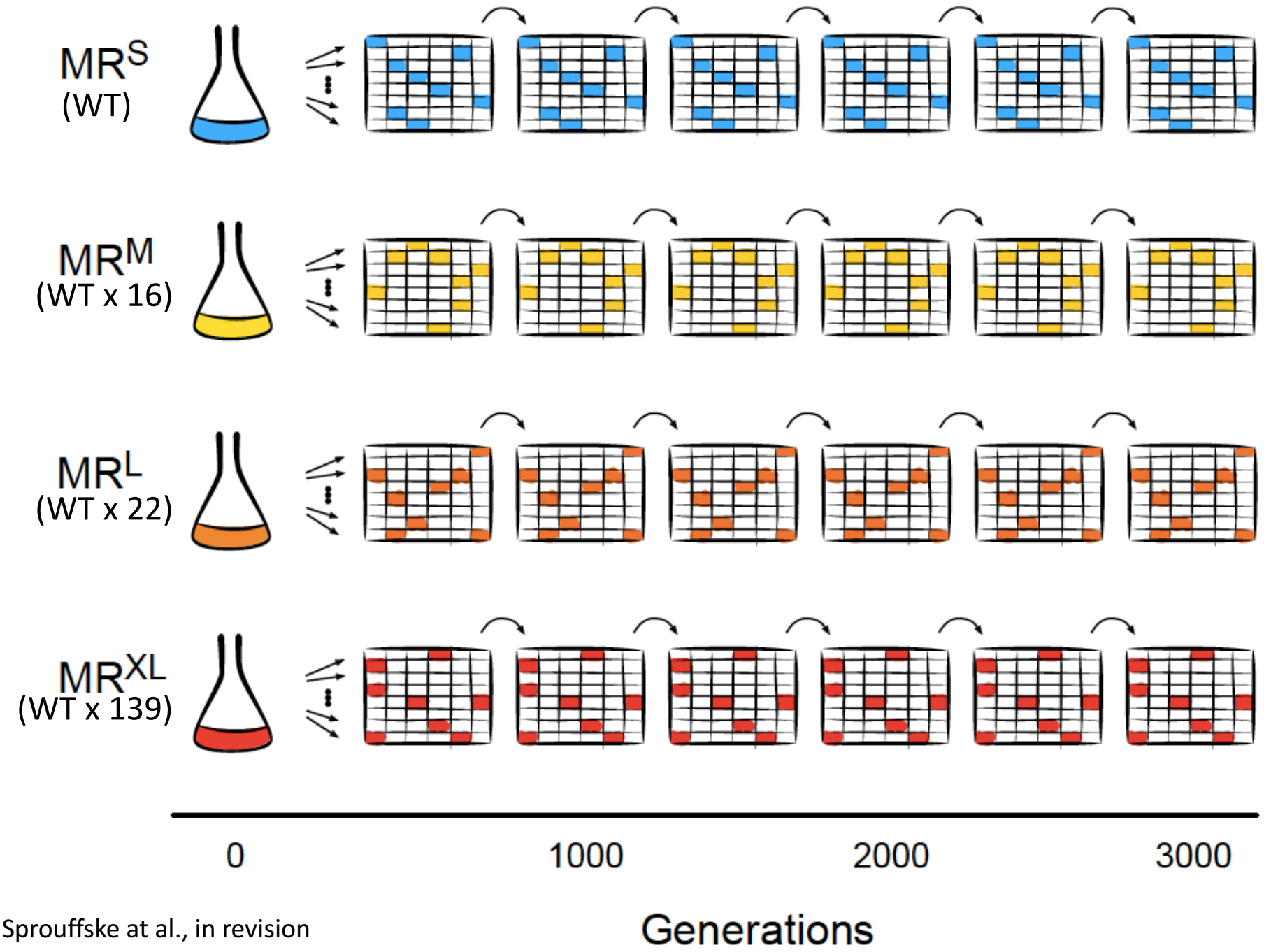
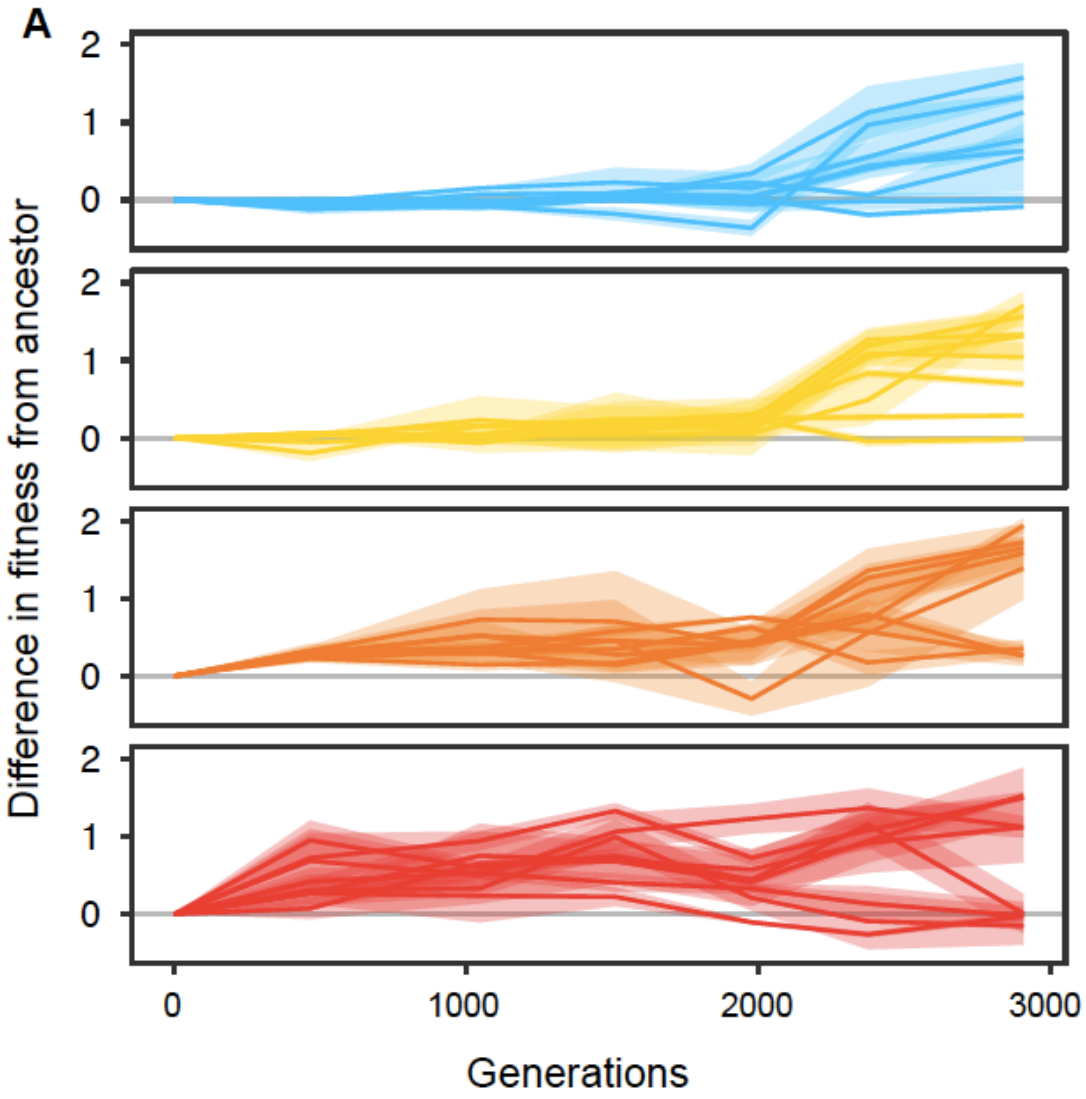
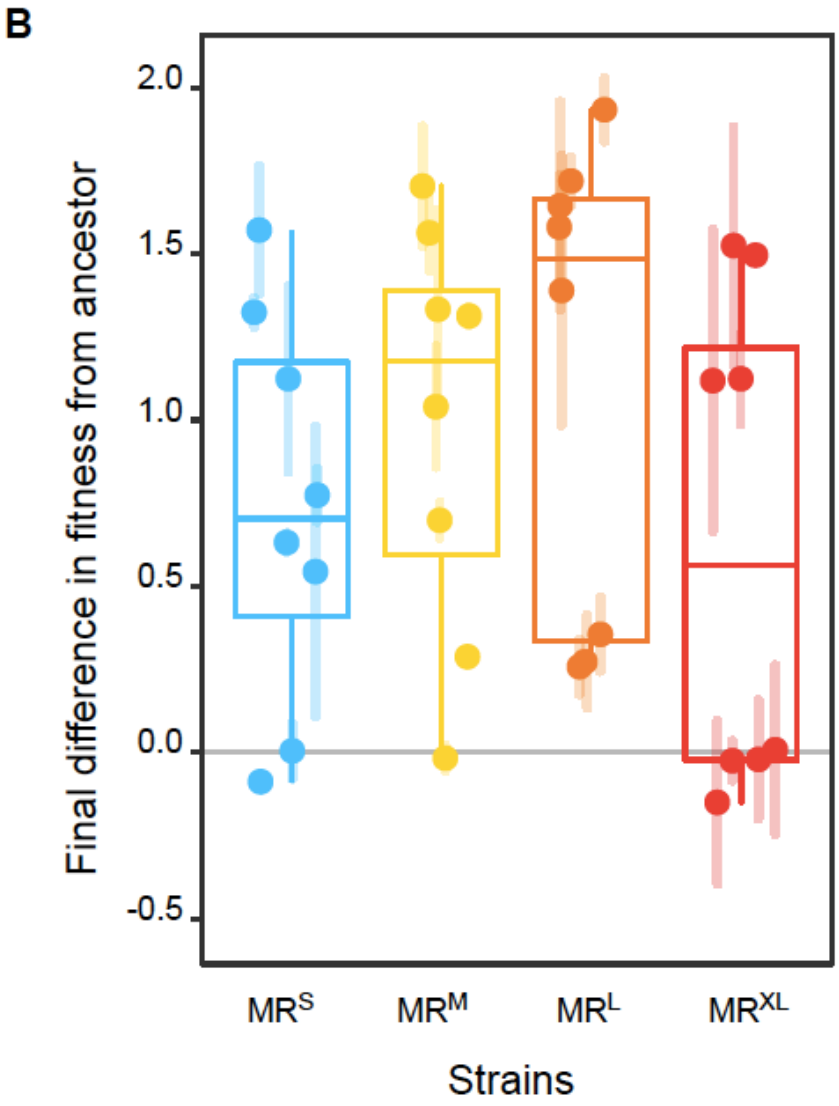
# Testing Effect of Mutation Rate on Adaptation in Large(ish) Populations




Sprouffske at al., in revision

# Fitness Evolution at Four Different Mutation Rates

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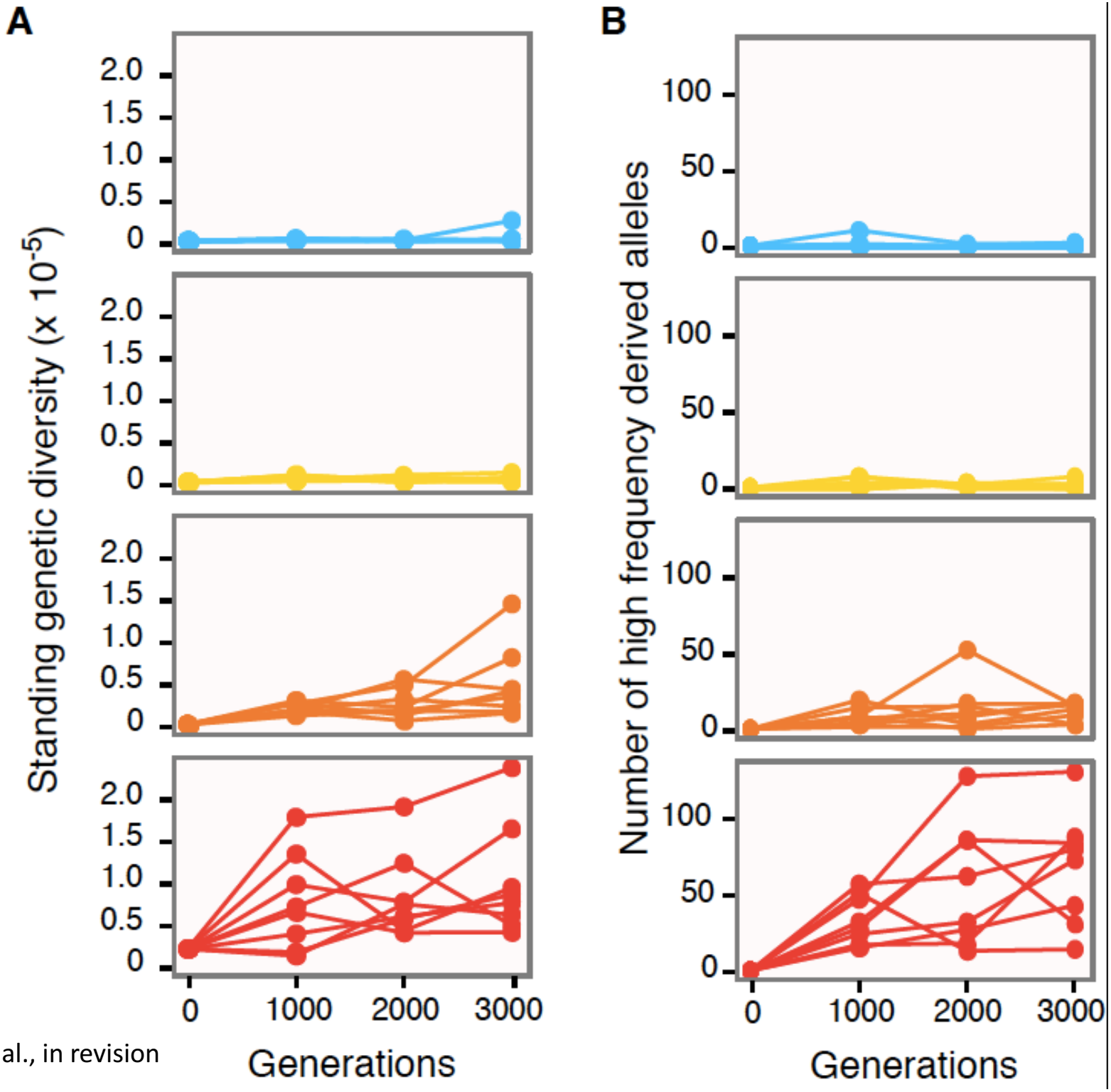


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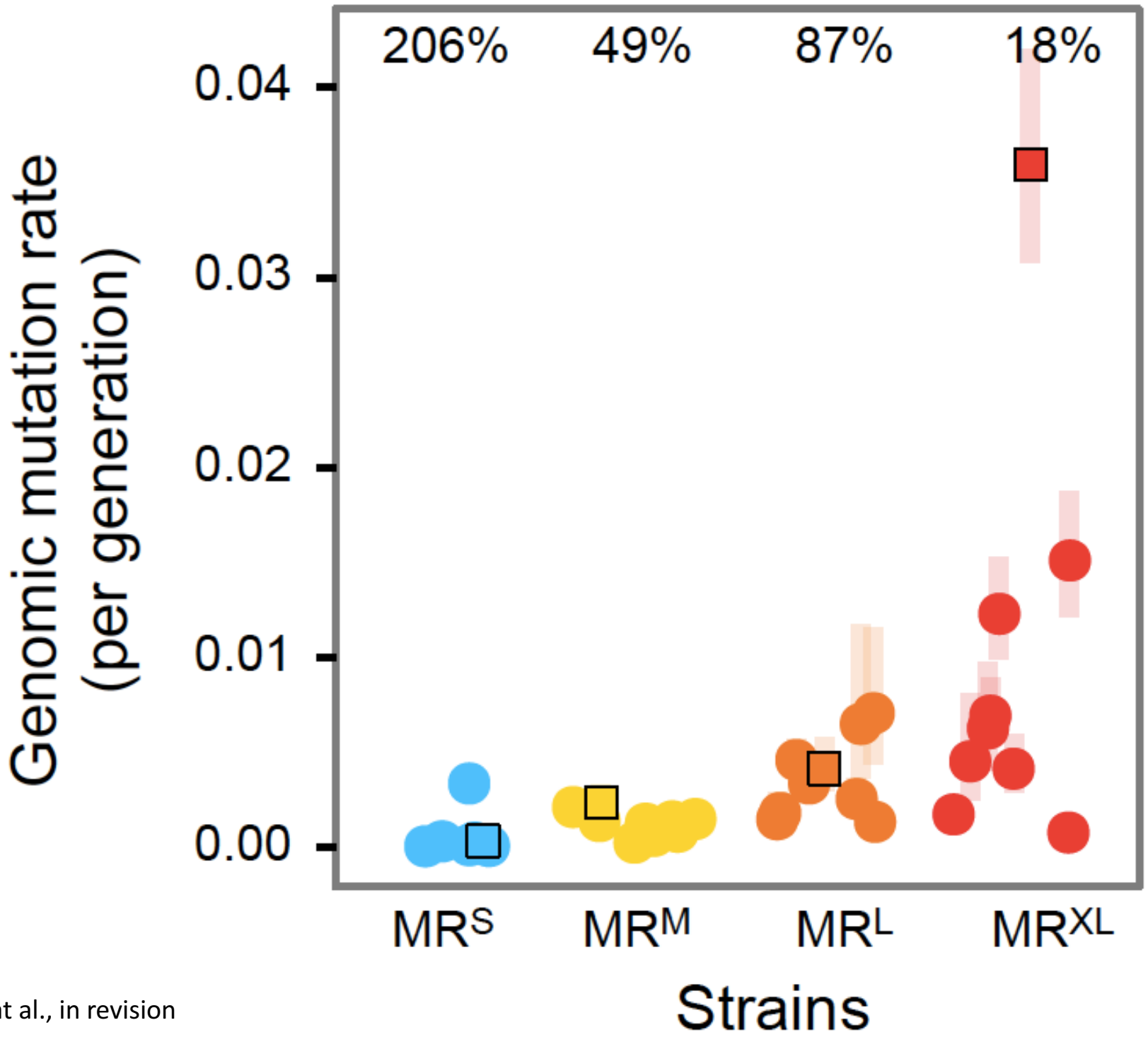


