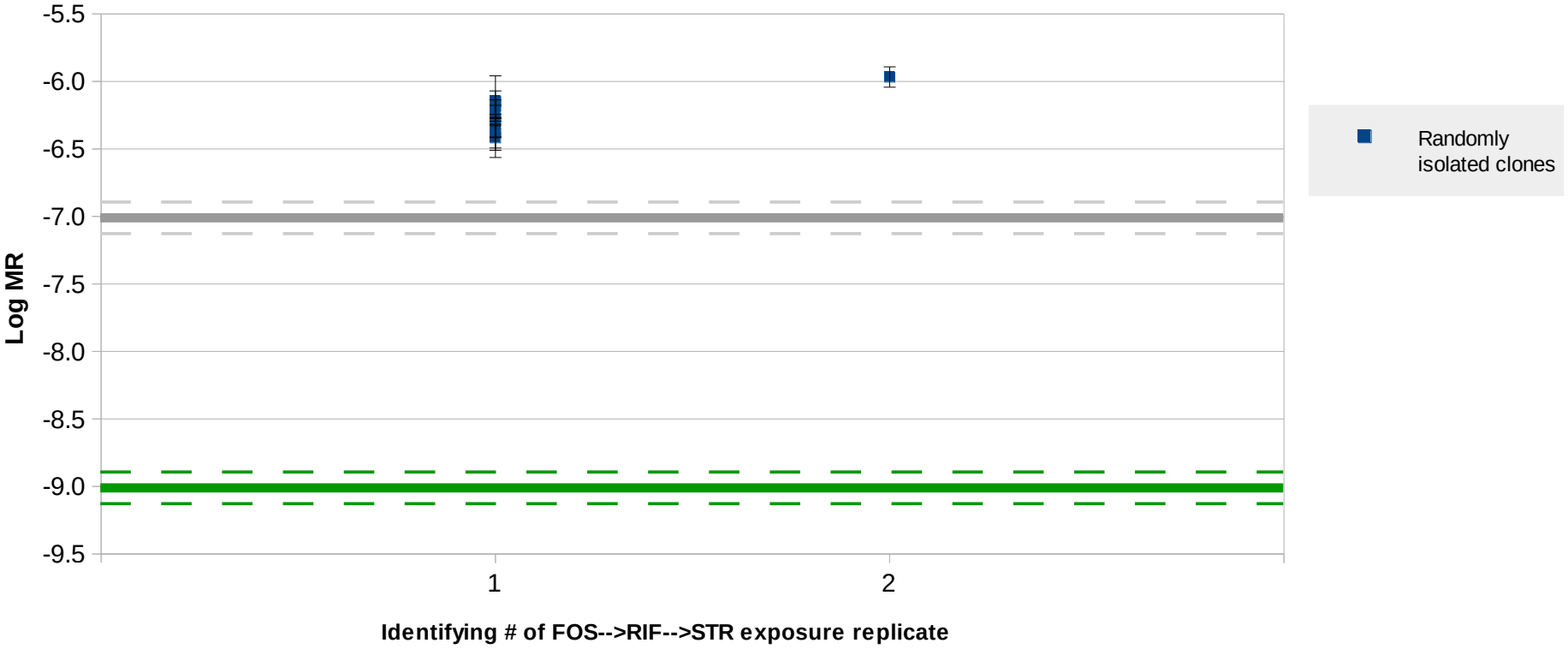
4) Replica-plate to **rifampicin**. Grow O/N. Colonies will grow.

5) Respread colonies with saline. Incubate O/N. A lawn will grow.

6) Replica-plate to **streptomycin**. Incubate O/N. Colonies will grow.

7) Harvest clones from strep plate. Assay mutation rates.

Result: repeated lethal selection enriches higher-order mutator genotypes in a population



*The mutator ancestor clone was exposed to **three rounds of antibiotics in succession: fos, rif, and str**.*

Nine clones were harvested from the str plate [Replicate 1].

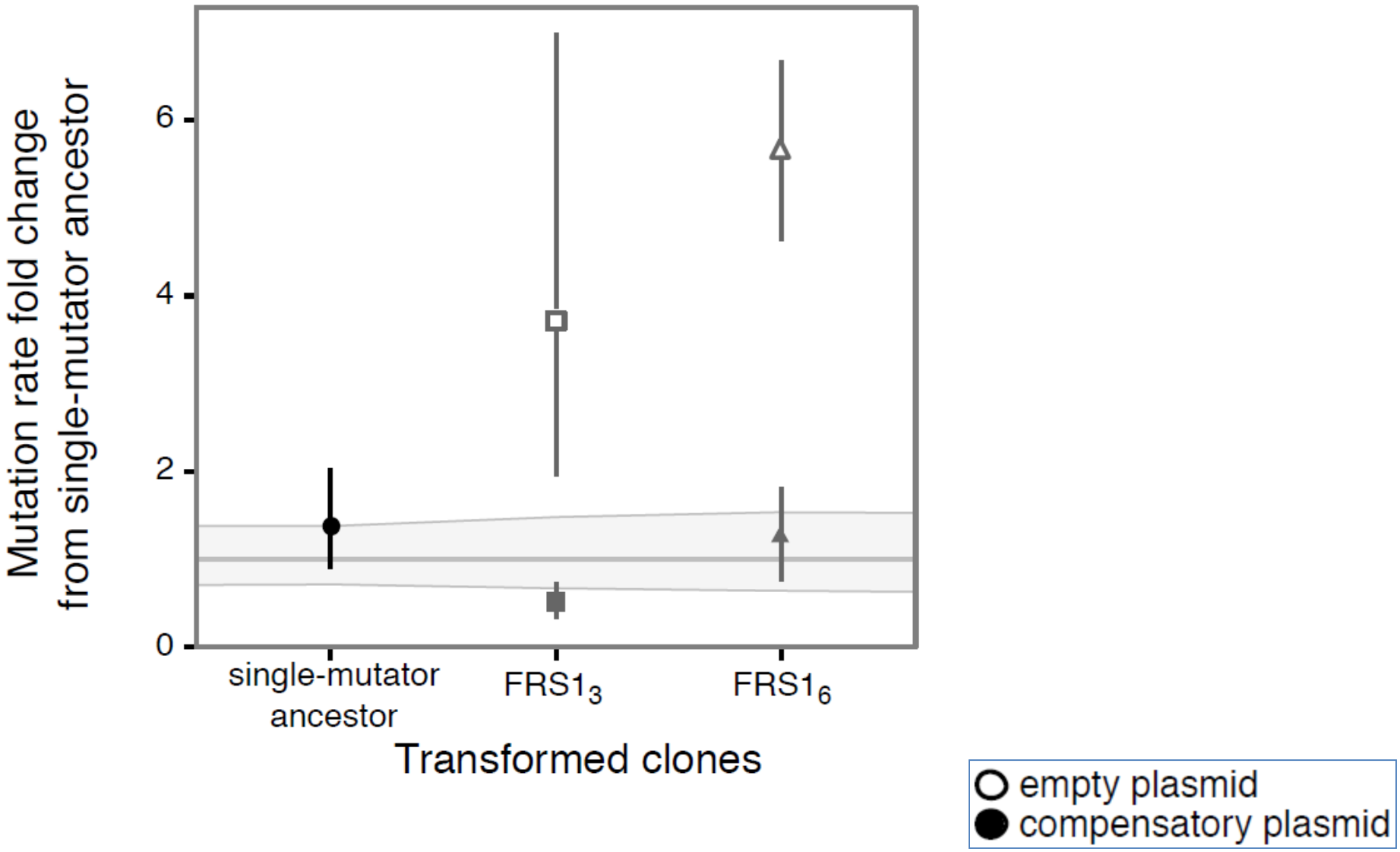
The same selection series was repeated on the same ancestral clone, but this time just one final clone was measured [Replicate 2].

Genomic sequencing of two endpoint hypermutator clones from lethal selection experiment shows that they now harbor *two* mutator alleles

- Both clones retain the *mutL* mutation from the mutator ancestor.
- Both clones also have a new frameshift mutation (1 bp insertion) in *mutT*, a known mutator locus.
- (Both clones have resistance mutations for all three selective antibiotics.)

