

The sounds of silence

Fitness effects of synonymous mutations in a gene
under selection

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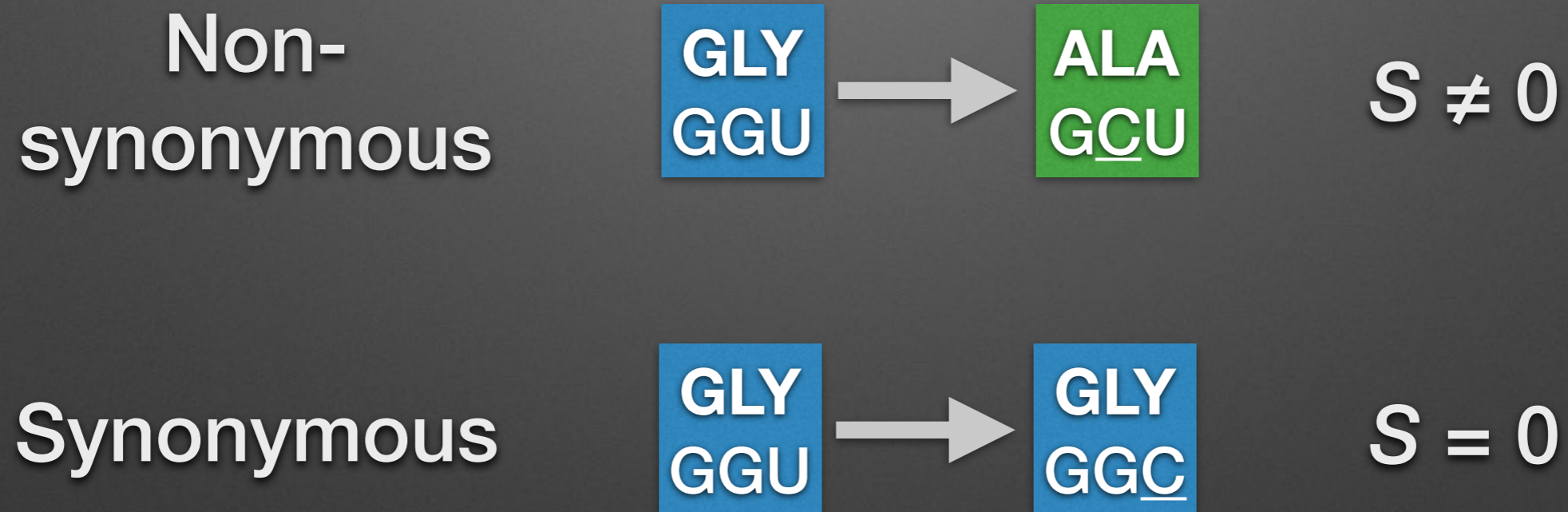
<http://www.cfccreates.com/productions/359-vectors>