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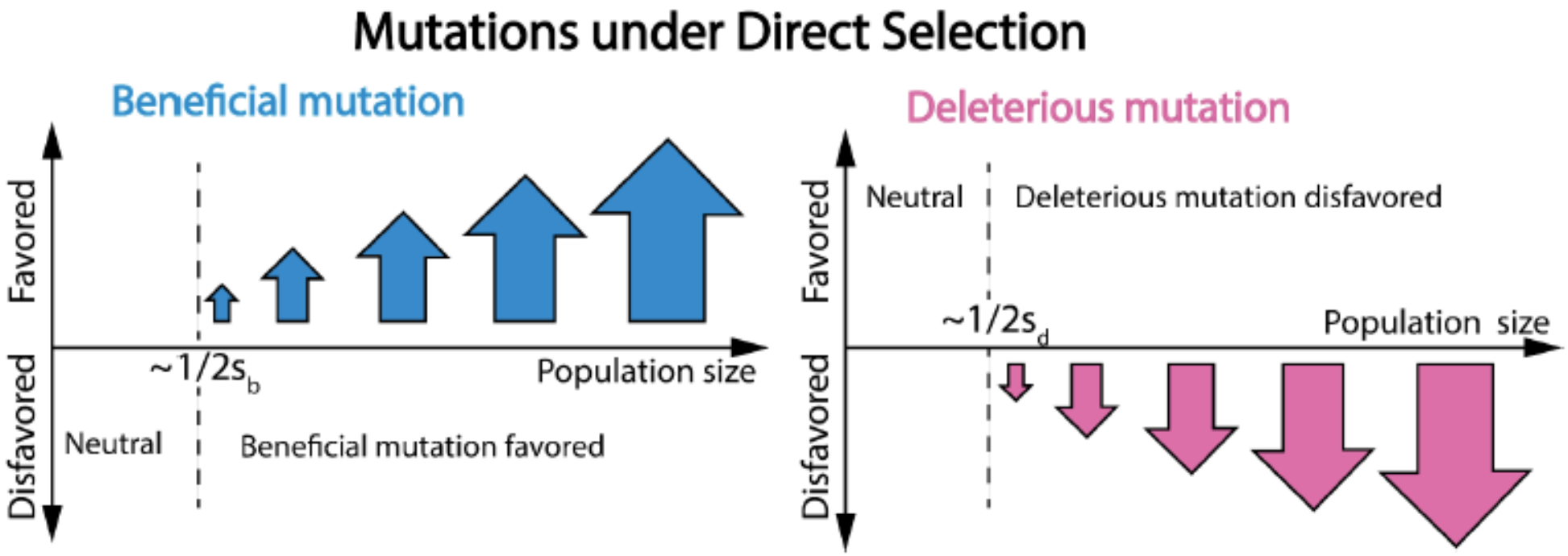
# Testing Effect of Population Size on Mutator Hitchhiking

## *Direct* Selection and *N*

- Population size (*N*) is known to modulate the effectiveness—but, not the sign—of direct selection, i.e. selection on allele's fitness effect
- As population size decreases the influence of random genetic drift becomes stronger overwhelming the influence of selection

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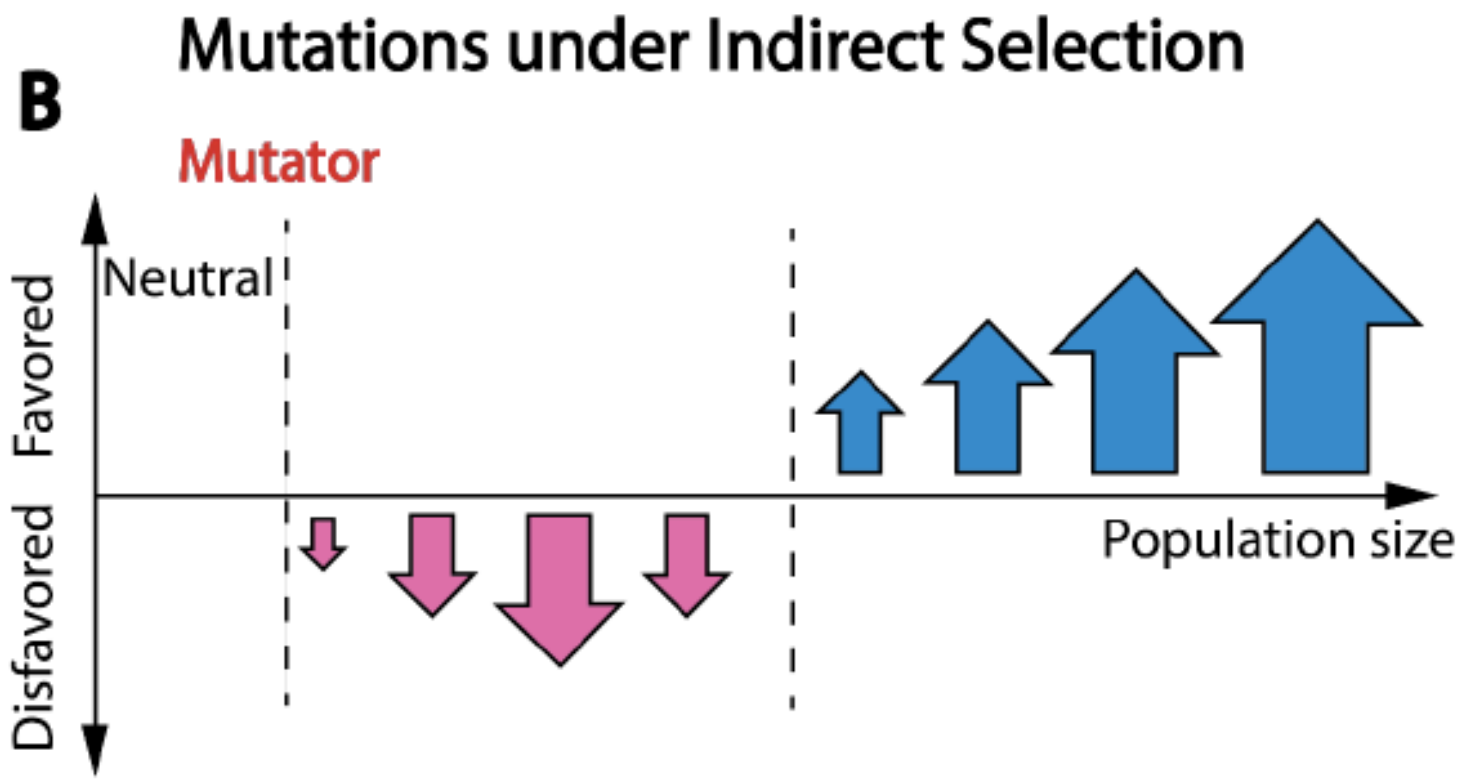
# Sign inversion in *indirect* selection

- Population size (*N*) determines the actual sign, and not just the effectiveness, of indirect selection

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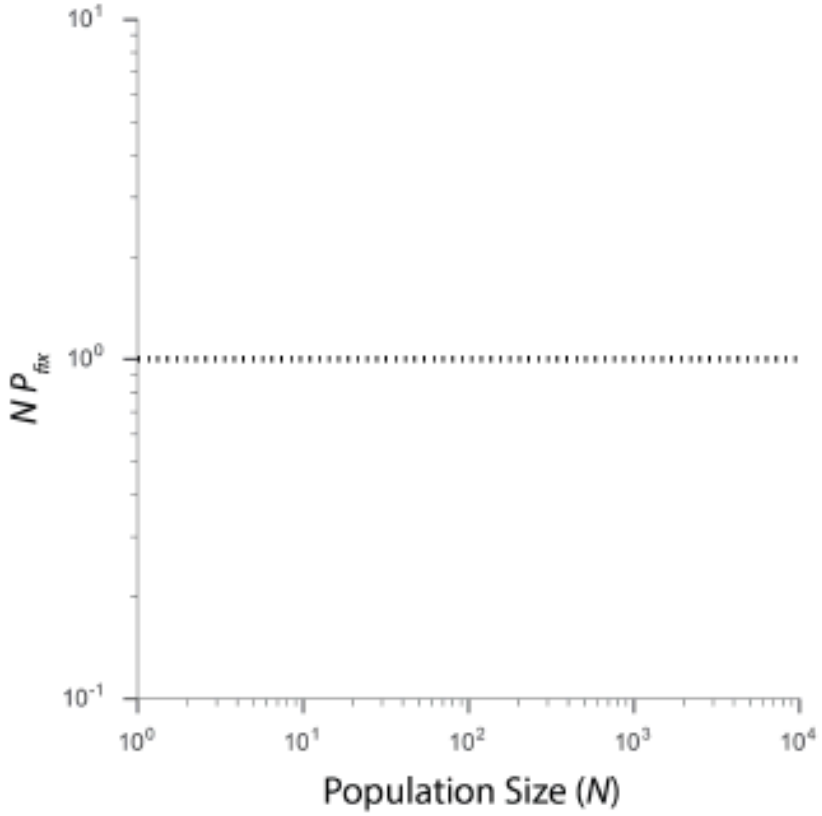
# Introduction: $NP_{fix}$

- $NP_{fix}$ is the fixation probability normalized by the neutral expectation:
  - $P_{fix}/\frac{1}{N} = NP_{fix}$
- For a neutral allele $NP_{fix} = 1$

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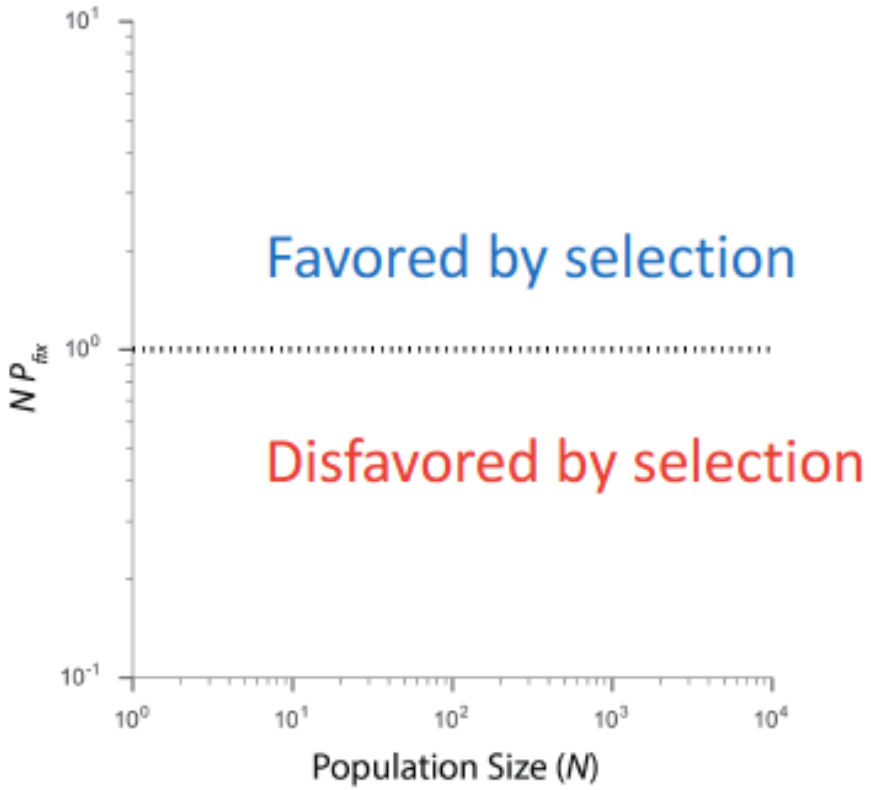
# Introduction: $NP_{fix}$

- $NP_{fix} = 1$ is the neutral expectation/threshold used to ascertain whether an allele is favored or disfavored by selection

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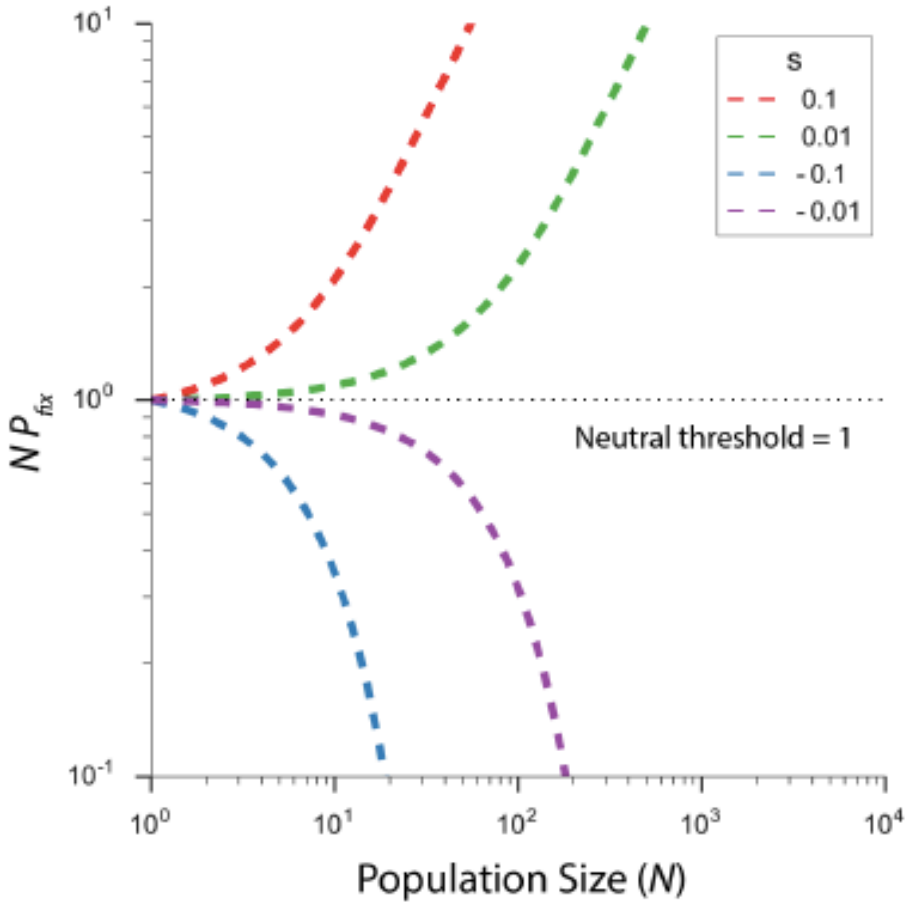
# Introduction: *Direct* Selection and *N*




$$P_{fix} = \frac{1 - e^{-2s}}{1 - e^{-2Ns}}.$$

Kimura (1962)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# Sign inversion in *indirect* selection

- Analytic model
- Stochastic computer simulations
- Empirical tests of the model in experimental yeast populations