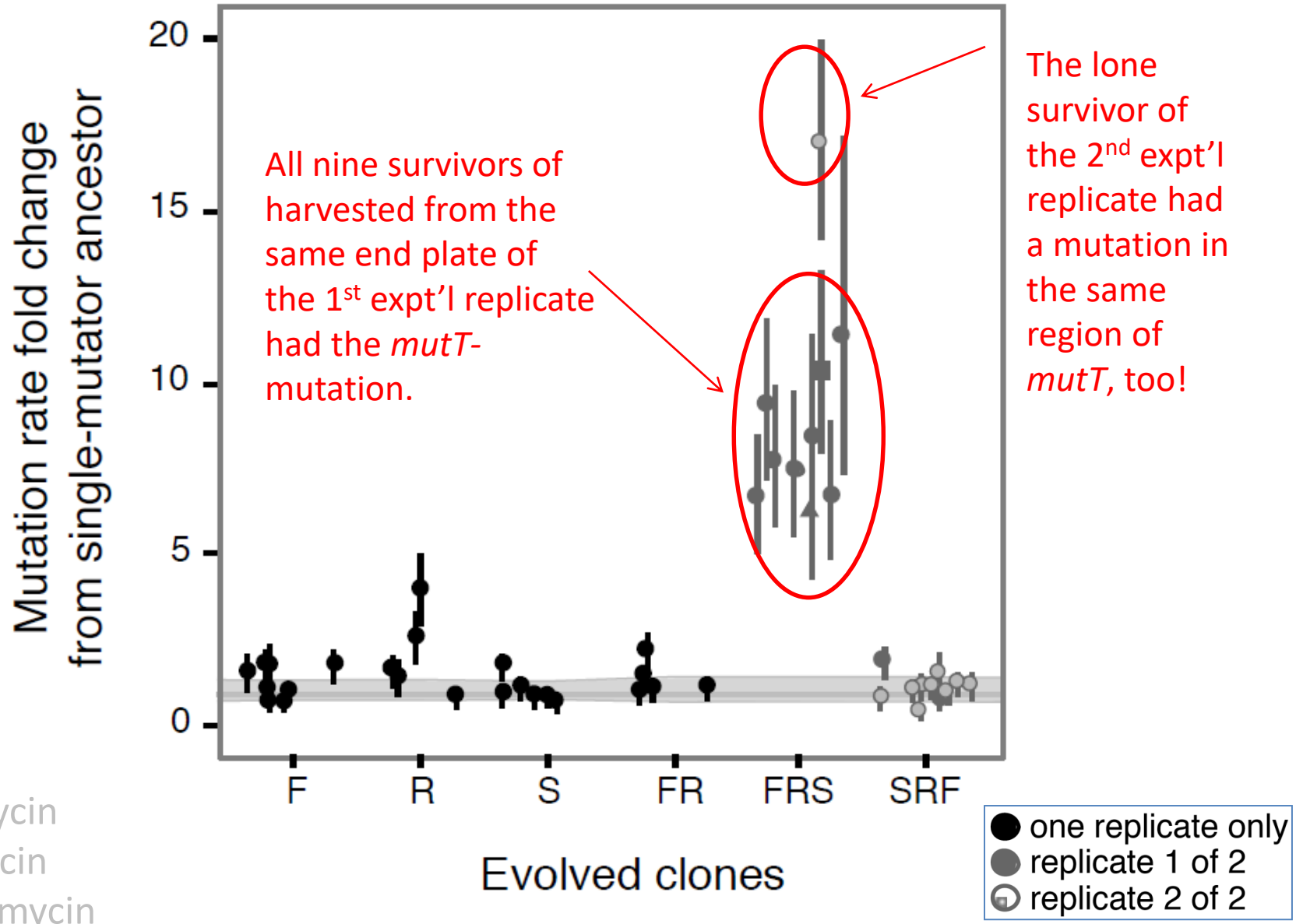
Plasmid complementation tests.

If the two *mutT*$^{-}$ hypermutator clones are transformed with *mutT*$^{+}$ plasmids, the ancestral mutator mutation rate is restored.



Mutation rates for all hard selection clones after antibiotic exposure.

Triple antibiotic selection does enrich for hypermutators -- *if* the least selective antibiotic is first in the series.



F = fosfomycin
R = rifampicin
S = streptomycin

mutT^{-} mutations outside of the Sniegowski lab

In Lenski's long-term evolution experiment, one of the evolving lines acquired a mutator phenotype due to a spontaneous 1-bp insertion in *mutT*, which elevated the mutation rate.

This mutation was in the same string of cytosines as in the mutation in our own experiment.

```
ATGAAAAAGC TGCAAATTGC GGTAGGTATT ATTCGCAACG AGAACAATGA
AATCTTTATA ACGCGTCGCG CAGCAGATGC GCACATGGCG AATAAACTGG
AGTTTCCCGG CGGTAAAATT GAAATGGGTG AAACGCCGGA ACAGGCGGTG
GTGCGTGAAC TTCAGGAAGA AGTCGGGATT ACCCCCCCAAC ATTTTTCGCT
ATTTGAAAAA CTGGAATATG AATTCCCGGA CAGGCATATA ACACTGTGGT
TTTGGCTGGT CGAACGCTGG GAAGGGGAGC CGTGGGGTAA AGAAGGGCAA
CCCGGTGAGT GGATGTCGCT GGTCGGTCTT AATGCCGATG ATTTTCCGCC
AGCCAATGAA CCGGTAATTG CGAAGCTTAA ACGTCTGTAG
```

mutT^{-} mutations outside of the Sniegowski lab

In an *E. coli* mutation accumulation experiment from the Foster lab, a 1-bp indel was found in *mutT.*

This mutation was in the same string of cytosines as in the mutation in our own experiment.

```
ATGAAAAAGC TGCAAATTGC GGTAGGTATT ATTCGCAACG AGAACAATGA
AATCTTTATA ACGCGTCGCG CAGCAGATGC GCACATGGCG AATAAACTGG
AGTTTCCCGG CGGTAAAATT GAAATGGGTG AAACGCCGGA ACAGGCGGTG
GTGCGTGAAC TTCAGGAAGA AGTCGGGATT ACCCCCCCAAC ATTTTTCGCT
ATTTGAAAAA CTGGAATATG AATTCCCGGA CAGGCATATA ACACTGTGGT
TTTGGCTGGT CGAACGCTGG GAAGGGGAGC CGTGGGGTAA AGAAGGGCAA
CCCGGTGAGT GGATGTCGCT GGTCGGTCTT AATGCCGATG ATTTTCCGCC
AGCCAATGAA CCGGTAATTG CGAAGCTTAA ACGTCTGTAG
```

Why was there convergent evolution of the *mutT*$^{-}$ mutation?

In general, frameshift mutations occur at greater frequency in repeat regions. (polymerase slippage)

Recall that in Lenski's long-term evolving lines, two of the mutator populations had insertions in the same repeat region of *mutL*, despite their independent evolution. (Shaver & Sniegowski 2003)

Are these "mutable mutators" evolvability adaptations?

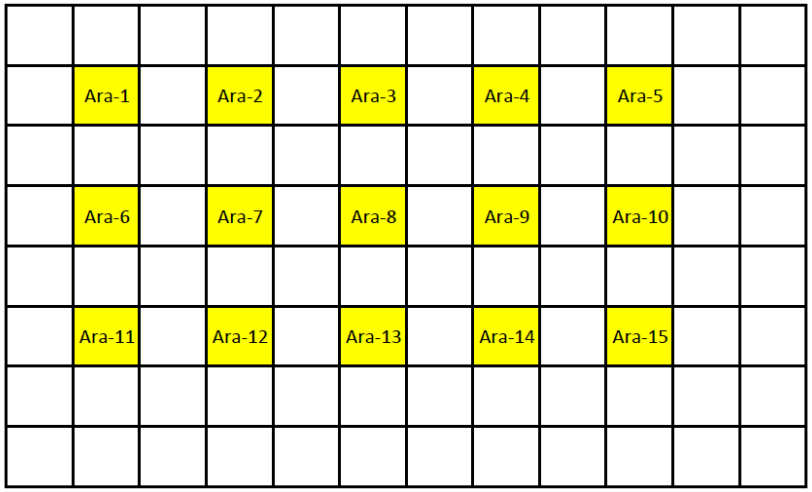
Testing for spontaneous evolution of higher-order mutator genotypes. I: long-term propagation of mutator asexual populations.

1. Start with 30 populations of single-mutator asexual *E. coli* (*mutS*-). All starting populations are isogenic, EXCEPT that 15 populations are Ara- & 15 populations are Ara+.

2. Grow the 30 populations in a liquid minimal growth environment in 96-well plates, each population in its own well.

3. Propagate the 30 populations daily via batch-transfer.

4. Freeze samples of each population every ~2 weeks.



Obtaining the Fluctuation Test Data for the Long-Term Evolving Populations

After ~900 generations of propagation, **150 clones** were randomly isolated from the 30 different populations. (That's **5 clones per population**.)

The mutation rate of each evolved clones was measured via fluctuation tests.