Artist: Eva Markvoort Photo: Cyrus McEachern
Courtesy of 65RedRose.com #4Eva

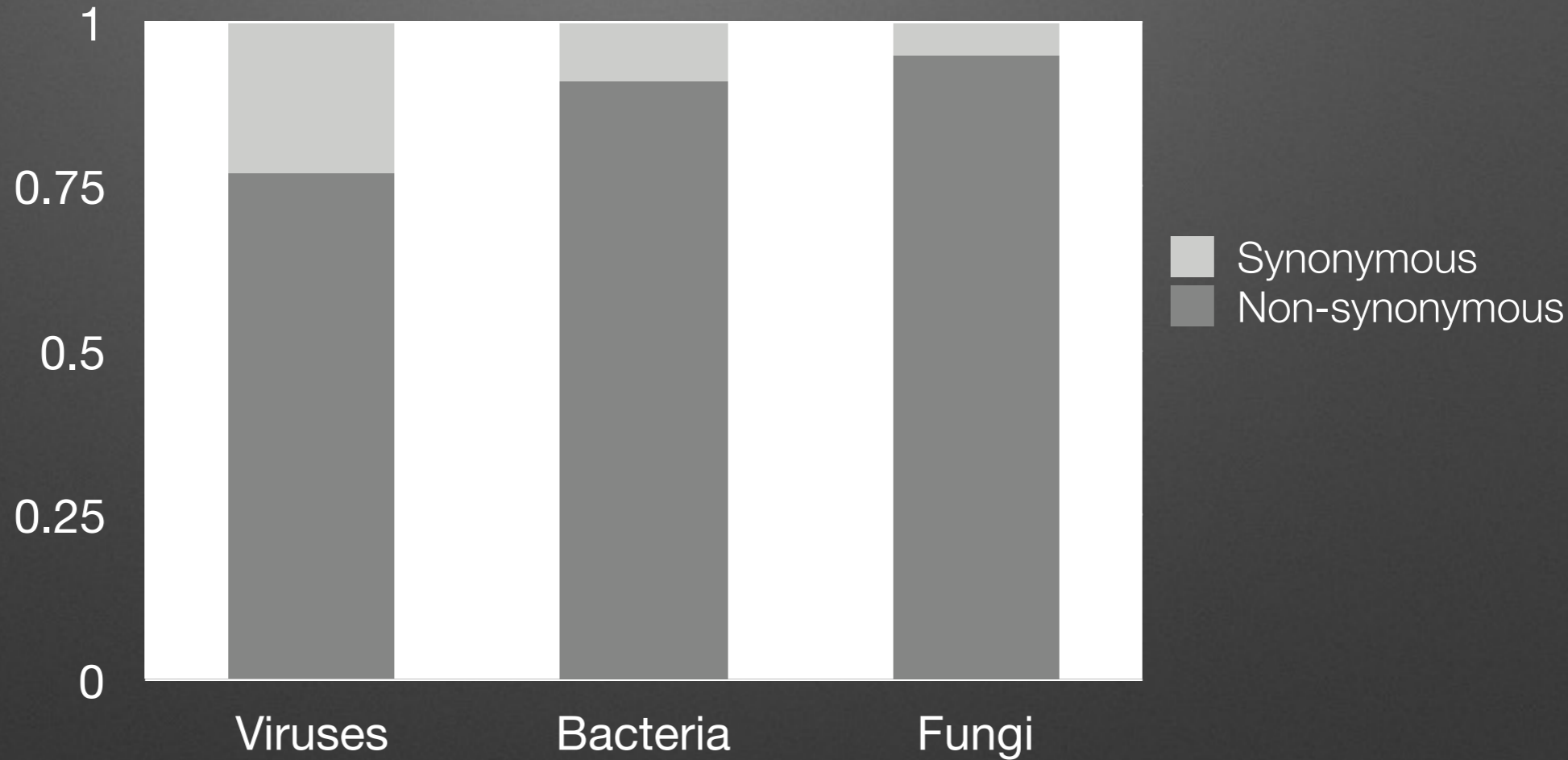
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The 'standard model' of evolutionary genetics*