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# Model

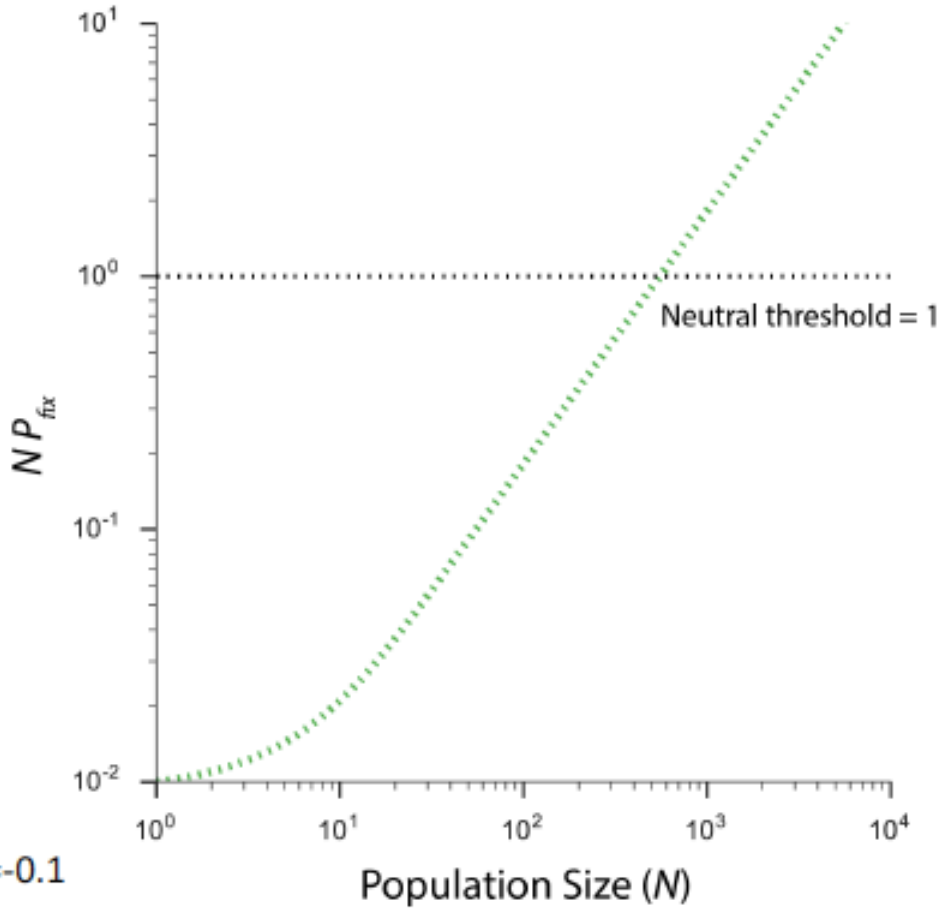
- Option A: Mutators can fix by hitchhiking.
- Must produce a beneficial mutation

$$\frac{U_{ben}}{U_{del}+U_{ben}} \approx \frac{U_{ben}}{U_{del}}$$

- The beneficial mutation has to survive drift and sweep to fixation

$$P_{fix}(s_{ben})$$

- $P_{fix}^{hitchhiking} \approx \frac{U_{ben}}{U_{del}} \cdot P_{fix}(s_{ben})$ (dotted line)




$U_{del}=10^{-4}$, $U_{ben}=10^{-6}$, $s_{ben}=0.1$, $s_{del}=-0.1$

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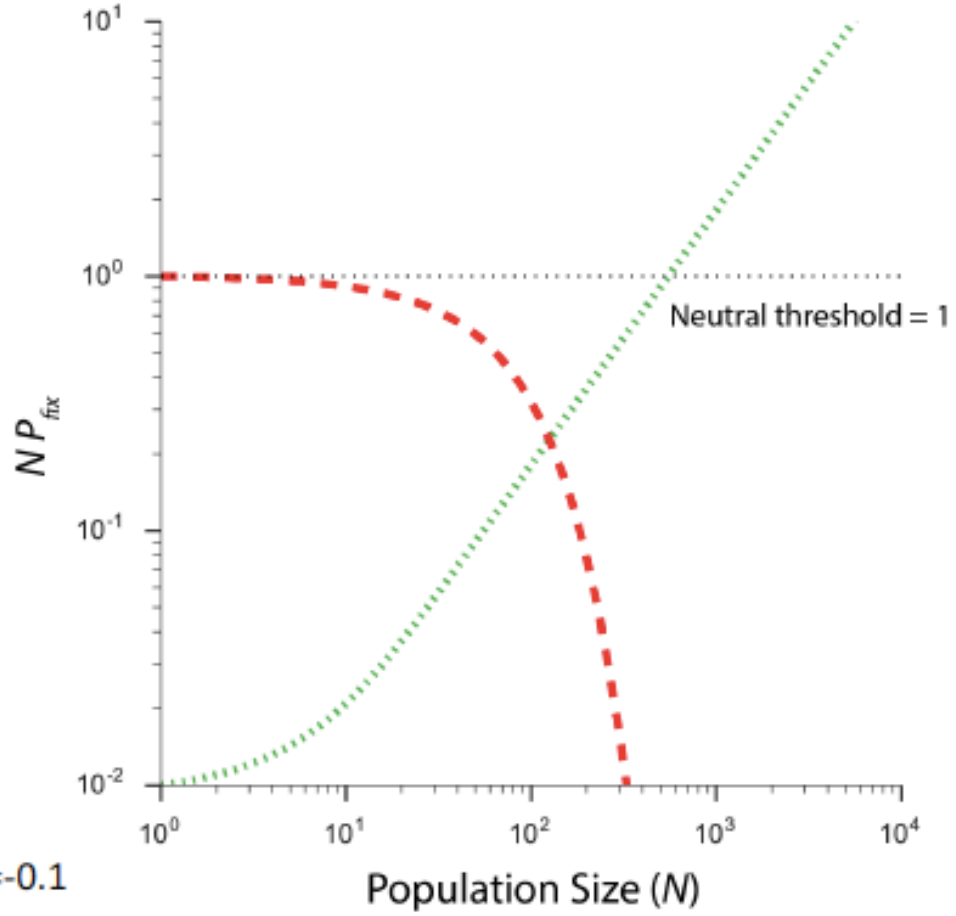
# Model

- Option B: Mutators can fix by random genetic drift.
- Drift must overpower indirect selection against the excess deleterious mutations (load)
  - ≈ the difference in $U_{del}$ between mutator and non-mutator

- $P_{fix}^{drift} \approx P_{fix}(-\Delta U_{del})$

  (*dashed line*)




$U_{del}=10^{-4}$, $U_{ben}=10^{-6}$, $s_{ben}=0.1$, $s_{del}=-0.1$

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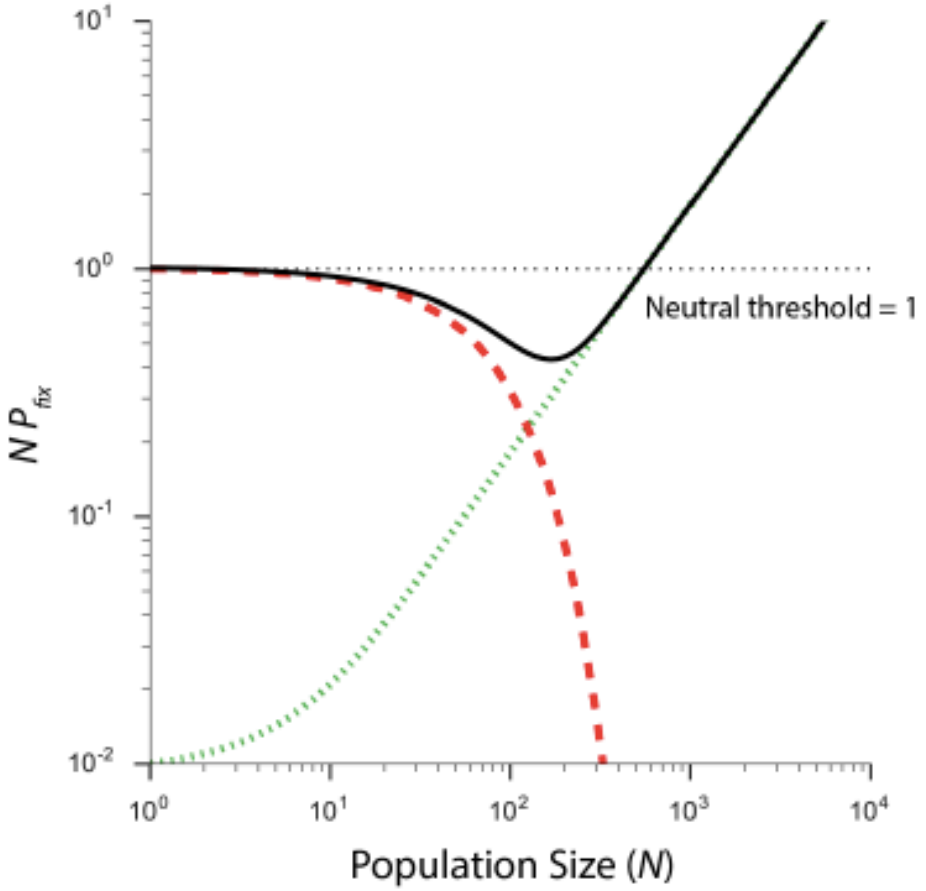
# Model

$$P_{fix}^{mutator} = P_{fix}^{drift} + P_{fix}^{hitchhiking}$$

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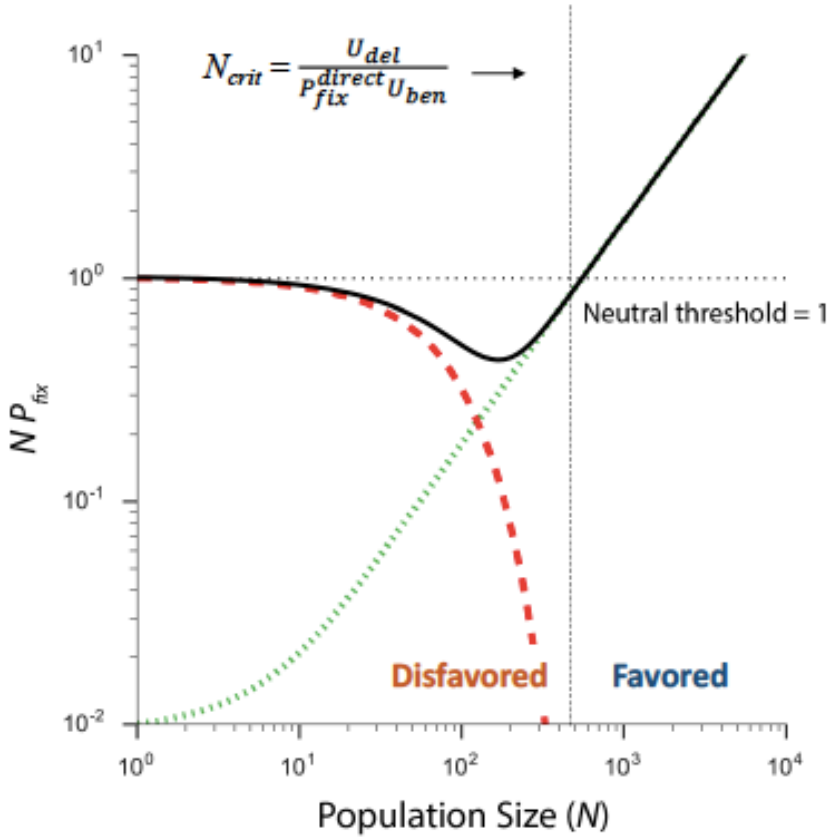
# Selection on Mutators changes sign as *N* declines: "Sign Inversion"

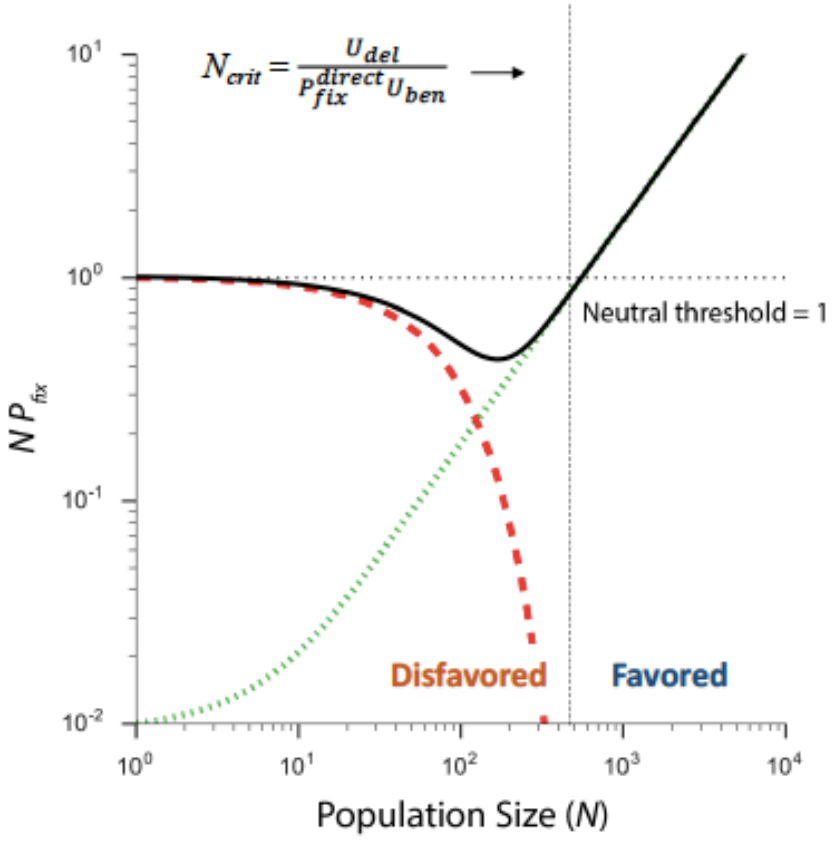
- $P_{fix}^{drift}$ is generally below the neutral threshold due to selection against deleterious mutations




Raynes et al. in revision

# Selection on Mutators changes sign as *N* declines: "Sign Inversion"

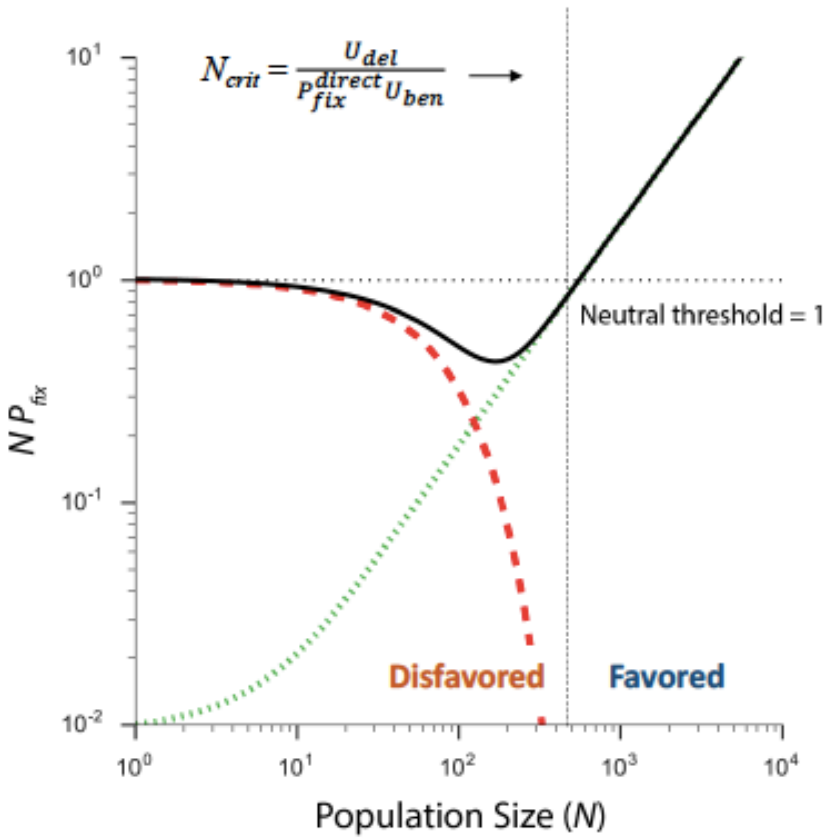
- $P_{fix}^{drift}$ is generally below the neutral threshold due to selection against deleterious mutations
- $P_{fix}^{hitchhiking}$ is depressed by the requirement to first generate a rare beneficial mutation. Crosses neutral threshold at $N_{crit.}$




Raynes et al. in revision

# Selection on Mutators changes sign as *N* declines: "Sign Inversion"

- $P_{fix}^{drift}$ is generally below the neutral threshold due to selection against deleterious mutations
- $P_{fix}^{hitchhiking}$ is depressed by the requirement to first generate a rare beneficial mutation. Crosses neutral threshold at $N_{crit.}$
- Once $P_{fix}^{hitchhiking}$ drops below the neutral threshold, selection against deleterious mutations keeps $\boldsymbol{P_{fix}^{mutator}}$ below the neutral threshold until the cost of the load is overpowered by drift