Overview of the Mutation Rate (MR) Data Collected From Fluctuation Assays

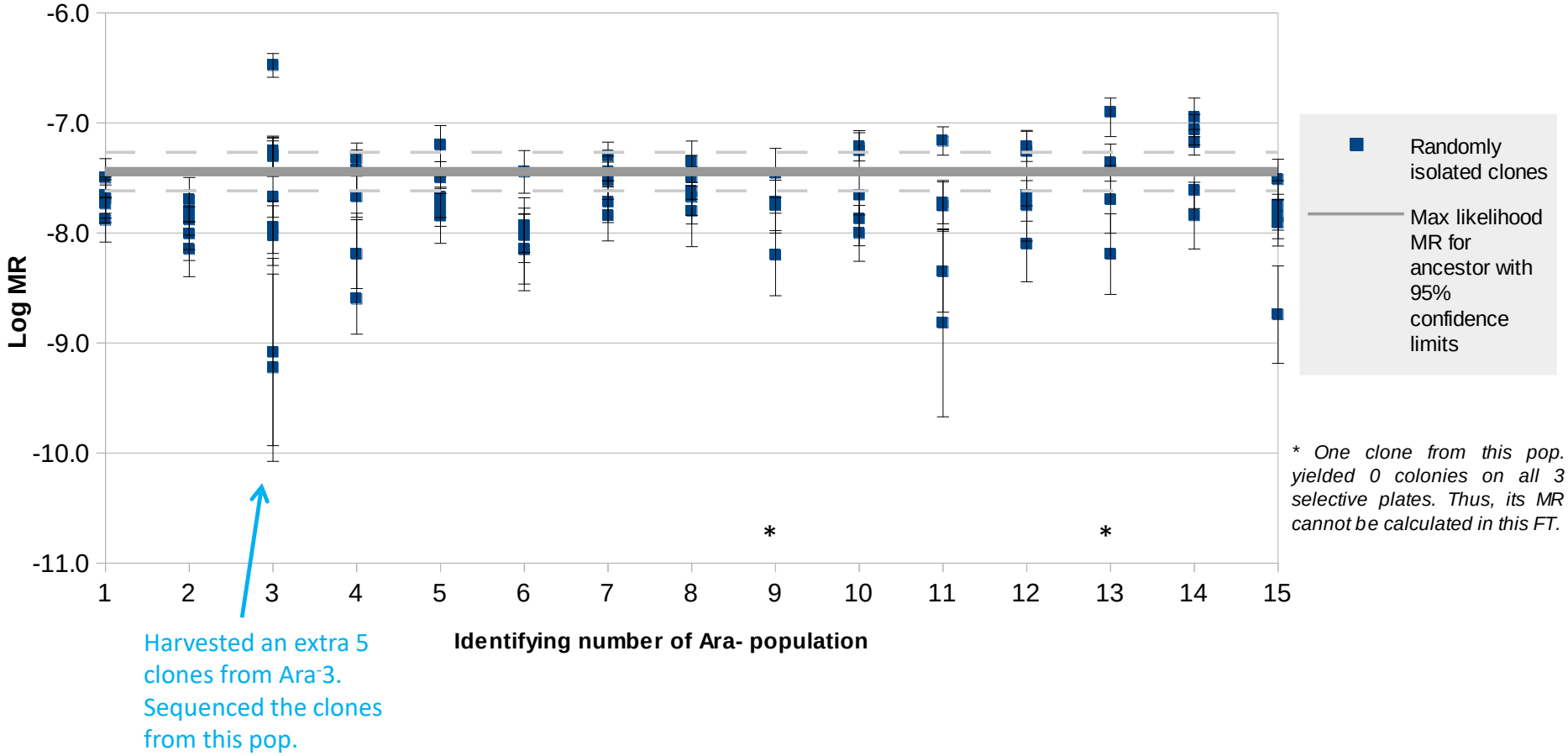
The MRs of the evolved clones were compared to the MR of their mutator ancestor (at generation 0), and assessed for overlap between the 95% confidence intervals:

9% of the clones had **higher** MRs than their mutator ancestor. (**14** clones)

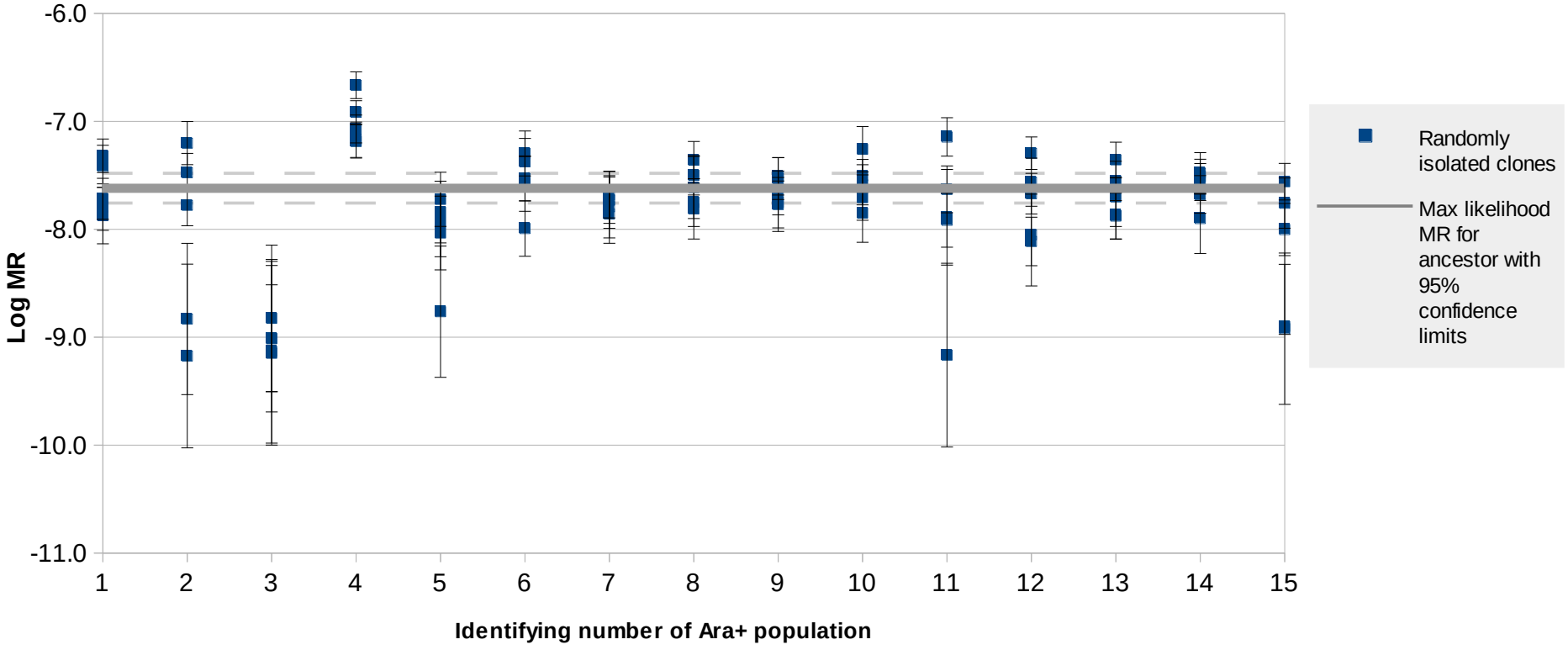
23% of the clones had **lower** MRs than their mutator ancestor. (**34** clones)

68% of the clones had **similar** MRs to their mutator ancestor. (**102** clones)