*Depends on N_e , of course

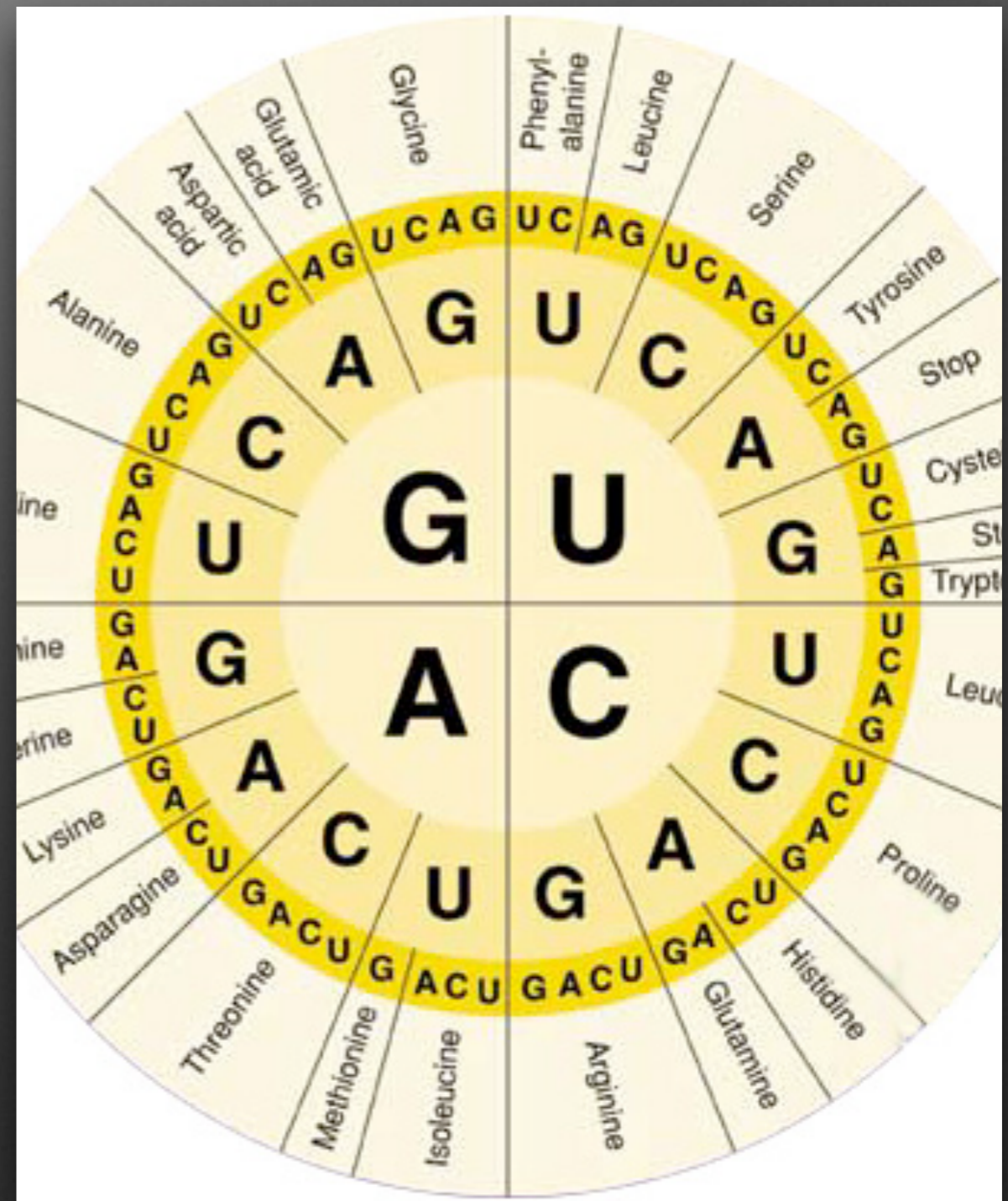
Most mutations recovered from selection experiments are non-synonymous