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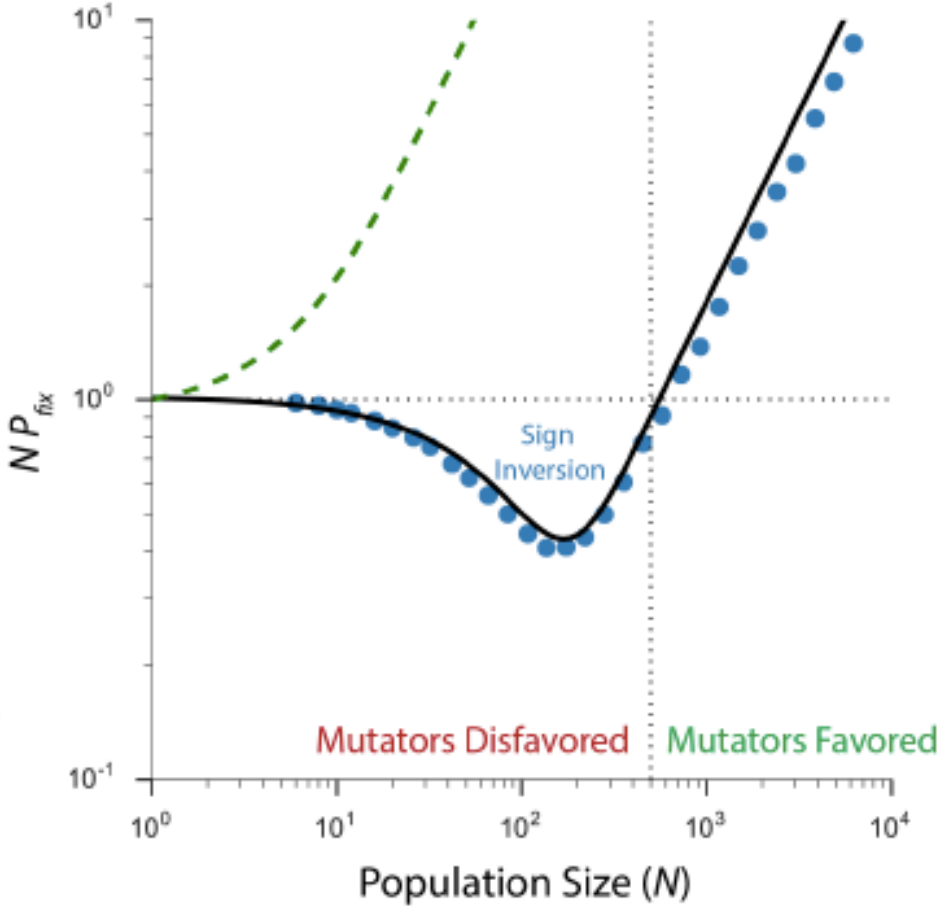
# Sign inversion is borne out in stochastic simulations




**Blue dots**: stochastic Wright-Fisher simulations
$10^6$ replicates, $U_{del}=10^{-4}$, $U_{ben}=10^{-6}$, $s_{ben}=0.1$, $s_{del}=-0.1$.
Mutators mutate 100× faster than non-mutators.
**Green dashed line**: $P_{fix}(s_{ben}=0.1)$, direct selection

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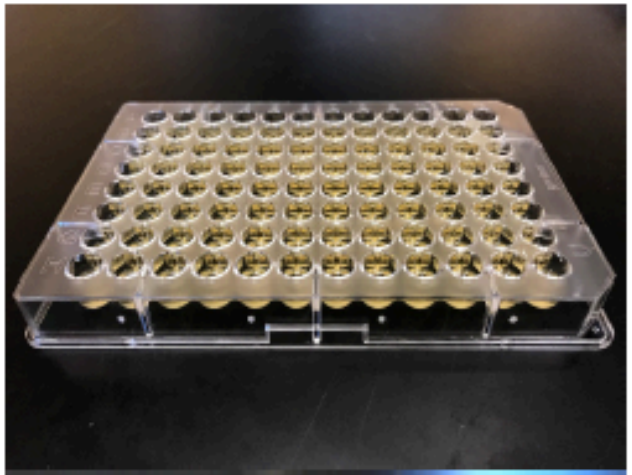
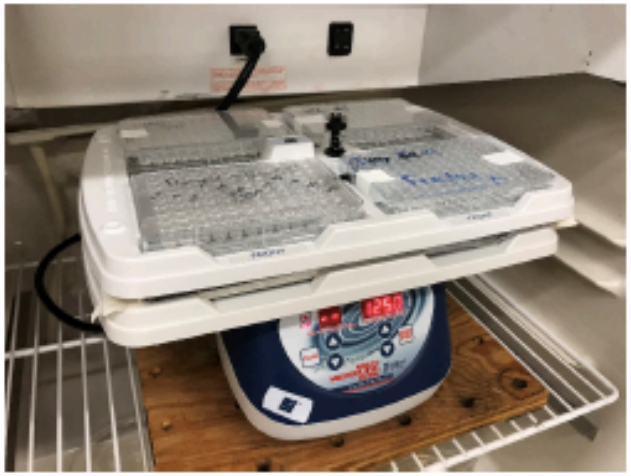
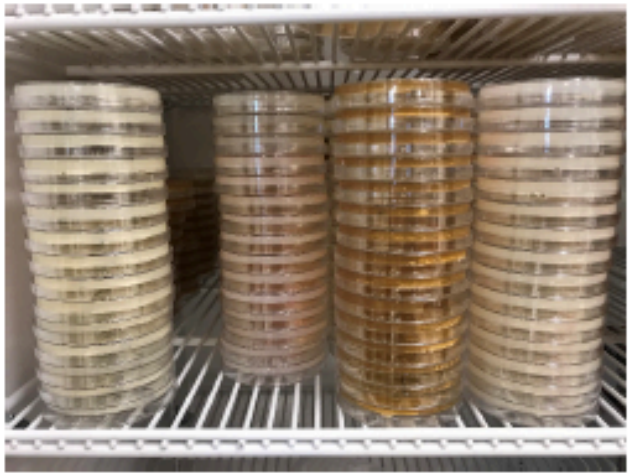
# Empirical test of sign inversion: model system

- *S. cerevisiae* yeast as a model system:
  - Unicellular eukaryote
  - Short generation times
  - Asexually reproduce by budding
  - Population size can be easily controlled







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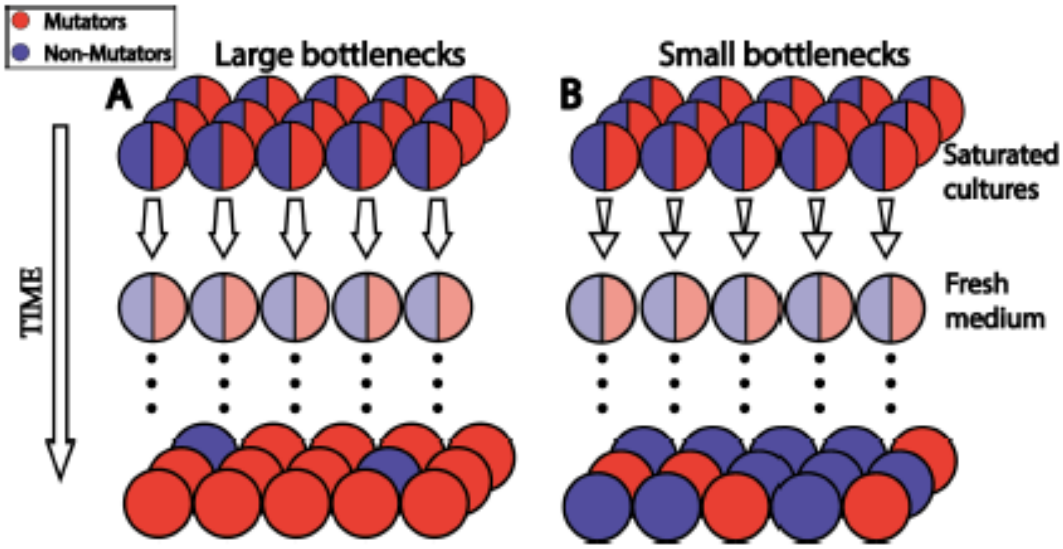
# Empirical test of sign inversion: experimental design

- Conducted competitions between mutator (≈100-fold) and non-mutator strains of yeast.
- Initiated competitions at approximately equal frequencies (neutral expectation ~0.5).
- Propagated by regular transfers into fresh medium (*N* controlled by the size of the transfer bottleneck)
- Followed mutator frequency with fluorescent markers

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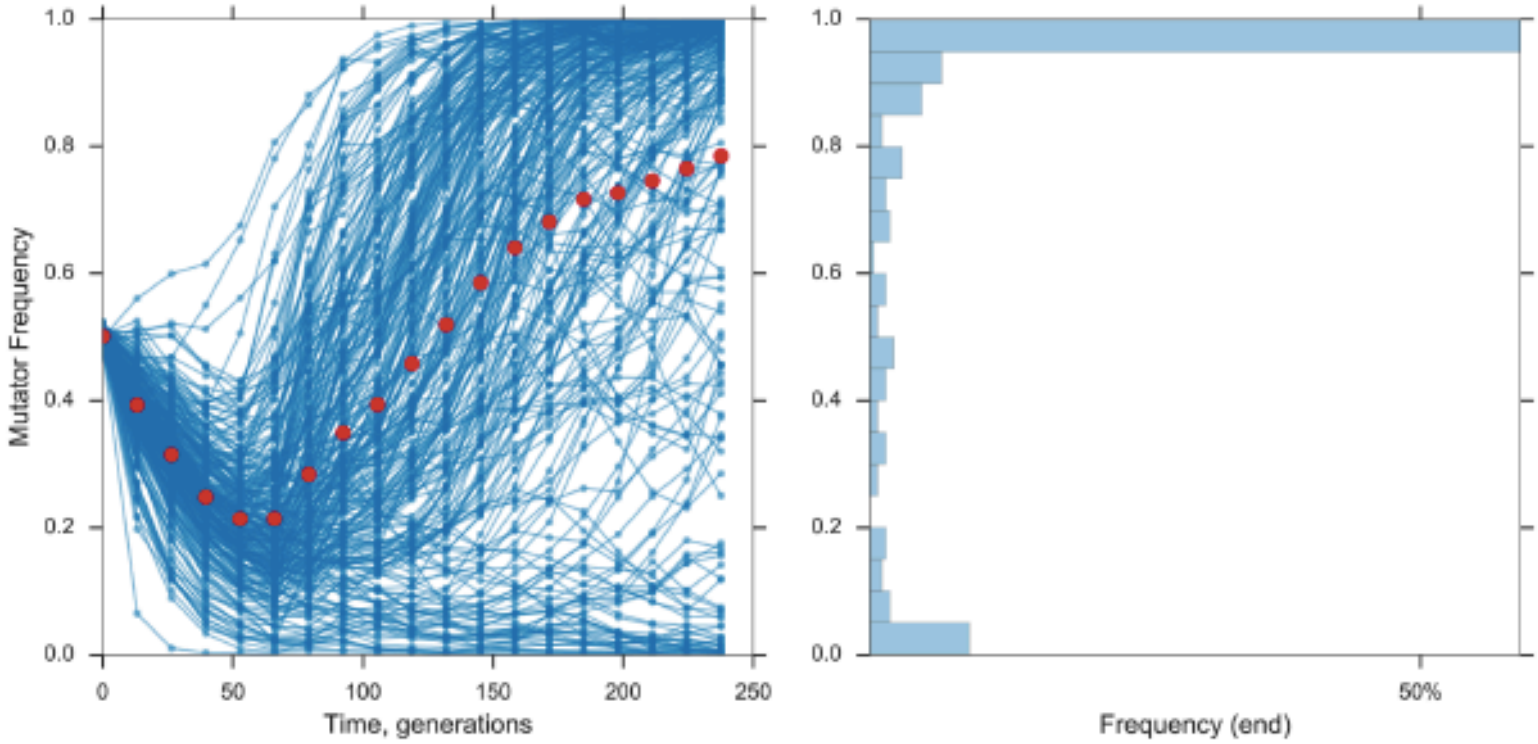
# Large populations: Mutators favored




Mutators won in 162 of the 276 competitions in the first ~250 generations (~58.7%) and lost in only 25.
The mean mutator frequency in the 89 unresolved competitions (~0.63) was significantly higher than the starting frequency

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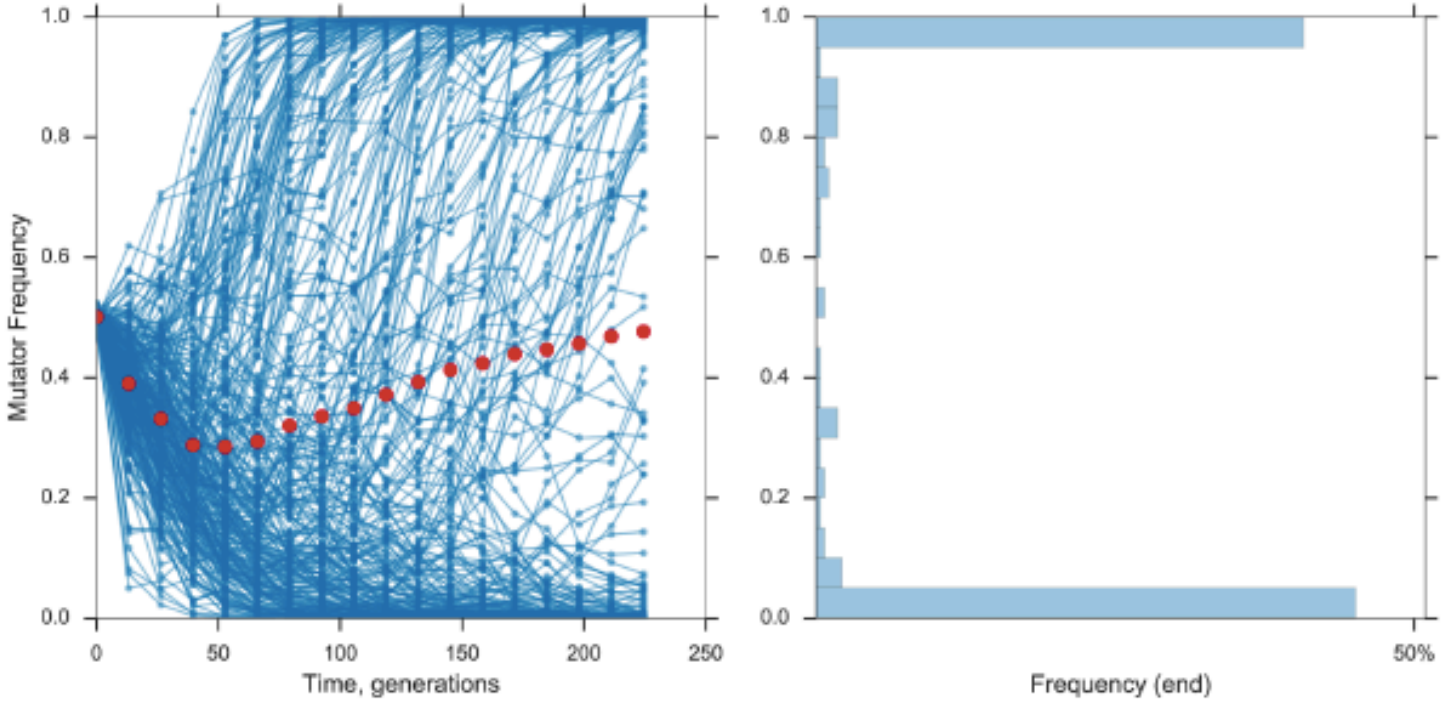
# Intermediate populations: Mutators disfavored?