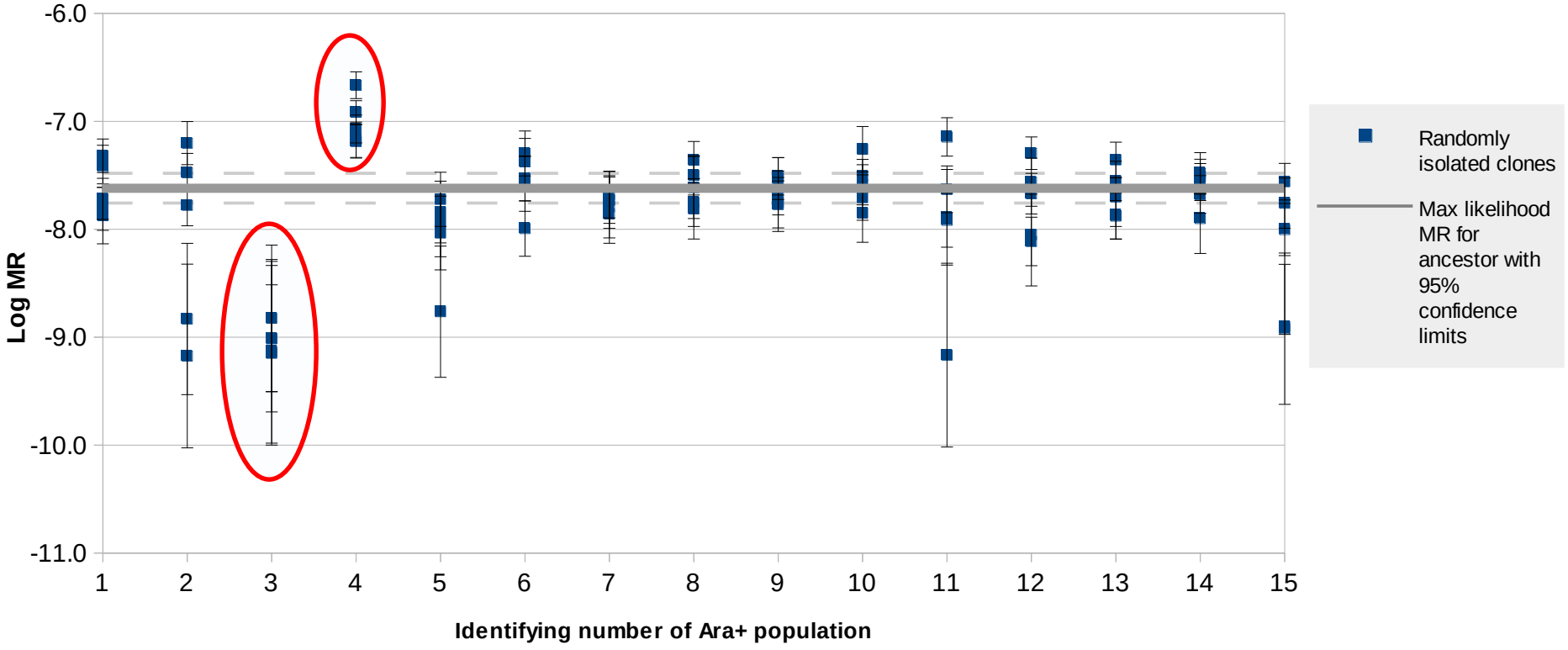
Mutation rates for the 80 evolved Ara$^-$ clones



Mutation rates for the 75 evolved Ara^{+} clones



Mutation rates for the 75 evolved Ara^{+} clones



So what can we make of these mutation rate measurements?

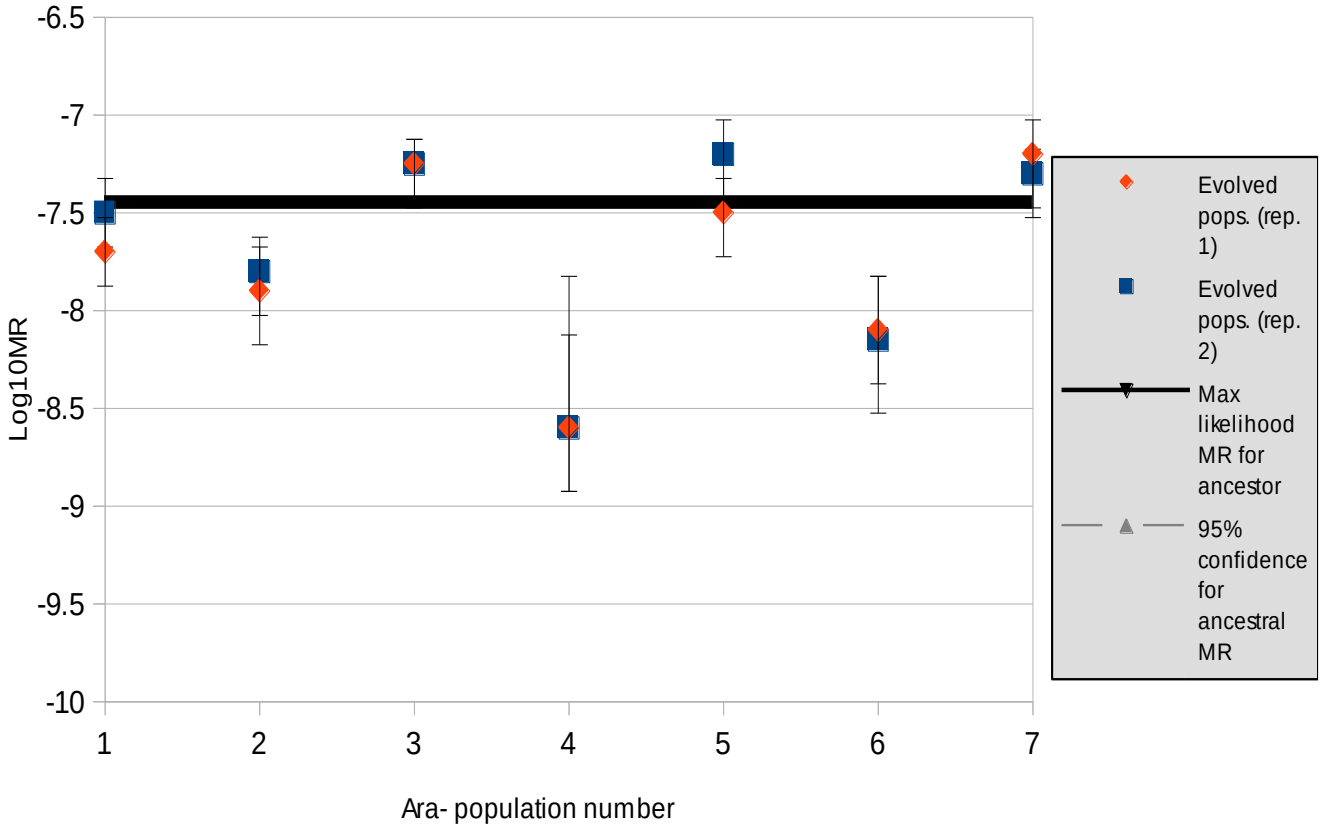
There is *variability* for mutation rate across evolved populations.

Most populations (21 out of 30) appear to be *polymorphic* for mutation rate. (But multiple testing concerns…)

However, some populations appear to be homogenous for mutation rate across all five clones:

- In these cases, usually homogenous for an *unchanged* mutation rate,
- But one population had all *elevated* mutation rates,
- And another population had all *lowered* mutation rates.

For a subset of the evolved clones, a second fluctuation assay replicate was performed…



What happened to the mutation rates after 900 generations of propagation under soft selection?

The increased mutation rates could have been caused by…

…mutator alleles spreading in the population, via mutator hitchhiking.

The decreased mutation rates could have been caused by…

…antimutator alleles that spread in the population, via:

A) conferring direct pleiotropic fitness advantages, and/or,

B) lowering the deleterious mutational load.

After 900 generations of propagation under soft selection, have the populations increased in fitness relative to ancestor?

- Uncontroversial hypothesis: yes, they have.
- We used the Ara^-/Ara^+ colony color differences as a way of determining relative fitness, through growth competitions.