Kassen (2014) *Experimental Evolution and the Nature of Biodiversity*. Roberts and Co., USA

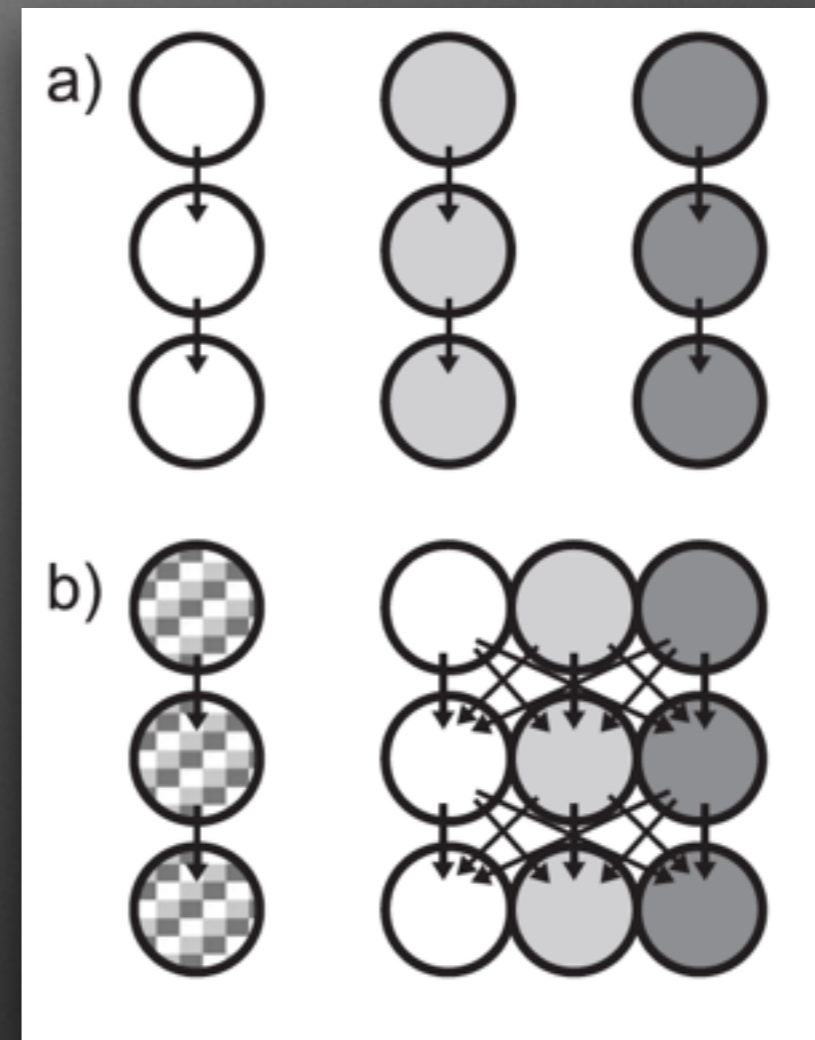
But synonymous mutations can sometimes be under selection