Mutators won in 112 of the 275 competitions in the first ~225 generations (~40.7%) and lost in 124 (~45.1%). The mean mutator frequency in the 39 unresolved competitions (~0.49) was not significantly different than the starting frequency

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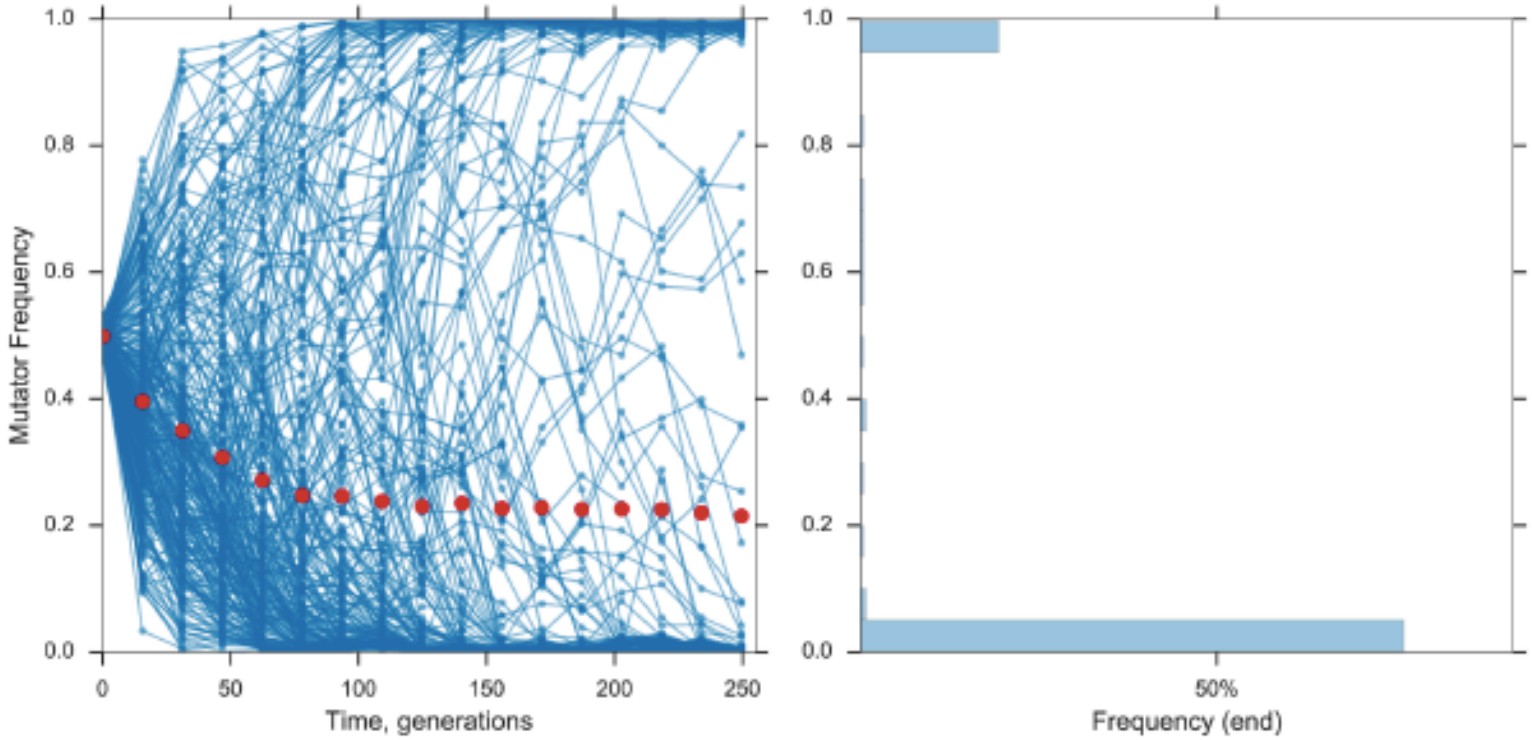
# Small populations: Mutators disfavored!




Mutators won in 53 of the 273 competitions in the first ~250 generations (~19.4%) and lost in 208 (~76.2%). The mean mutator frequency in the 12 unresolved competitions (~0.43) was not significantly different than the starting frequency

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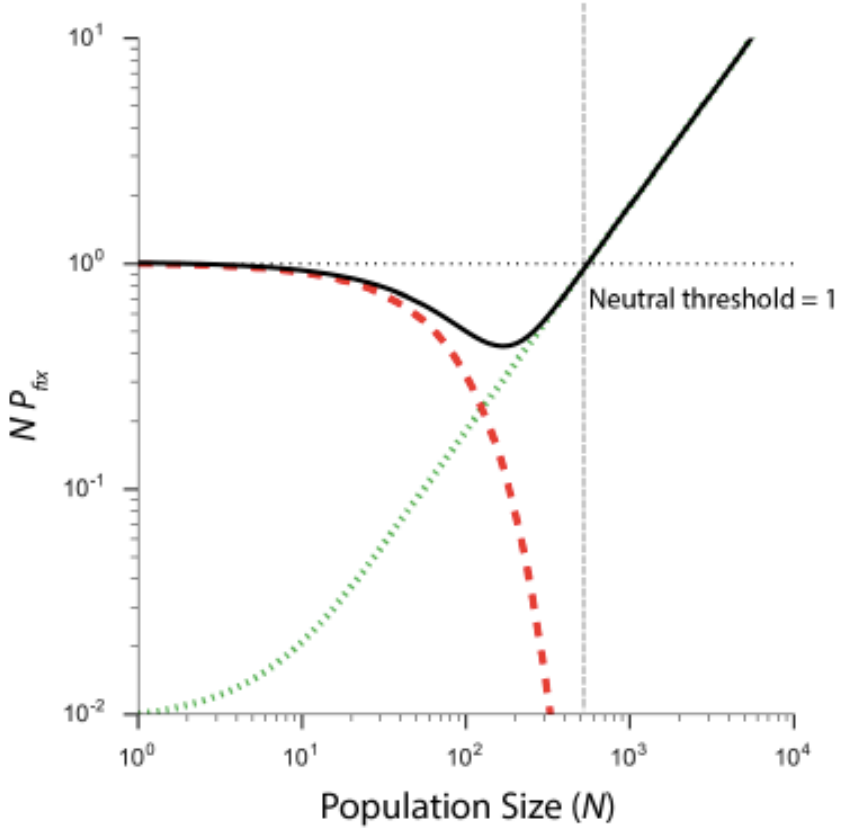
# Model: Mutator fixation probability

$$P_{fix}^{mutator} = P_{fix}^{drift} + P_{fix}^{hitchhiking}$$

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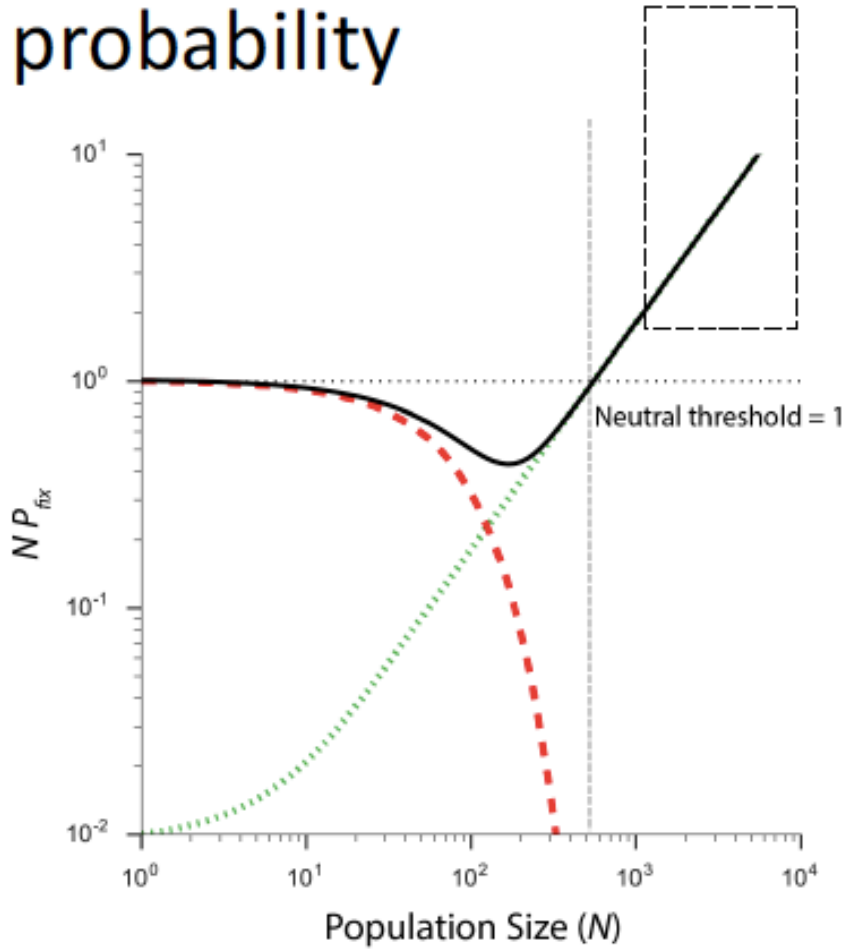


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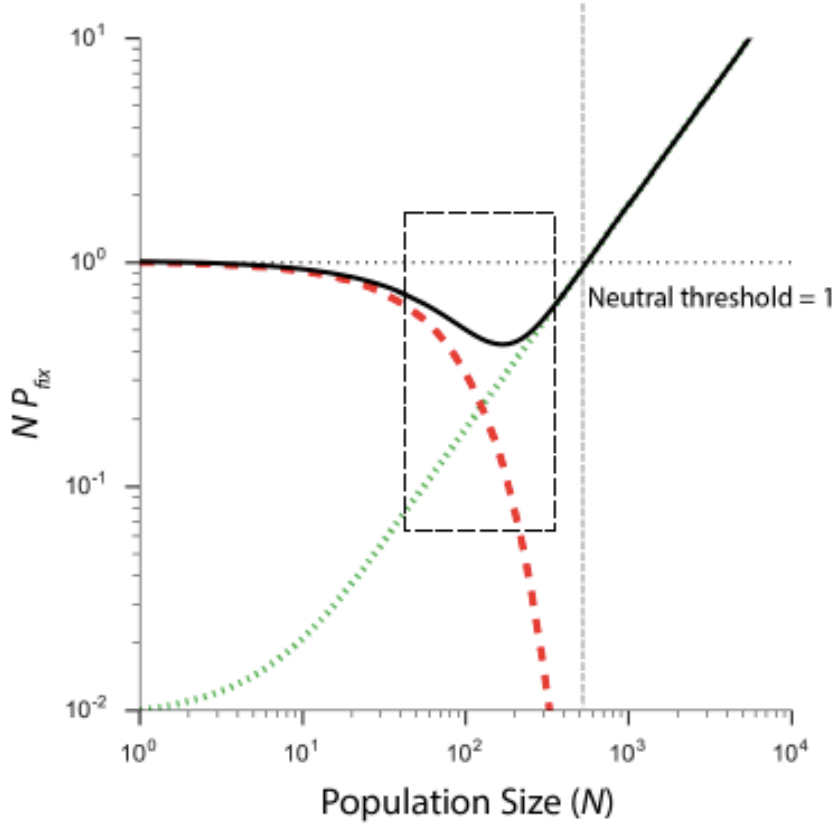


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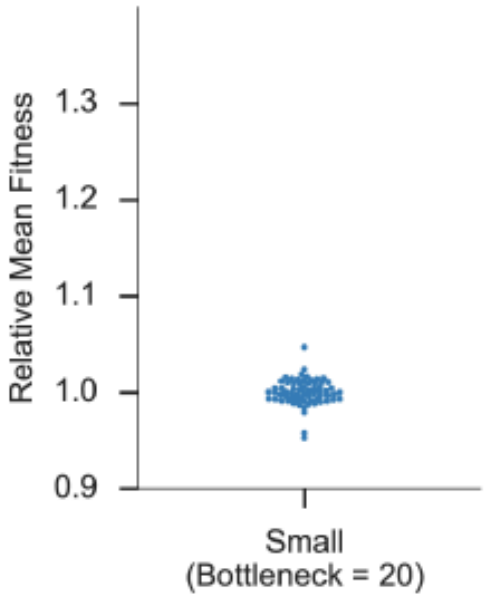
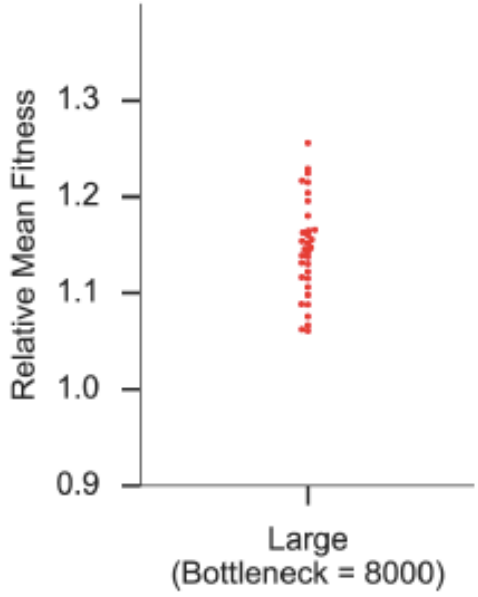
# Fitness measurements of competition winners confirm model predictions

**Small *N***: non-mutators win by outlasting the mutators lost to deleterious load

**Large *N***: mutators win by hitchhiking with beneficial mutations

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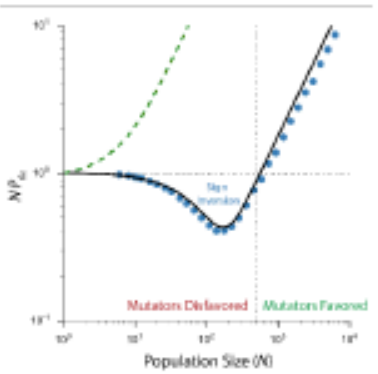
# Summary

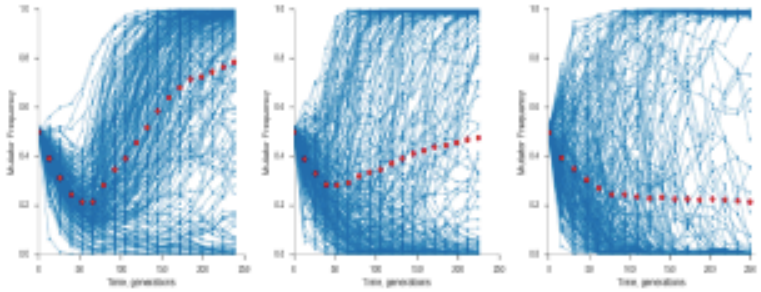
- Simple analytic model for the fixation probability of a mutator includes two well-understood processes – hitchhiking and drift

$$P_{fix}^{mutator} = P_{fix}^{drift} + P_{fix}^{hitchhiking}$$

- Most surprising prediction: the *N*-dependent inversion in the sign of selection
- Model is borne out in computer simulations




- Sign inversion and its mechanism are confirmed in experiments with yeast



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# Summary


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# Implications for indirect selection

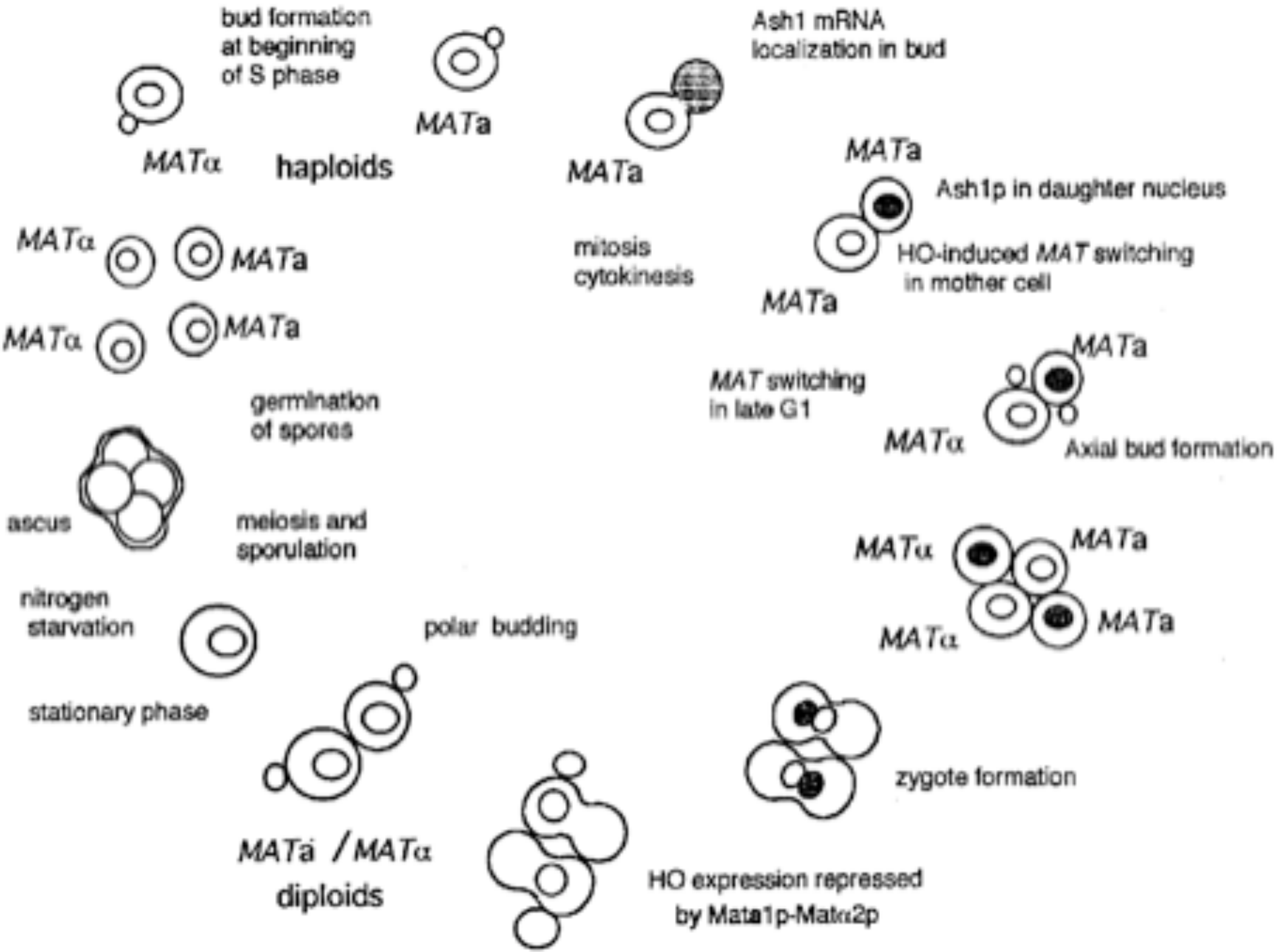
- The mechanistic basis of sign inversion may not be unique to mutators
- It is likely that any mechanism that introduces random genetic or phenotypic variation is more likely to negatively affect fitness than to improve it
- Could other indirectly selected modifiers of variation, such as modifiers of recombination, dominance, ploidy be similarly susceptible to sign inversion?
- Sign inversion may reveal an important new role of population size in evolution by indirect selection

# What about recombination?

- Recombination is expected to inhibit mutator hitchhiking because recombination erodes linkage disequilibrium (= nonrandom association) between mutators and beneficial mutations.