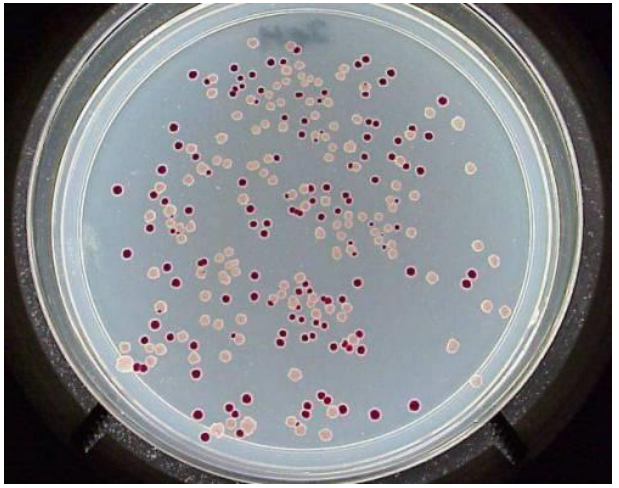


Image credit: Lenski lab

Fitness Competition Assays: Methodology

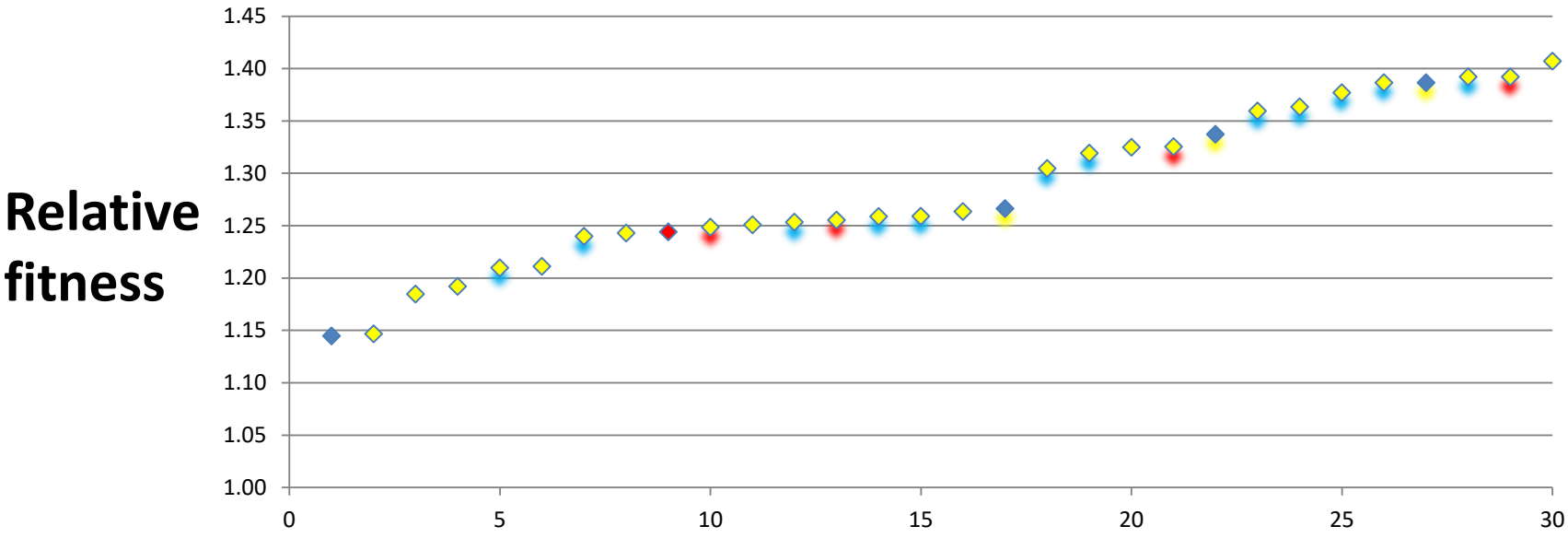
EVOLVED POPULATIONS	VERSUS	AN ANCESTRAL MUTATOR CLONE
 Ara-3 population	VS.	 Ara^{+} ancestral clone
 Ara+4 population	VS.	 Ara^{-} ancestral clone
…and so forth.		

Fitness assays for each of the populations were performed in triplicate.

Ara^{-} and Ara^{+} colonies are distinguishable by color on tetrazolium arabinose (TA) plates.

Relative fitness has increased in all evolved populations, but is there any detectable relationship between fitness and mutation rate?

The 30 evolved populations' relative fitnesses, rank-ordered:



LEGEND:

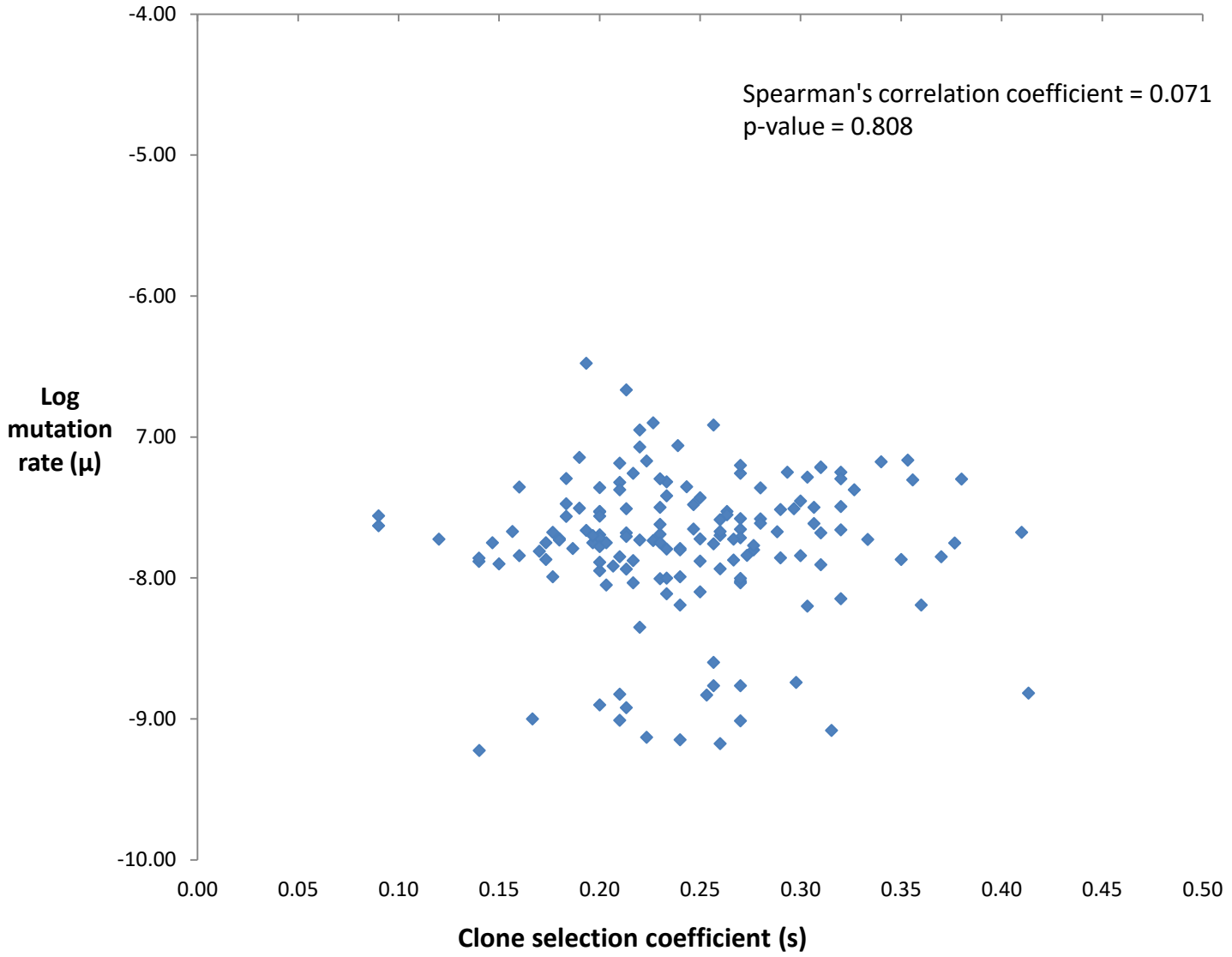
Blue means the MRs of the isolated clones of this population are *lower* than ancestor.

Red means the MRs are *higher* than ancestor.

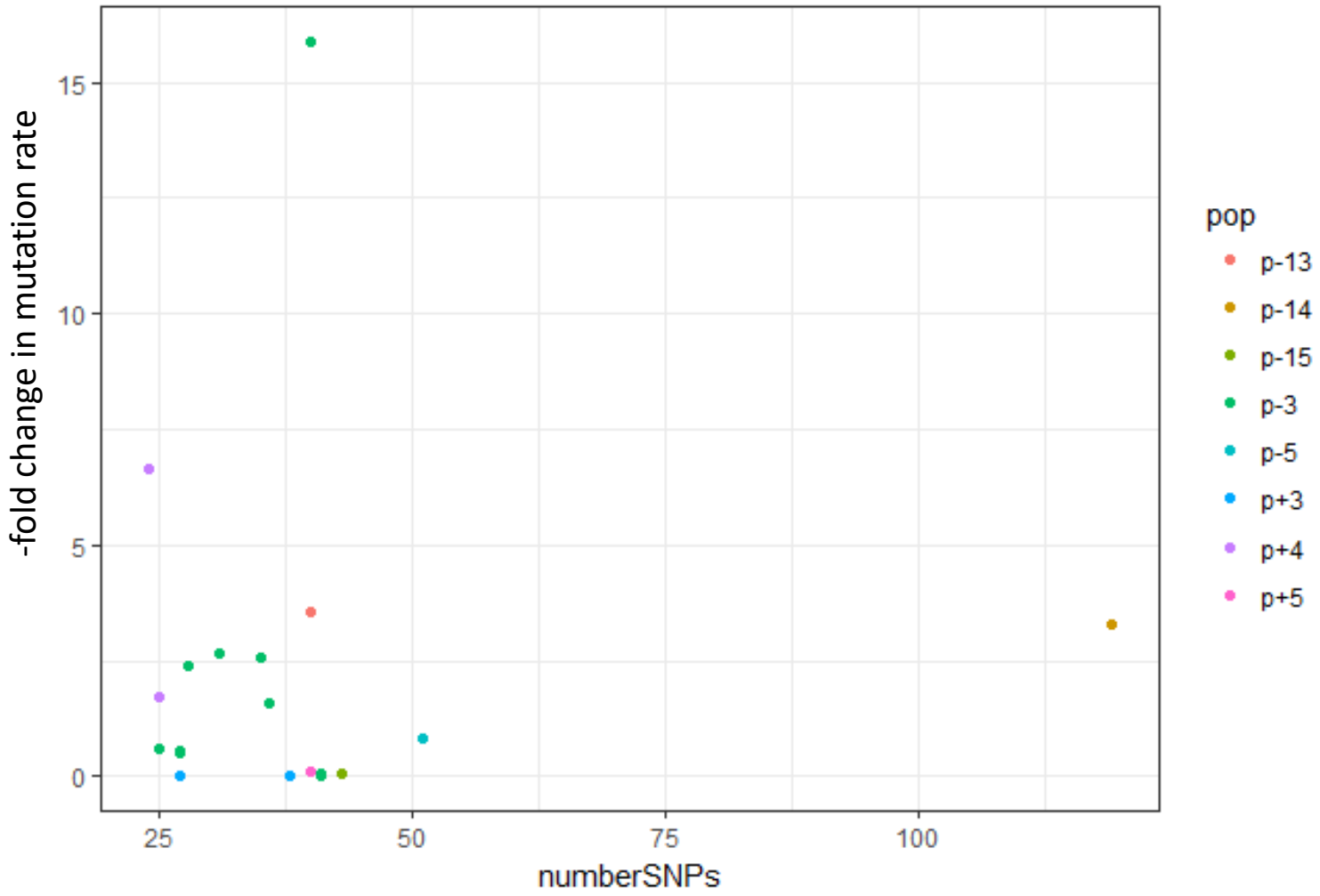
Yellow means the MRs *similar* to ancestor.