- Codon bias
- Associated with human disease
- Substantial experimental evidence they can change protein expression
- Some comparative evidence suggesting weak purifying selection



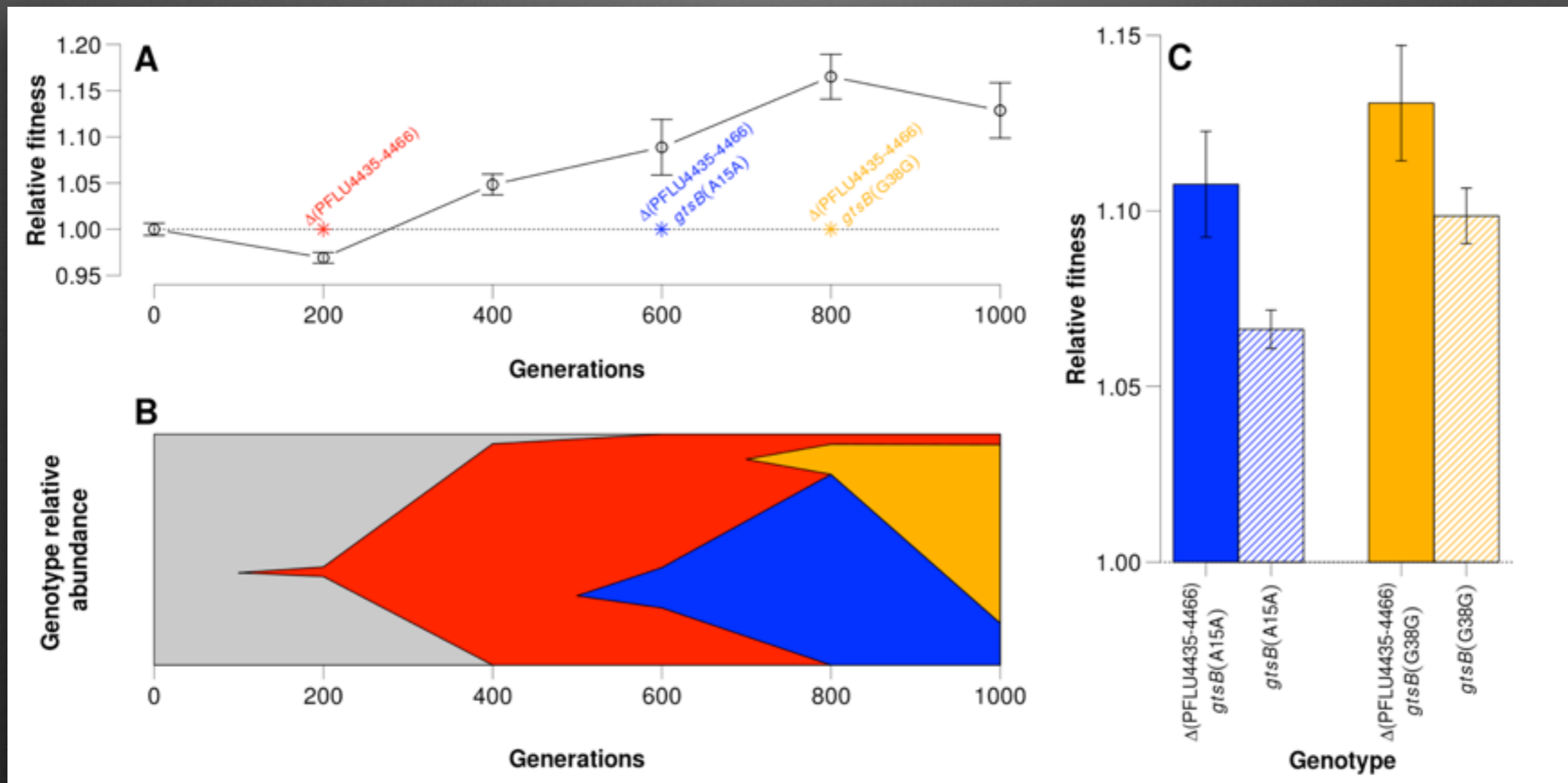
Pseudomonas fluorescens evolving in spatially heterogeneous environments