- There has been relatively little theoretical work on the effect of recombination on genomic mutation rate evolution.

- There has been very little experimental work in this area. Will briefly discuss two projects: one published, one in limbo but promising.




Source: Herskowitz, I. 1988. *Microb. Rev.* 52, 536-553

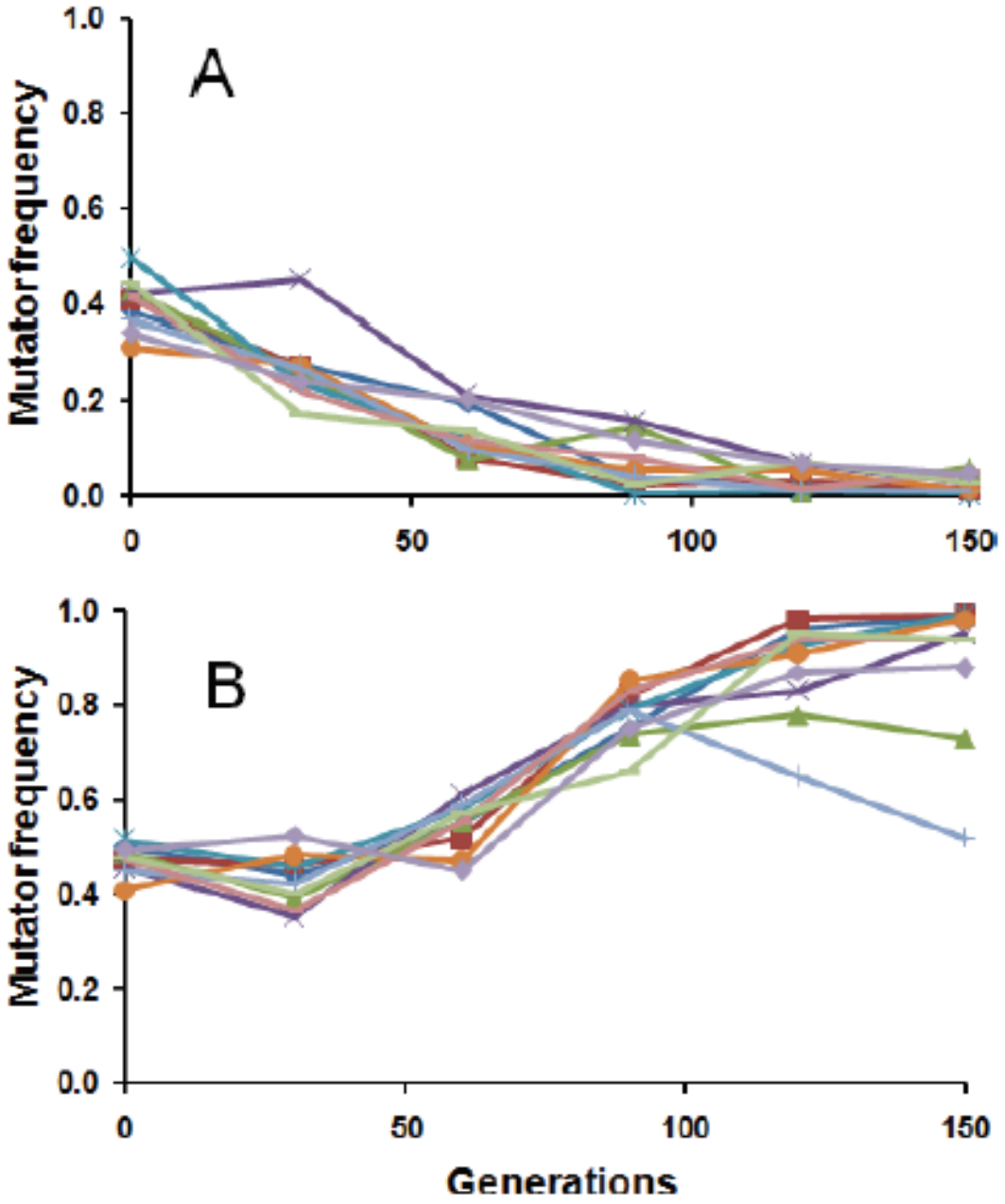



**Figure 1 Mutator dynamics in asexual populations**. Mutator strains decline toward extinction in all ten haploid populations **(A)**. Mutator strains hitchhike to higher frequencies in all ten of the diploid populations, approaching fixation in seven of them **(B)**.

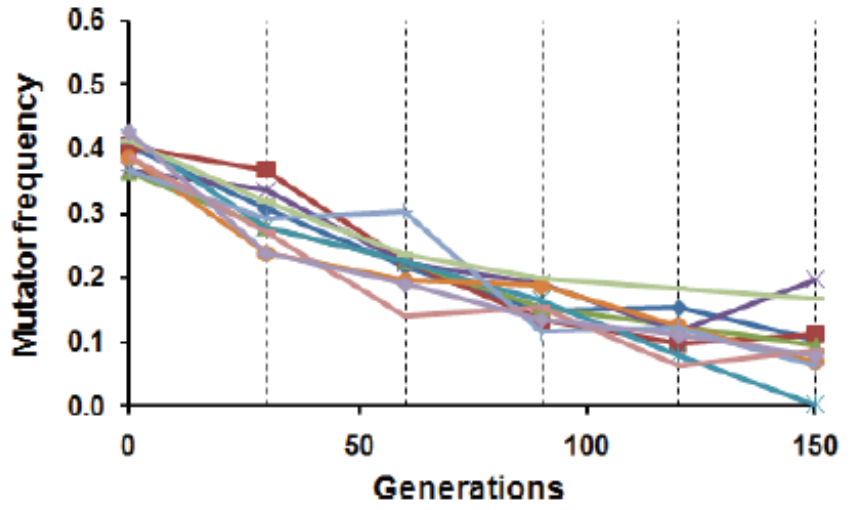


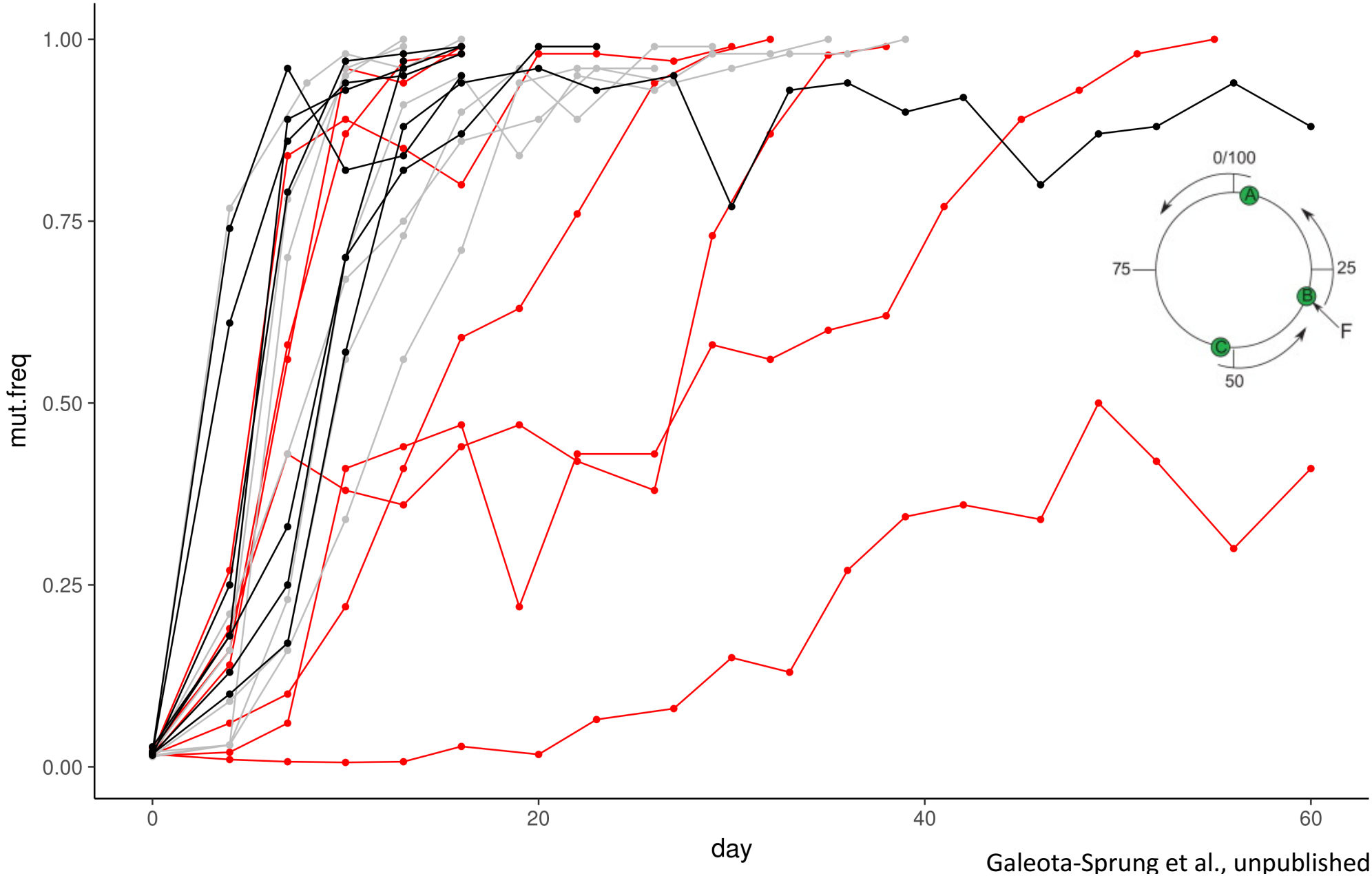

**Figure 2 Mutator dynamics in sexual populations**. The mutator allele declines in frequency in all sexual diploid populations. Dashed vertical lines indicate the times when sporulation was induced and yeast cells were allowed to mate randomly.

Does recombination suppress mutators? It should, but this has been difficult to demonstrate conclusively. In this yeast experiment mutators were disfavored in sexual populations, but this could have been because of selection against recessive deleterious mutations associated with mutators during the haploid phase of propagation in the protocol.

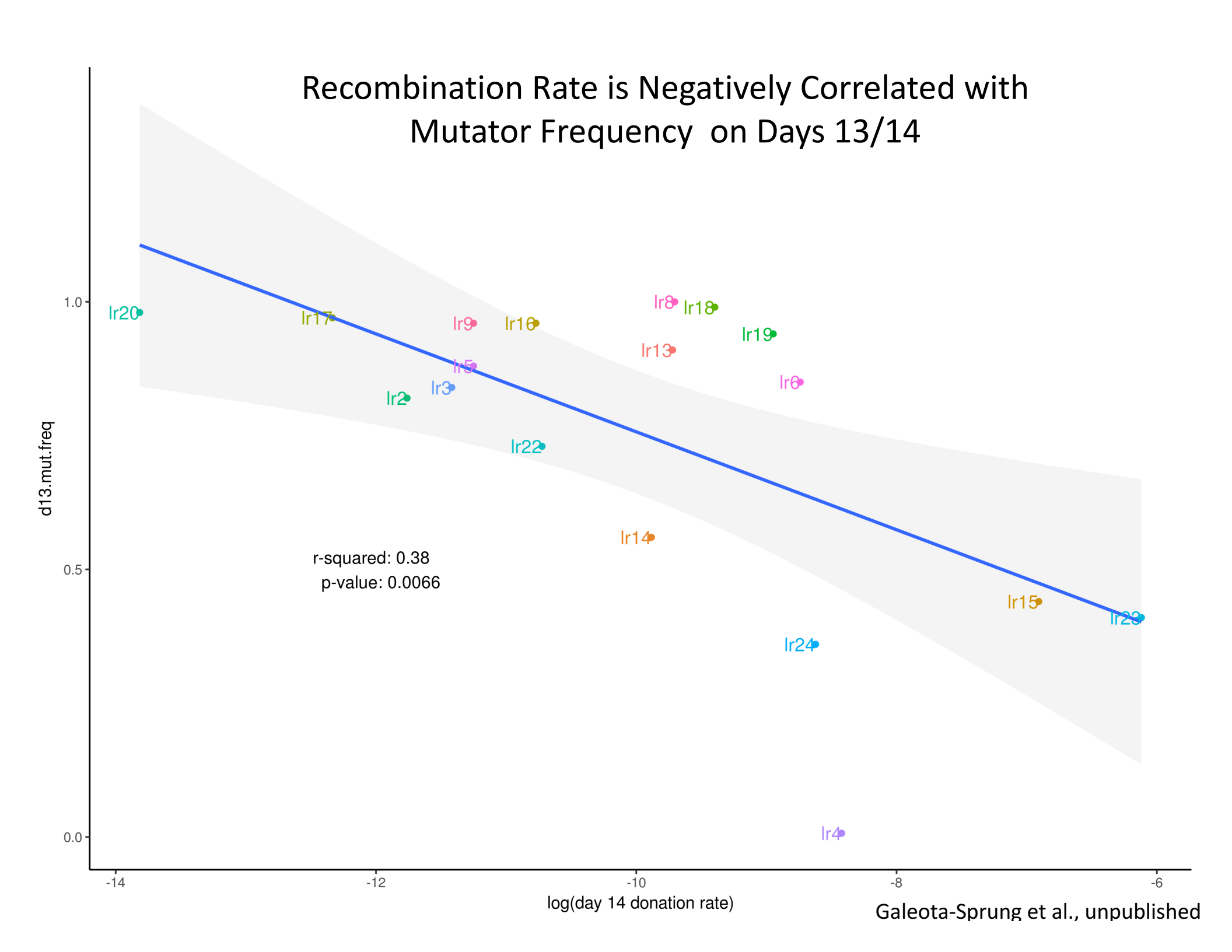
Raynes et al. 2011

# Joint Evolution of Mutation, Recombination Rates in Experimental *E. coli* Populations

(red, gray, black) = (highest, middle, lowest) donation rates




Galeota-Sprung et al., unpublished


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)
lr20
lr17
lr9
lr16
lr8
lr18
lr19
lr13
lr5
lr3
lr2
lr6
lr22
lr14
lr15
lr23
lr24
lr4
r-squared: 0.38
p-value: 0.0066
Galeota-Sprung et al., unpublished

# Why should sex and recombination evolve?

- Sex/recombination can certainly be thought of as potential evolvability adaptations. As such, the same problem applies as with mutation rate evolution: how does a modifier spread within a population?

- There are costs associated with both sex (male/female reproduction) and recombination.

- We will take a look at the costs and briefly examine conditions under which a modifier of recombination might be favored, neglecting the cost of sex (males) per se.