Populations appearing to be *heterogeneous* for MR have a colored shadow behind them.

In all evolved clones, fitness increased relative to ancestor – as expected. However, no relationship between fitness and mutation rate was detected.



Perhaps surprisingly, no correlation was found between mutation rate of the sequenced clones and the number of new mutations.



The Spearman's correlation coefficient (r_s) was -0.06, with a p-value of 0.60.

Perhaps surprisingly, no correlation was found between mutation rate of the sequenced clones and the number of new mutations.

Possible reason for this:

The acquisitions of new mutation rate modifiers could have occurred recently, in which case these changes in mutation rate might not be reflected in the current total number of mutations.

Genomic sequencing

- Is the ancestral *mutS*$^{-}$ mutation retained in the evolved clones?
- What new mutations might be responsible for the changes in mutation rate?

Sequenced the genomes of 20 of the evolved clones, which were selected to represent a variety of mutation rates and populations.

Brief summary of genomic findings

- The *mutS*$^{-}$ ancestral mutator mutation was retained in all of the 20 sequenced clones.
- There were a number of non-conservative mutations in known mutation rate modifier loci, including:
  - *miaA* (mutation found in clone with highly elevated mutation rate)
  - *radA*, *nrdE* (mutation found in clone with strongly lowered mutation rate)
  - *recC, topA* (mutations in various residues found in both elevated & lowered clones)

…. although no clear pattern of causality emerged.

Conclusions

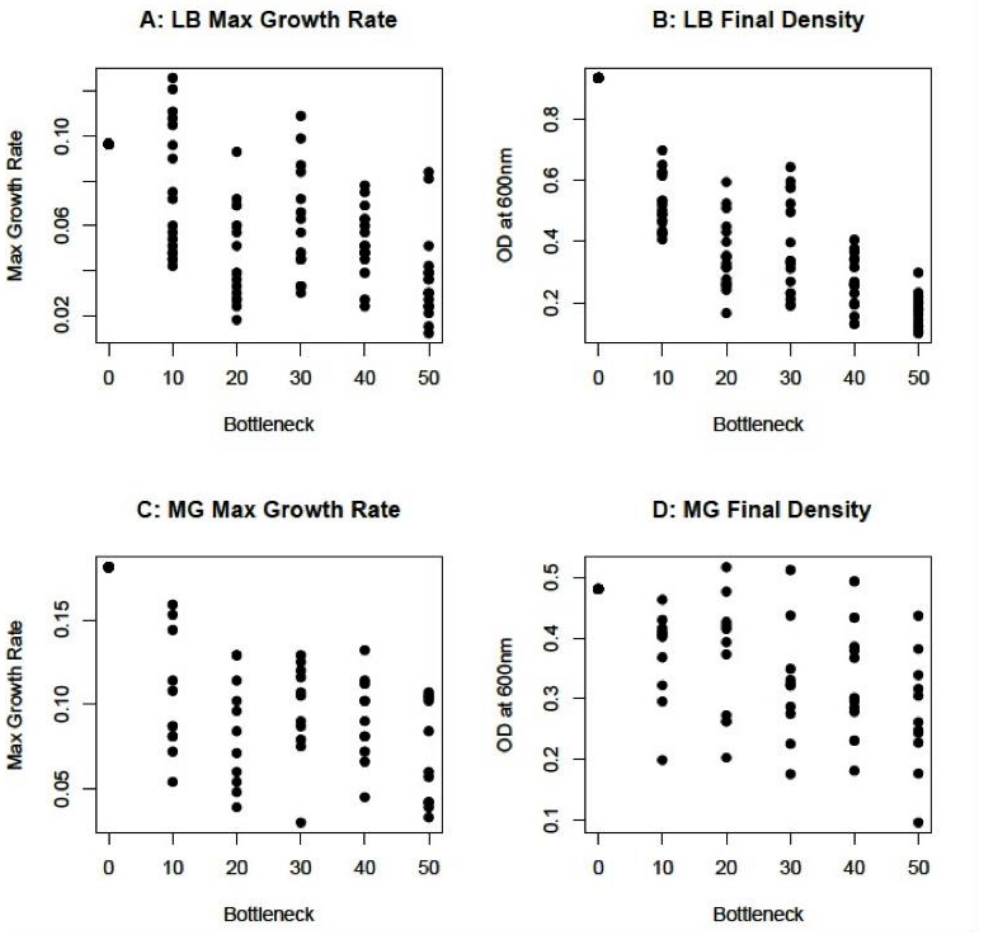
- In one population, all isolated clones underwent a further increase in mutation rate -- offering support to theoretical predictions of repeated mutator hitchhiking.
- Mutation rate instability (both upward *and* downward) may be more pervasive in asexuals than assumed.
- In several evolved individuals, mutation rates appeared to have been affected by (anti)mutators that have not, thus far, been identified in the literature.

Testing Fitness and Mutation Rate Evolution in Very Small Populations with Extremely High Mutation Rates

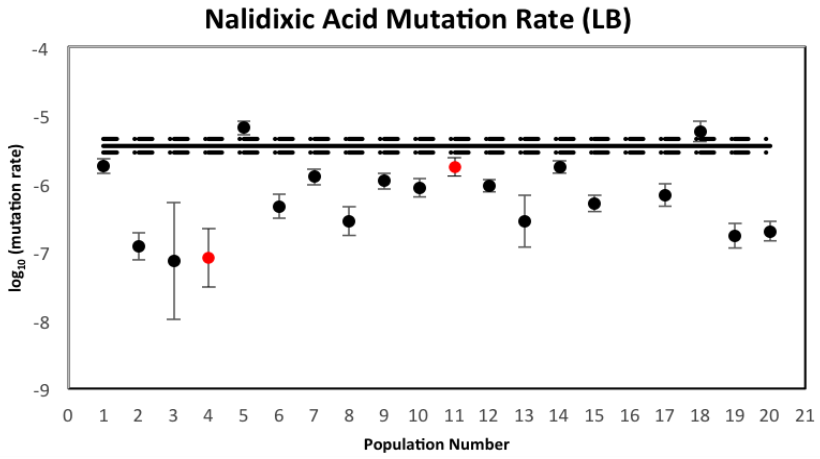
EXPERIMENTAL SETUP

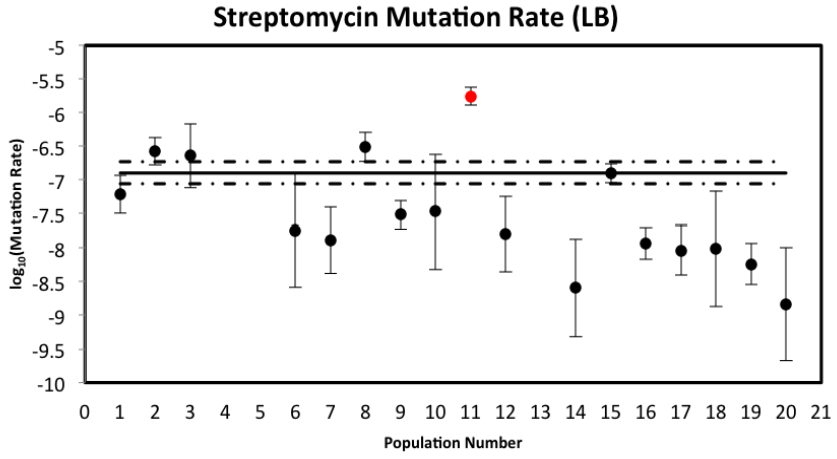
- An *E coli* strain possessing a genomic mutation rate ~4500 times higher than wild type *E coli* was propagated by dilution streaking to induce single cell bottlenecks for ~1250 generations.
- 20 replicate lines were propagated on 2 types of media: LB (rich medium) and MG (minimal glucose).
- Clones were isolated and frozen every 5 days of propagation, over a 50-day propagation period.
- A expected, fitness declined: 2 populations went extinct among LB lines and 9 populations went extinct among MG lines.
- **Mutation rates evolved during the experiment.**