- 100x serial transfer daily for ~1000 generations
- Stationary phase density $\sim 2 \times 10^7$ cells ml⁻¹
- 3 replicate lines per treatment
- Competitive fitness assays
- Whole genome-sequencing of evolved populations

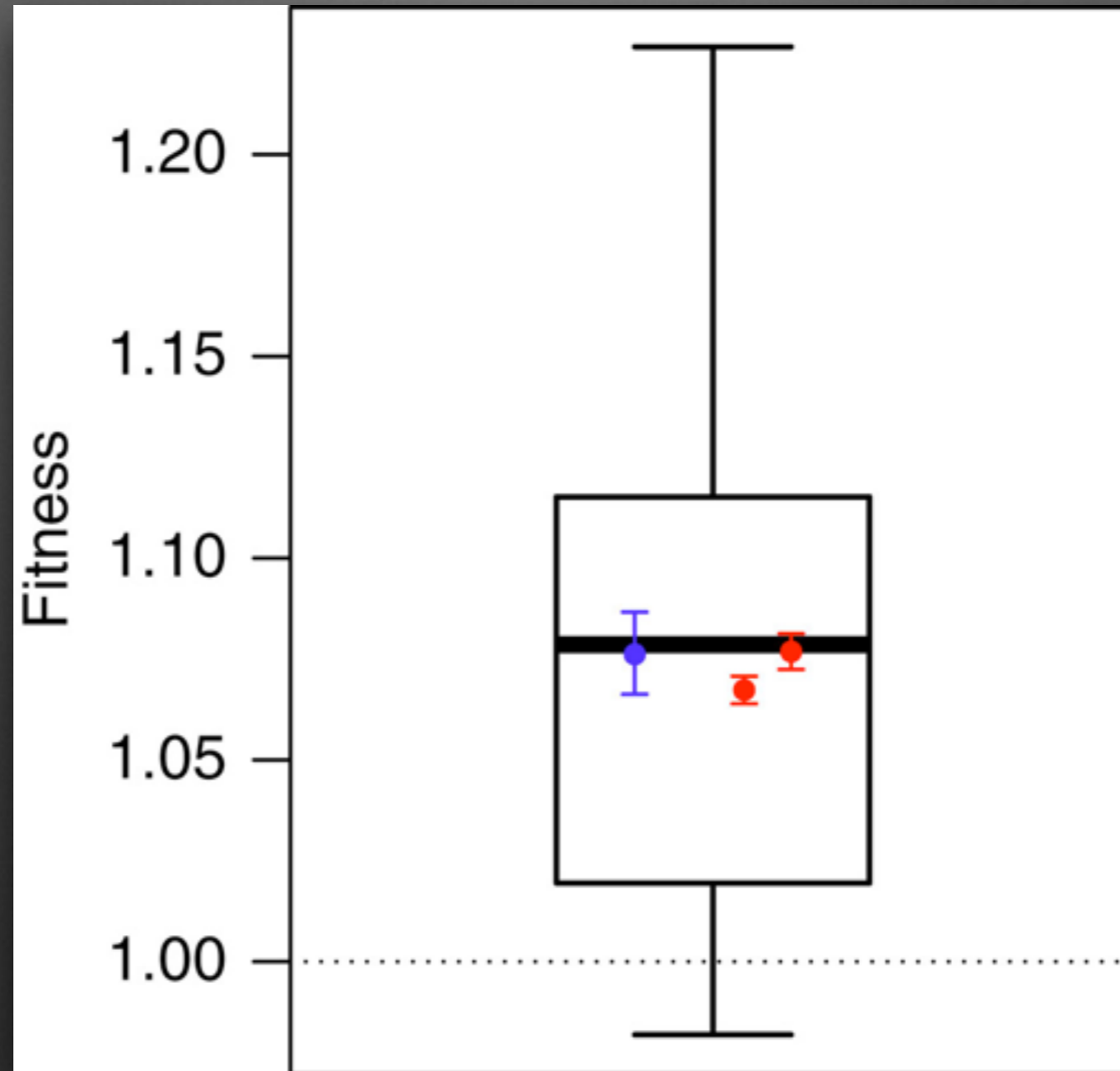


Bailey & Kassen (2012) *Am Nat* 180: 270-283
Schick, Bailey, & Kassen (2015) *Am Nat* 186: S48-S59
Bailey et al. (2015) *MBE* doi: 10.1093/molbev/msv033