# Evolutionary costs of sex

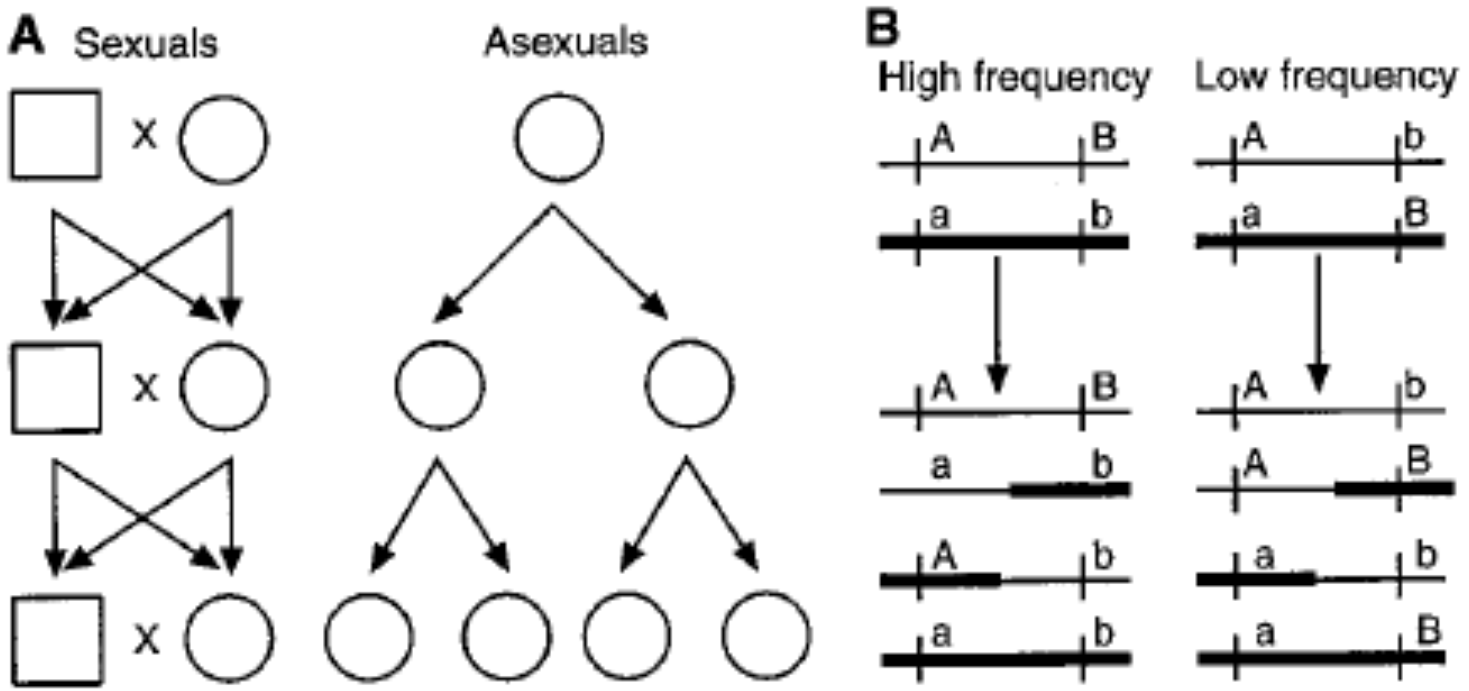



**Fig. 2.** (**A**) The cost of sex in a species with males (squares) and females (circles). If asexual females have the same family size as sexuals, but produce only daughters, their numbers relative to sexual females will double each generation. (**B**) Recombination load. If the two loci shown interact in their effects on fitness, such that allele A interacts well with B but poorly with b, and vice versa for a, the frequency of the double heterozygote AB/ab (in which recombination reduces the frequency of AB and ab) will be greater than that of Ab/aB (where recombination has the reverse effect) in a randomly mating population. Recombination will thus have the net effect of reducing the frequency of the favored gamete types, AB and ab, and so will reduce the mean fitness of an equilibrium population.

Barton, NH and B Charlesworth. 1998. Science 281, 1986-1990

# The Fisher-Muller hypothesis for the advantage of sex

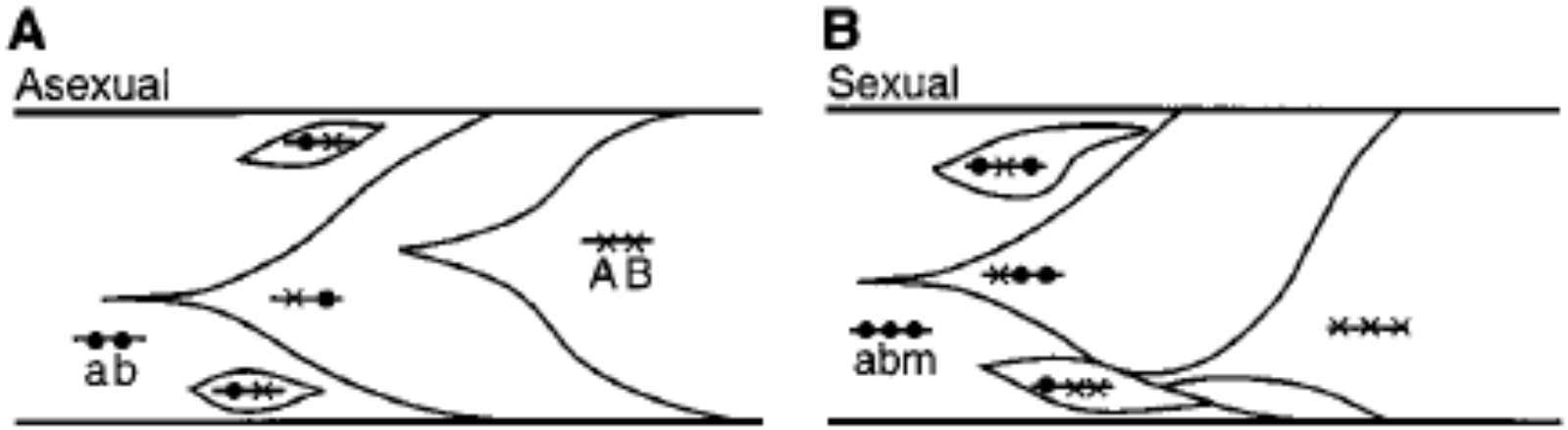
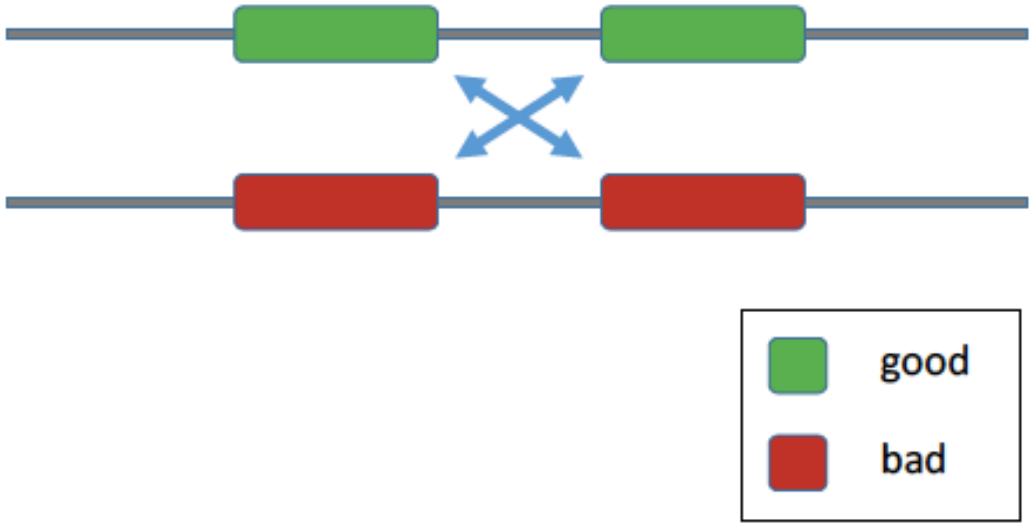



**Fig. 4.** (**A**) With asexual reproduction, favorable mutations must be established sequentially. For example, if allele A is destined to replace a, then any favorable alleles that occur at other loci (B, for instance) can only be fixed if they occur within a genome carrying A (*30*, *46*). (**B**) With sexual reproduction, favorable mutations at different loci can be combined; this leads to an advantage to modifiers that causes sex and recombination. A favorable allele B that occurs with the unfavorable allele a can only be fixed if it can recombine into association with A; if this requires that a modifier allele M be present, then that modifier will also tend to increase by hitchhiking (*48*, *72*).

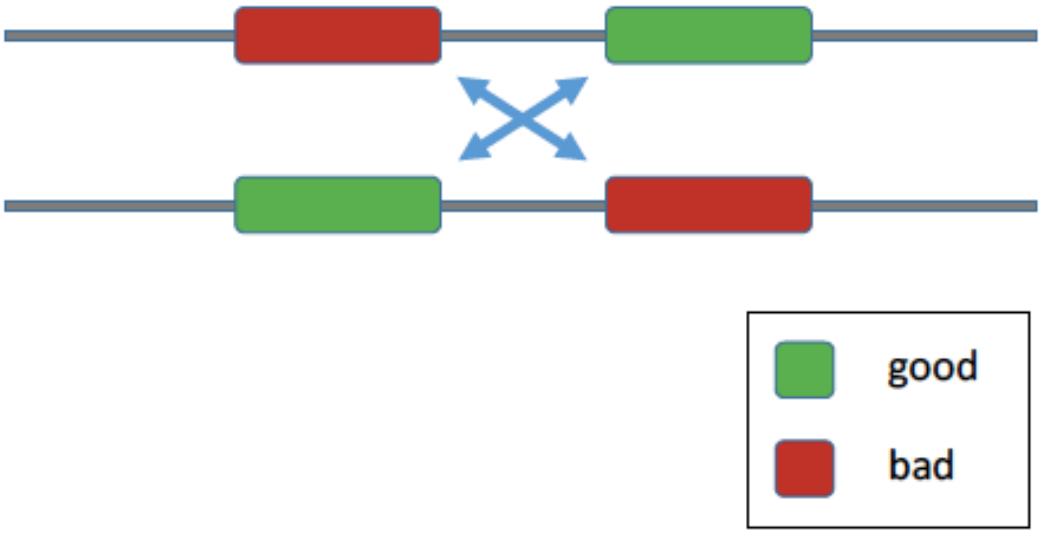
Barton, NH and B Charlesworth. 1998. Science 281, 1986-1990

# When sex is bad




- Sex breaks up good combinations that would otherwise fix efficiently.
- Sex breaks up bad combinations that would otherwise be purged efficiently.

# When sex is good




- Sex assembles good combinations that can fix efficiently.
- Sex assembles bad combinations that can be purged efficiently.

# MATHEMATICALLY SPEAKING

Let $X$ and $Y$ denote fitness contributions of two different genes.

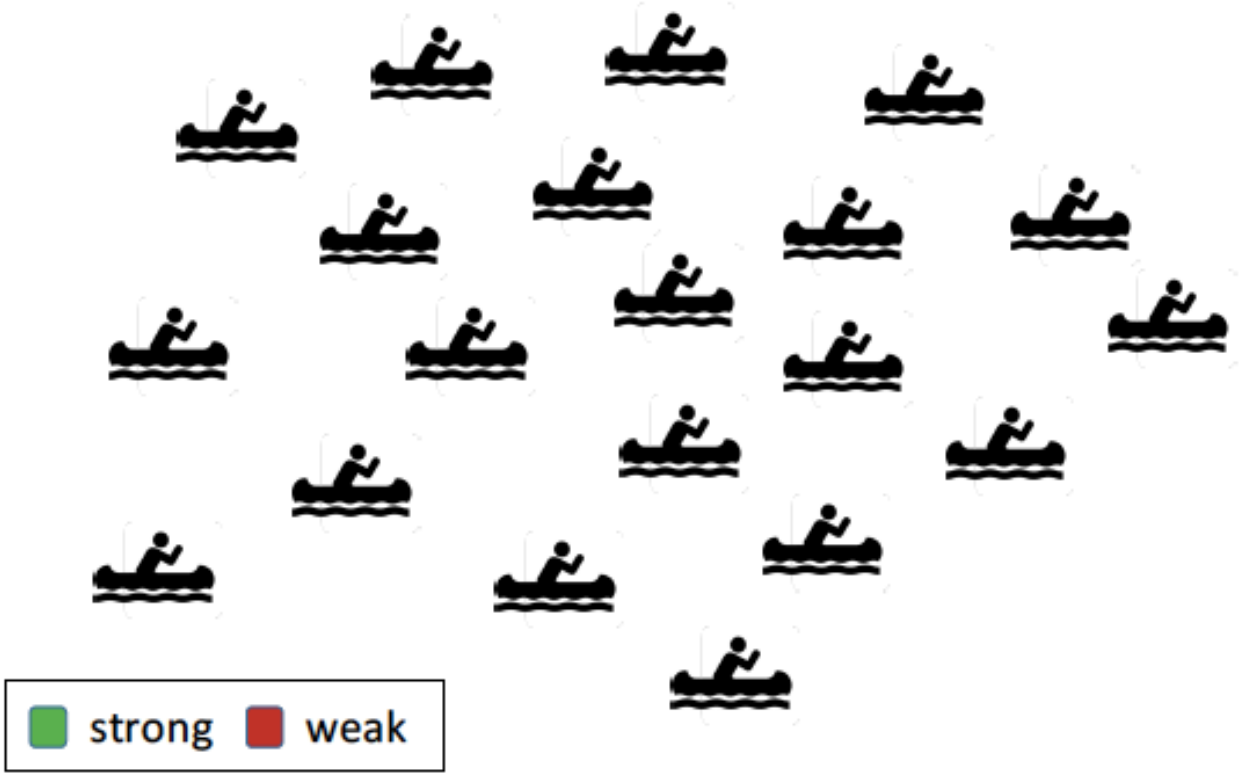
- Sex is bad when:

$$Cov(X, Y) > 0$$

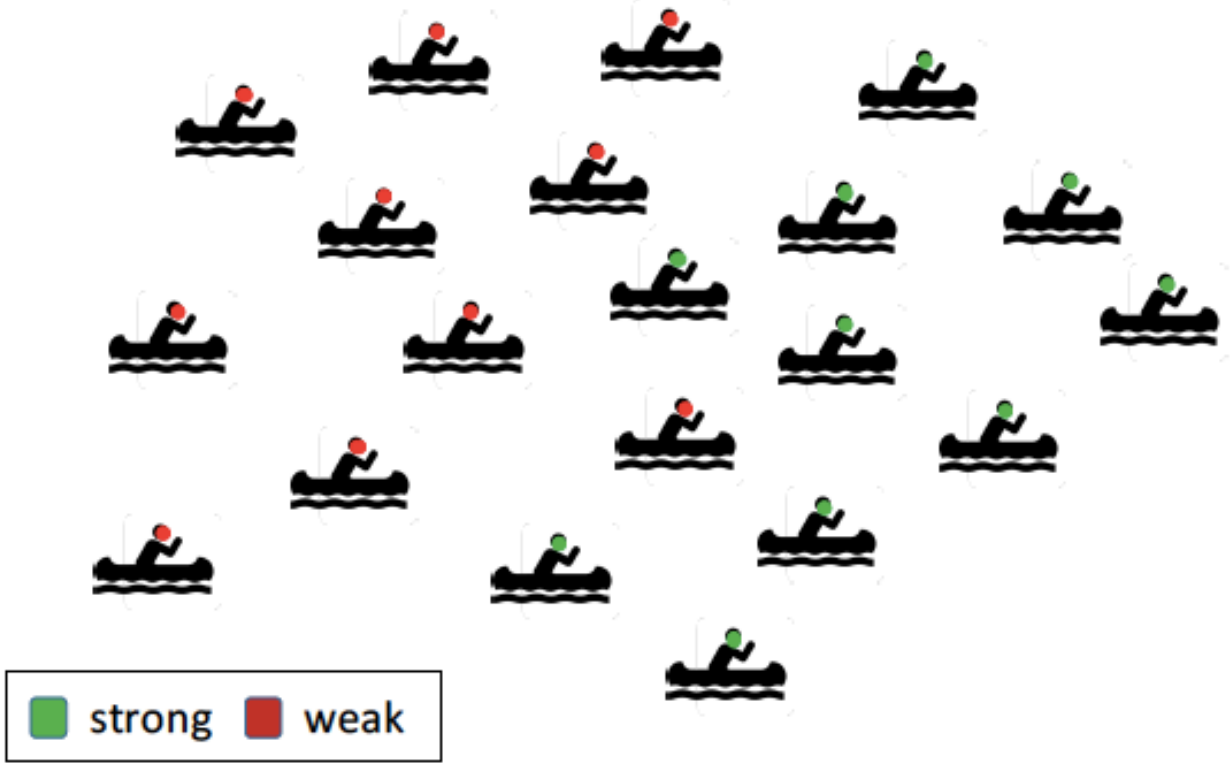
- Sex is good when:

$$Cov(X, Y) < 0$$

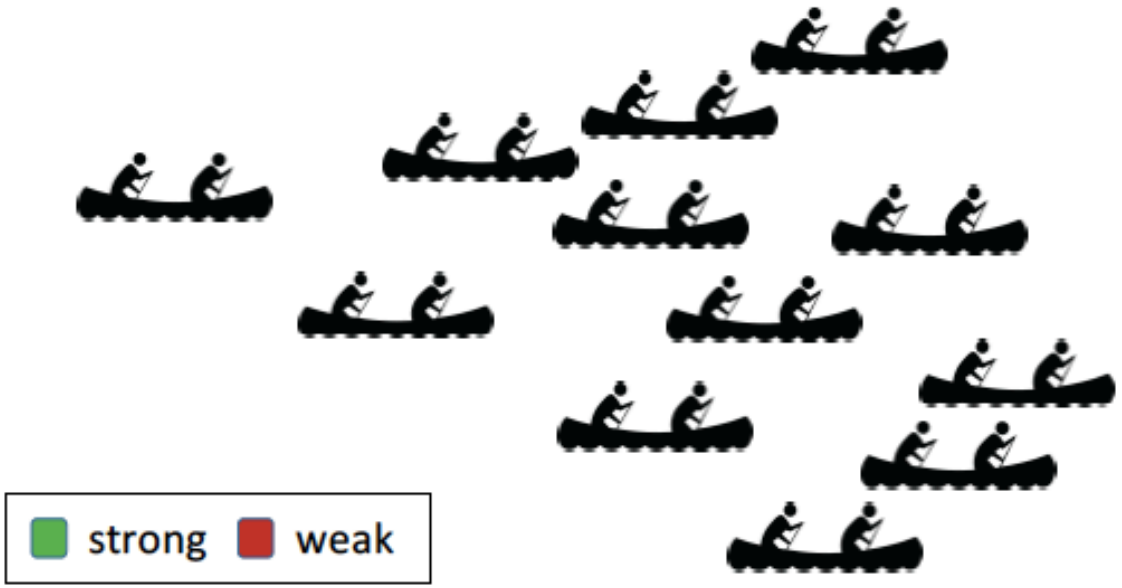
# CANOE RACE: NATURAL SELECTION ON **ONE** GENE (ONE PADDLER)