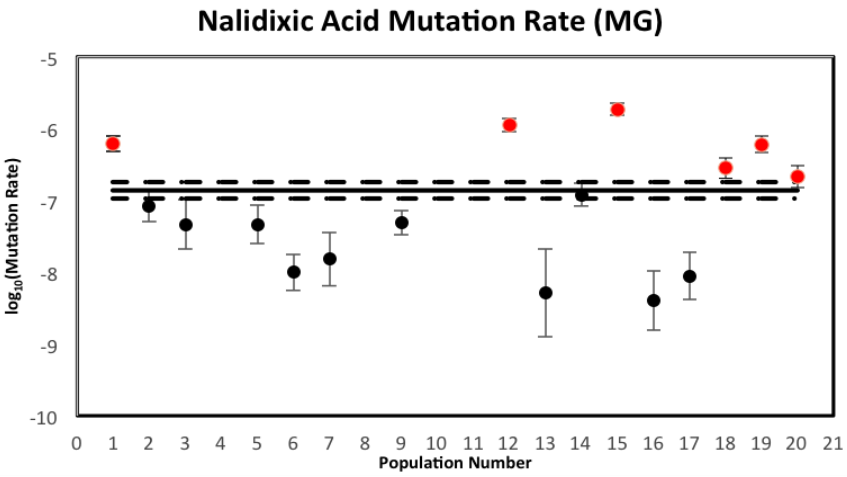
Results: Fitness proxies declined

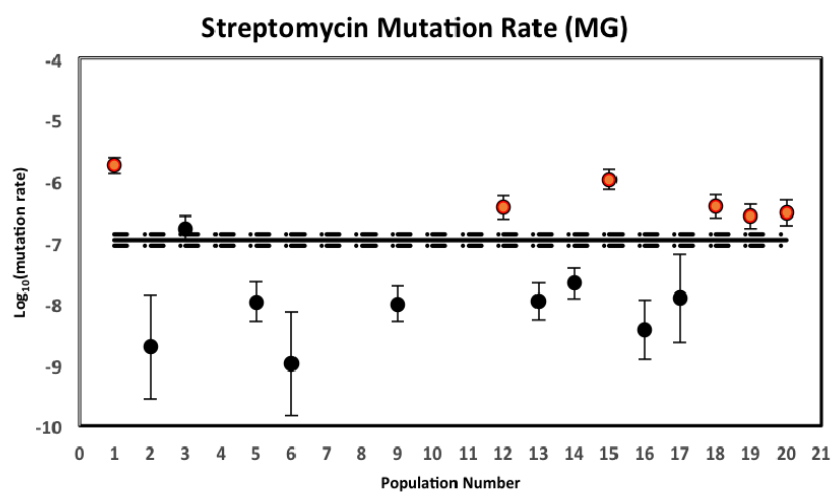


Mutation rates evolved







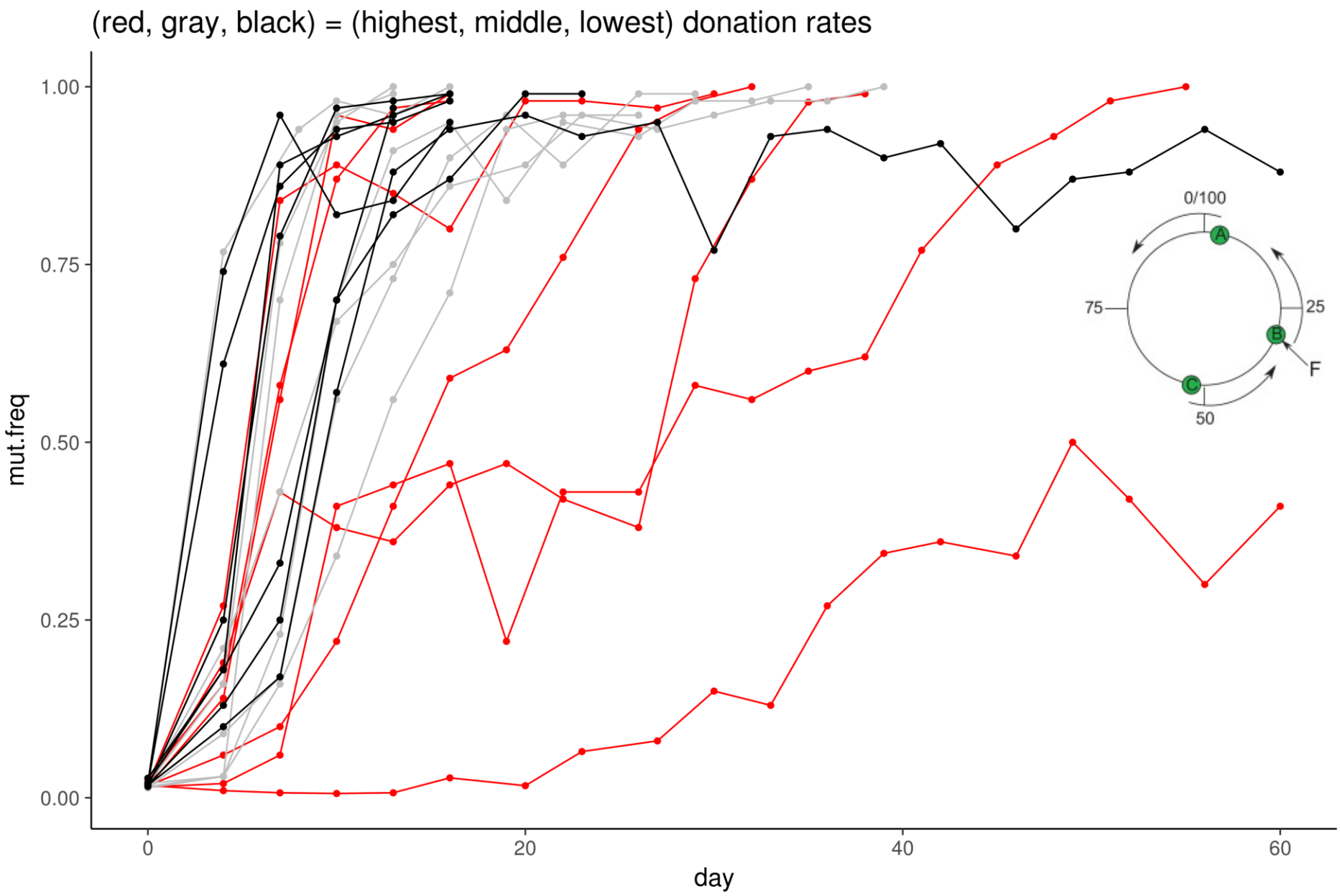


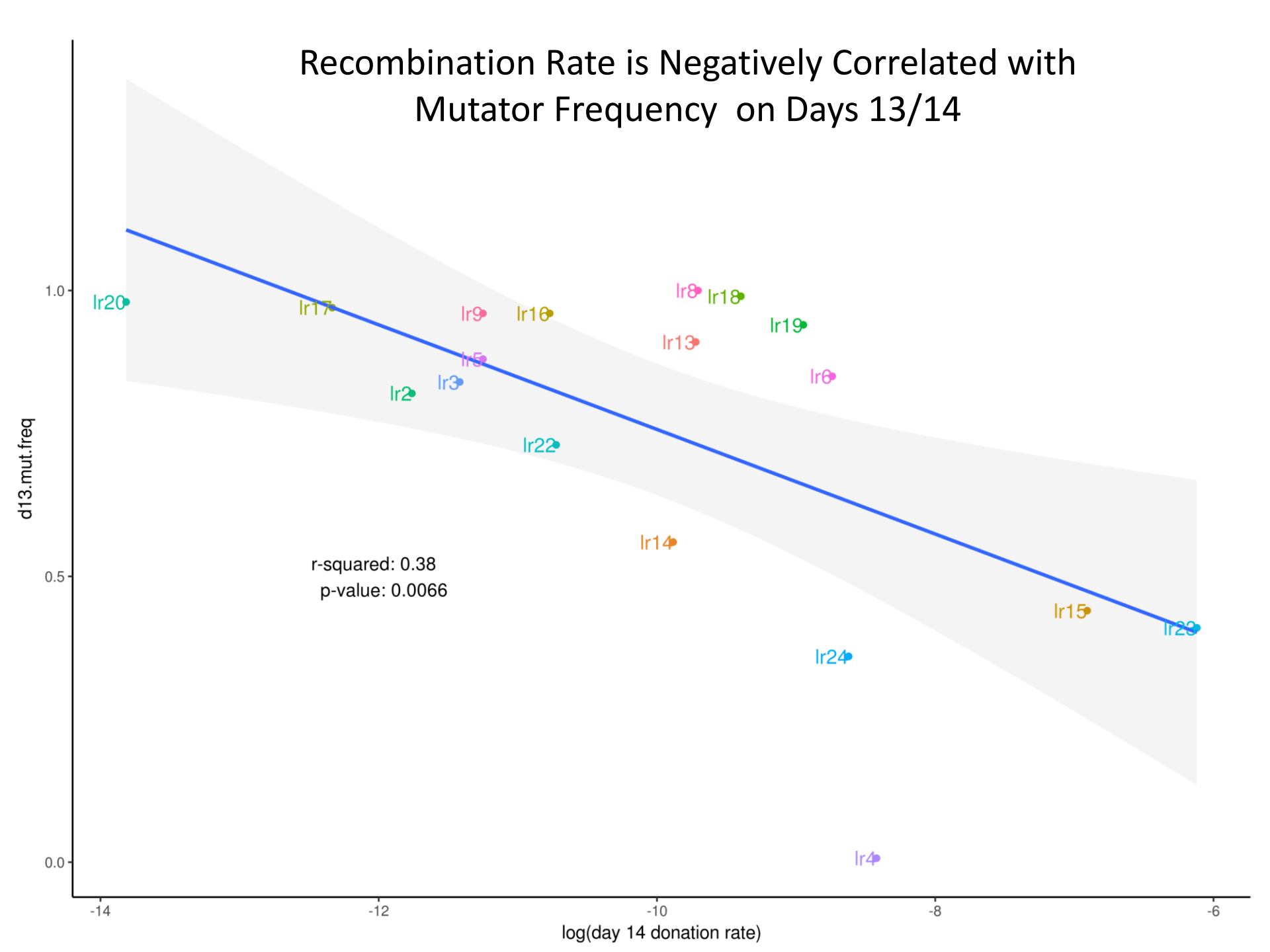
Black markers – Surviving Populations
Red markers- Extinct Populations

Conclusions from mutation accumulation in mutators

- As expected from theory and from previous experiments of this kind, fitness declined and some populations went extinct.
- Evolution of higher mutation rates has been observed previously in mutation-accumulation experiments, but not evolution of lower mutation rates.
- The observed relationship between population persistence and the direction of mutation rate evolution is also novel and may be explained by
  - Mutation pressure in the case of extinct populations that had evolved higher rates
  - Selection in the case of populations that persisted and had evolved lower rates.

Joint Evolution of Mutation, Recombination Rates in Experimental Populations




Recombination Rate is Negatively Correlated with Mutator Frequency on Days 13/14
d13.mut.freq
1.0
0.5
0.0
-14
-12
-10
-8
-6
log(day 14 donation rate)
r-squared: 0.38
p-value: 0.0066
lr20
lr17
lr9
lr16
lr8
lr18
lr19
lr13
lr5
lr6
lr3
lr2
lr22
lr14
lr15
lr23
lr24
lr4

Ongoing Work

Fate of beneficial mutations at extremely high mutation rates.

Predicting the evolution of fitness and the evolution of the distribution of fitness effects (DFE) of mutations in real time using experimental evolution data.

How *much* recombination is sufficient to stop elevation of the mutation rate? (After all, mutation rates are generally low…)

Summary

- **Experimental microbial evolution is a powerful approach for investigating both evolutionary process in general and the specific evolutionary changes that occur in defined environments, though it does have some inherent limitations that should be acknowledged.**
- **Experimental studies of mutation rate evolution have inspired new theory, which has in turn stimulated new experimental work on the consequences of asexuality for genomic mutation rates and the persistence of populations.**
- **(Phenomenology)[10] may allow characterization of DFE and the prediction of fitness evolution in experimental populations.**

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- Jude Dartey (high school intern)
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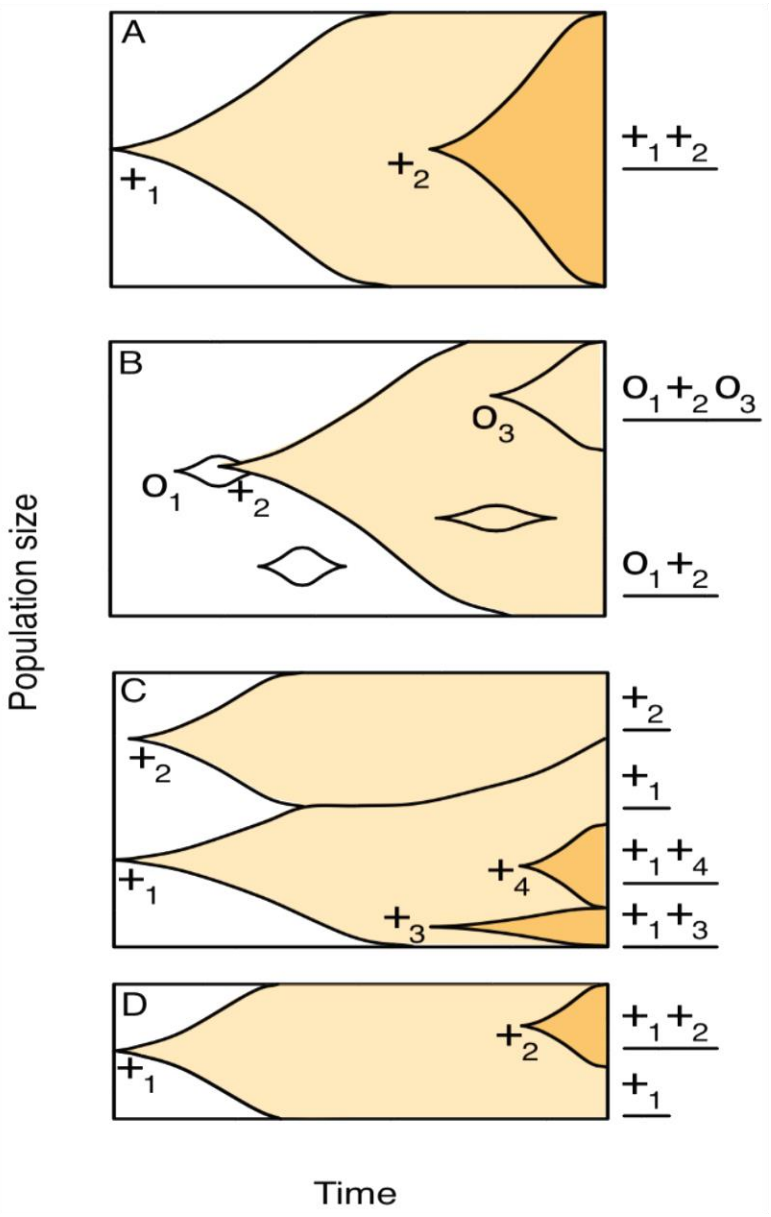
- Vaughn Cooper, U Pitt (genomic sequencing)
- *The Alfred P. Sloan Foundation, University of Pennsylvania Research Foundation, NSF, NIH, NASA (funding)*

And to you, for listening!



Visualizations of evolution in asexual (clonal) populations.
+ = beneficial mutation
O = neutral mutation

The central fact of evolution in clonal populations is that genetic variants (mutations) that have arisen on different genetic backgrounds cannot be combined into a single background as in a recombining population. A clonal population can only give rise to such combinations via mutation on a common genetic background.

A. Beneficial mutations rare.
B. Neutral mutations can “hitchhike” with beneficials.
C. When common, beneficial mutations will compete: “clonal interference”.
D. In a small population, the waiting time between beneficial mutations will longer for the same mutation rate.

Sprouffske et al., Current Biology 2012

Beneficial Mutations

- Central role in evolution, but tiny fraction of all mutations- hard to identify and investigate
- Low probability (2*s*, *s* = selective advantage) of substitution of beneficial allele due to drift and clonal interference:
  - one billion mutation events predicted per population
  - 50 neutral substitutions predicted per population
  - 10 - 20 beneficial substitutions predicted per population
- Reconstruction of entire adaptive evolutionary history of these populations possible?

More Recent Experimental Work

- *Spontaneous* evolution of higher-order mutator genotypes.
- Fitness evolution and mutation rate instability in very small populations with extremely high mutation rates.
- Joint evolution of mutation and recombination rates.

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