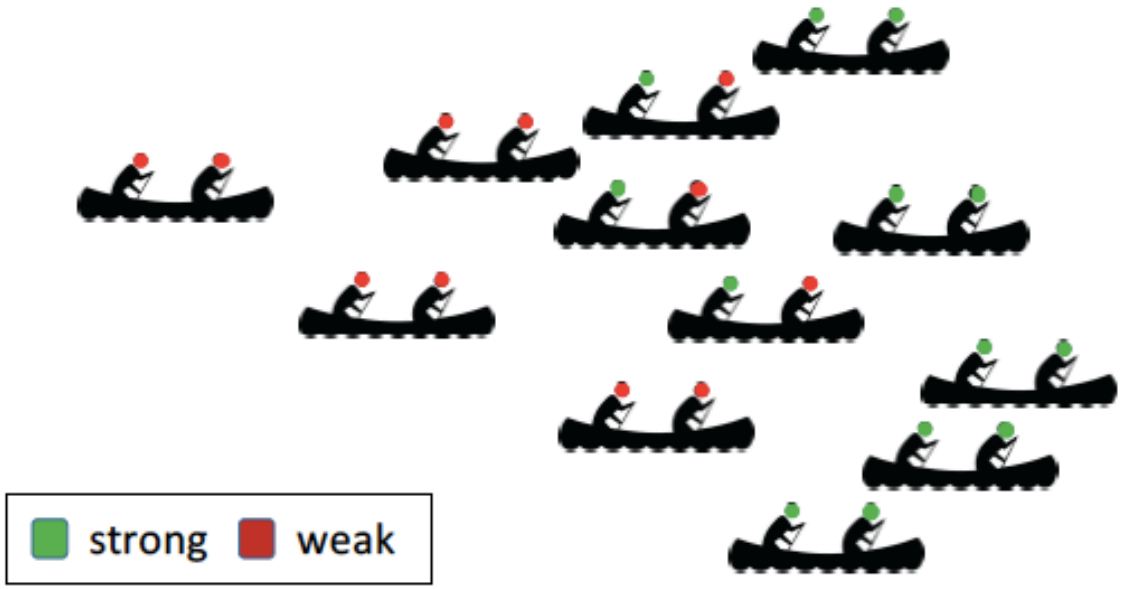
# CANOE RACE: NATURAL SELECTION ON **ONE** GENE (ONE PADDLER)



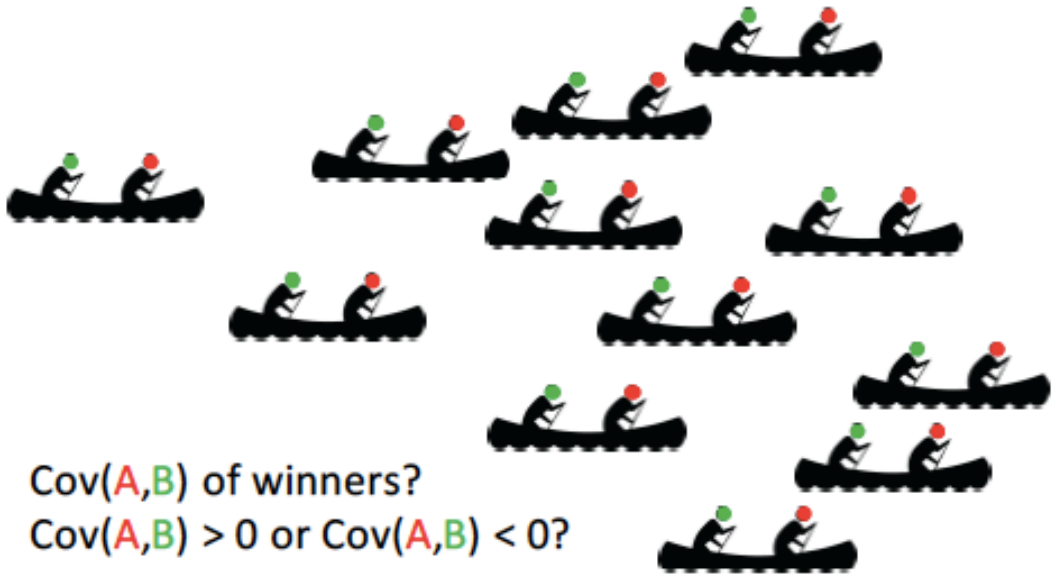
# CANOE RACE: NATURAL SELECTION ON **TWO** GENES (TWO PADDLERS)



# CANOE RACE: NATURAL SELECTION ON **TWO** GENES (TWO PADDLERS)



# CANOE RACE: NATURAL SELECTION ON **TWO** GENES (TWO PADDLERS)

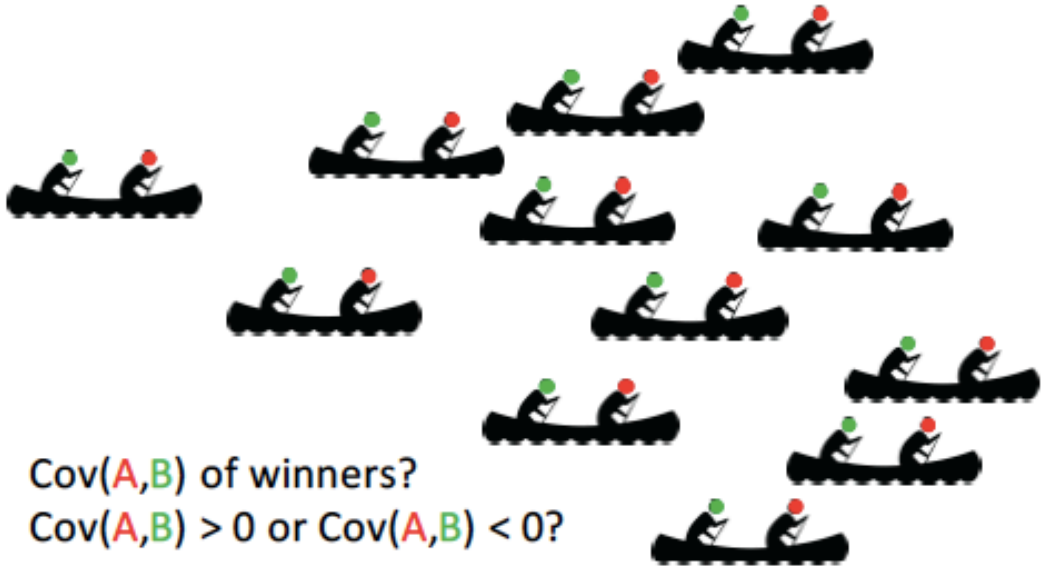


Cov(A,B) of winners?
Cov(A,B) > 0 or Cov(A,B) < 0?

PADDLER STRENGTH

| A | B | total |
|---|---|---|
| 1.34 | 1.12 | 2.46 |
| 0.95 | 1.42 | 2.37 |
| 1.17 | 1.13 | 2.30 |
| 1.03 | 0.92 | 1.95 |
| 1.04 | 0.78 | 1.82 |
| 0.89 | 1.01 | 1.90 |
| 1.22 | 1.36 | 2.58 |
| 0.73 | 0.79 | 1.52 |
| 1.11 | 0.88 | 1.99 |
| 1.04 | 0.98 | 2.02 |

# CANOE RACE: NATURAL SELECTION ON **TWO** GENES (TWO PADDLERS)



Cov(A,B) of winners?
Cov(A,B) > 0 or Cov(A,B) < 0?

**ANSWER: Cov(A,B) < 0 (!!)**

PADDLER STRENGTH

| A | B | total |
|---|---|---|
| 1.34 | 1.12 | 2.46 |
| 0.95 | 1.42 | 2.37 |
| 1.17 | 1.13 | 2.30 |
| 1.03 | 0.92 | 1.95 |
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| 1.22 | 1.36 | 2.58 |
| 0.73 | 0.79 | 1.52 |
| 1.11 | 0.88 | 1.99 |
| 1.04 | 0.98 | 2.02 |

# IMPLICATION

$$Cov(X, Y) = \mathbb{E}(XY) - \mathbb{E}(X)\mathbb{E}(Y) < 0$$

- Implies that a random gene swap should improve fitness!

# Many thanks to...


- **Philip Gerrish (theory)**
- Sophie Pénisson (mathematical collaborator)
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- Jude Dartey (high school intern)
- Katy Kao, Texas A&M (recombining strains)
- Vaughn Cooper, U Pitt (genomic sequencing)
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And to you, for listening!

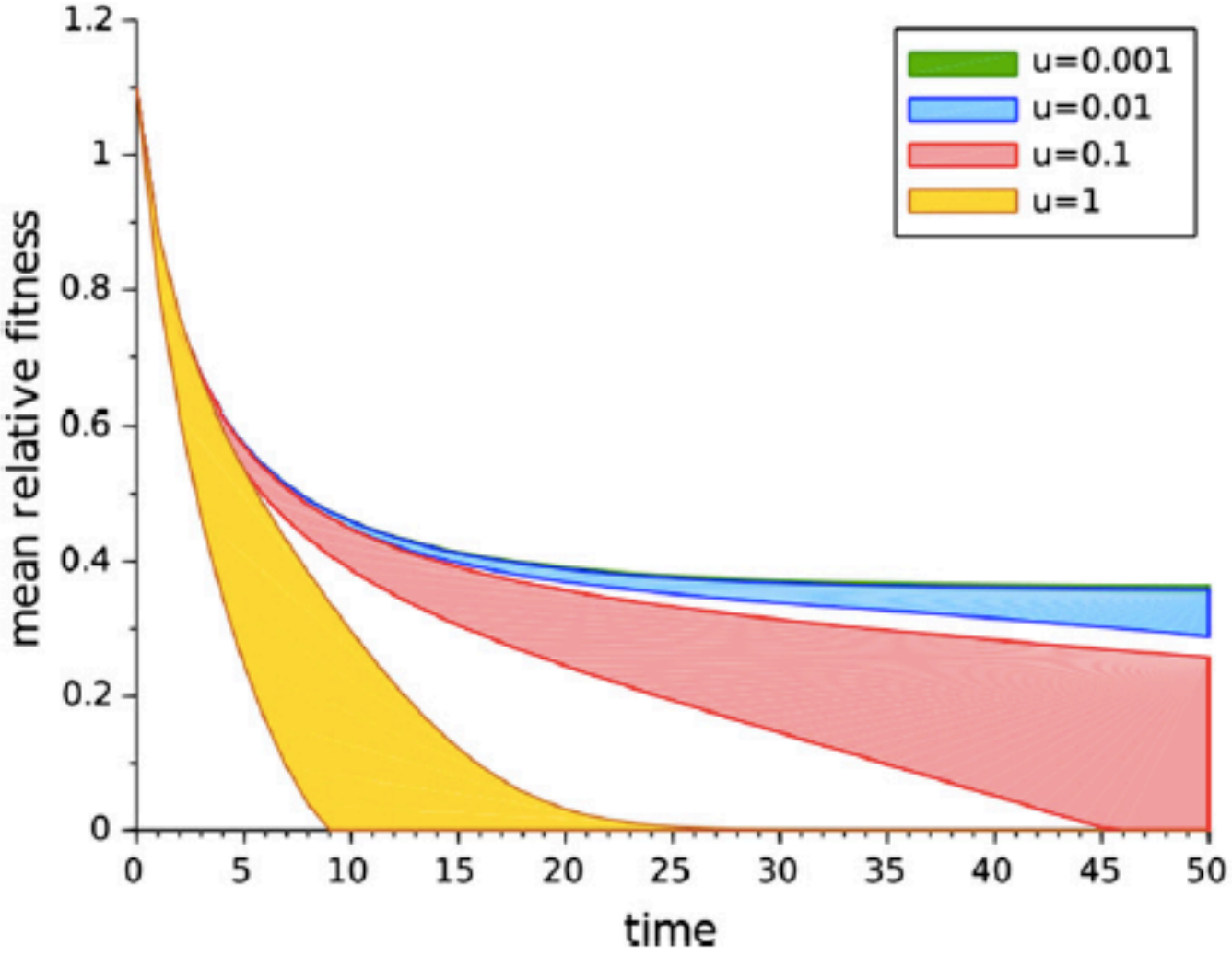
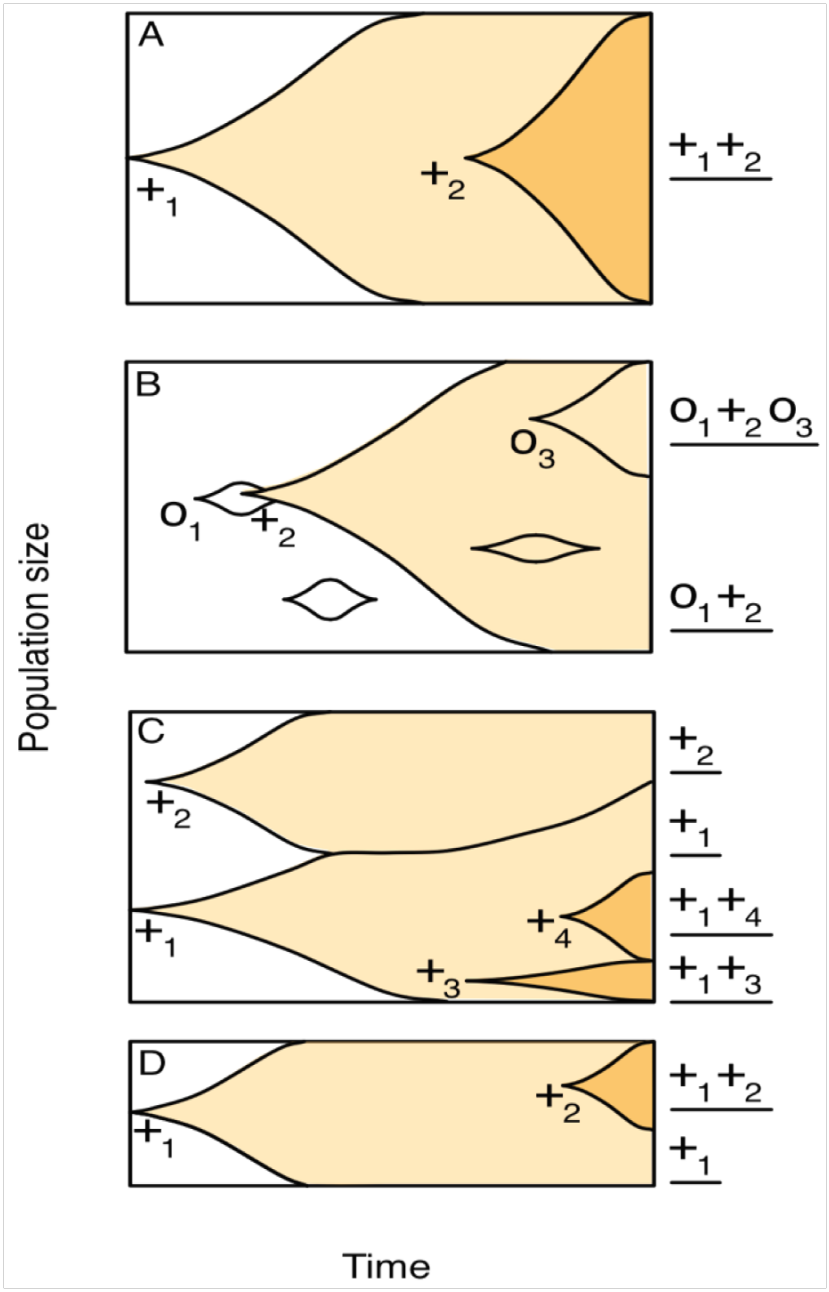



**Figure 2** Evolution over time of the mean relative fitness of a single beneficial lineage ($s_b = 0.1$) in a large wild-type population, initially homogeneous, with $s_d = 0.03$. Upper and lower bounds were obtained as described in File S1. The decrease in relative fitness is caused by the comparatively rapid accumulation of deleterious mutations owing to the small size of emerging beneficial lineages, *i.e.*, lineage contamination.

Pénisson et al. 2017

# Sniegowski Lab




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