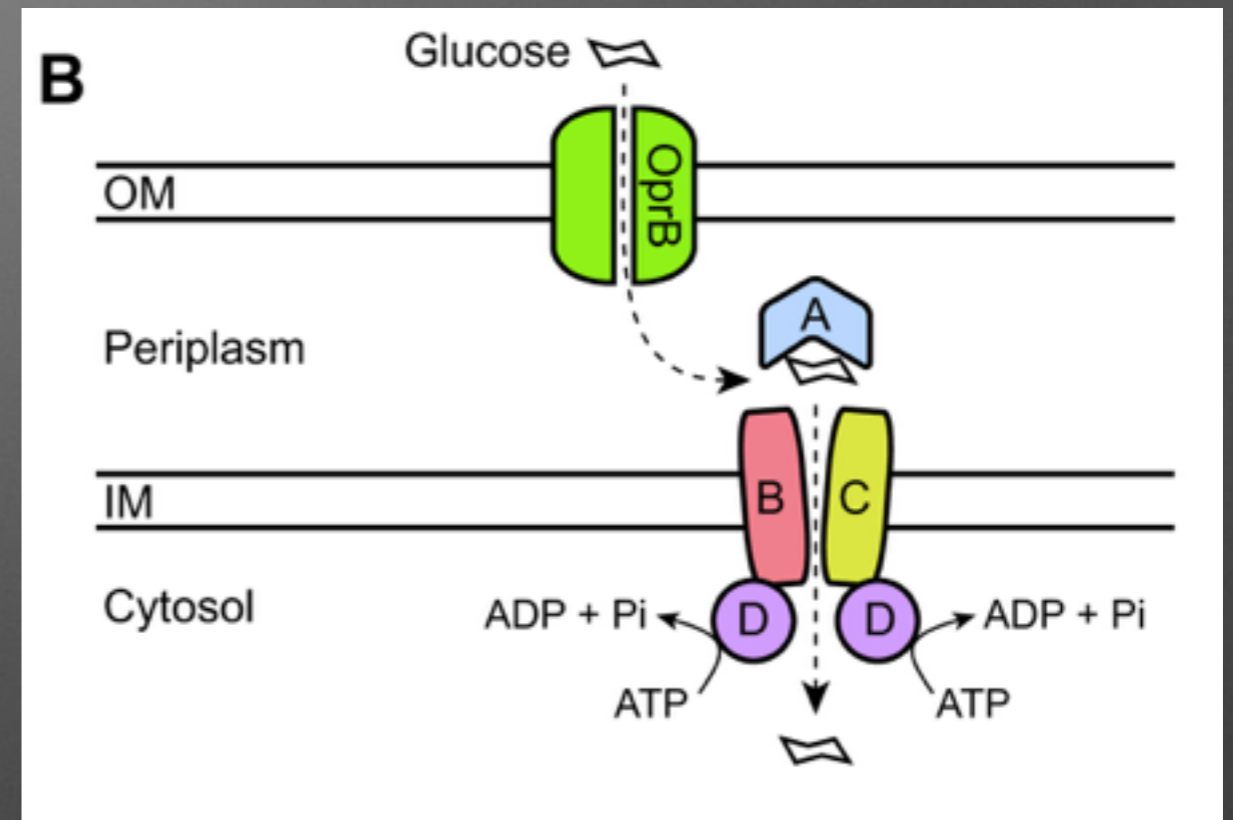
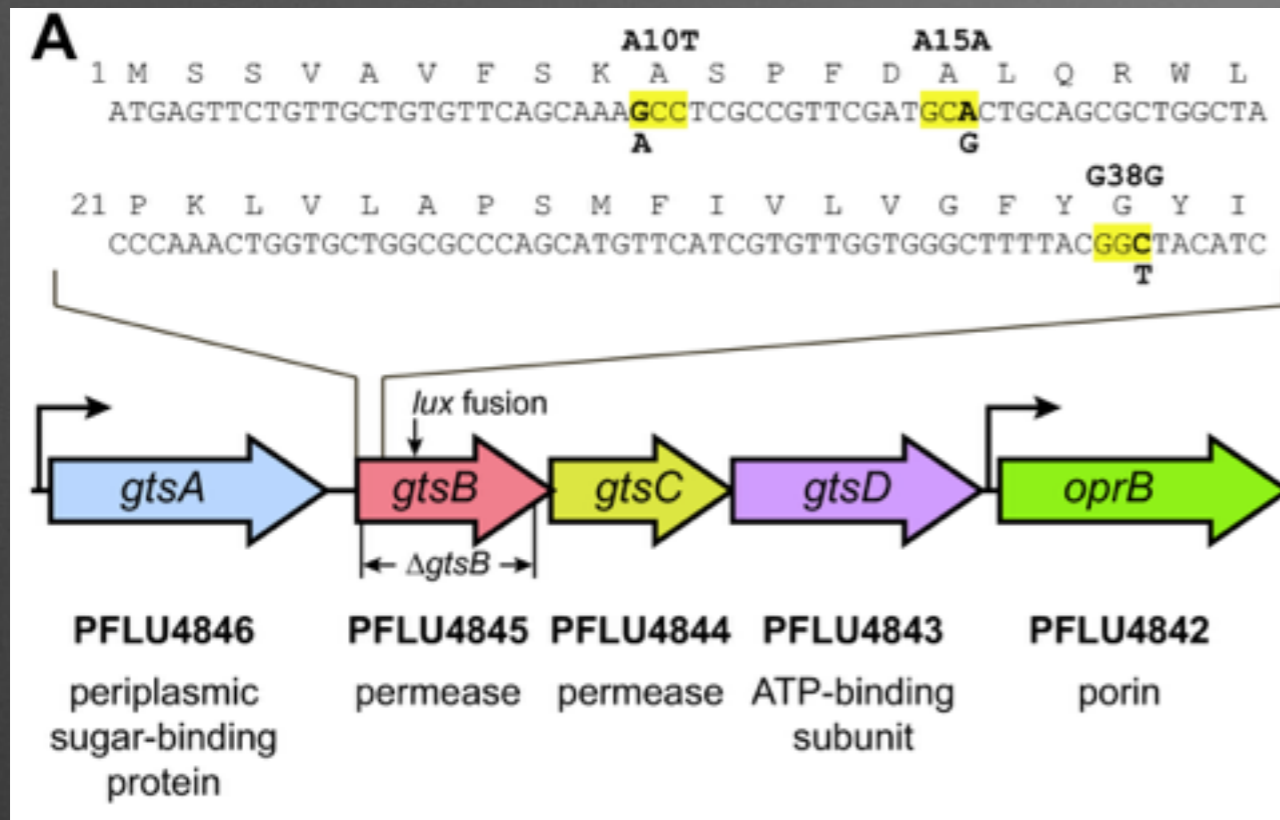
Adaptive synonymous mutations in a glucose-limited population



Fitness effects of fixed mutations

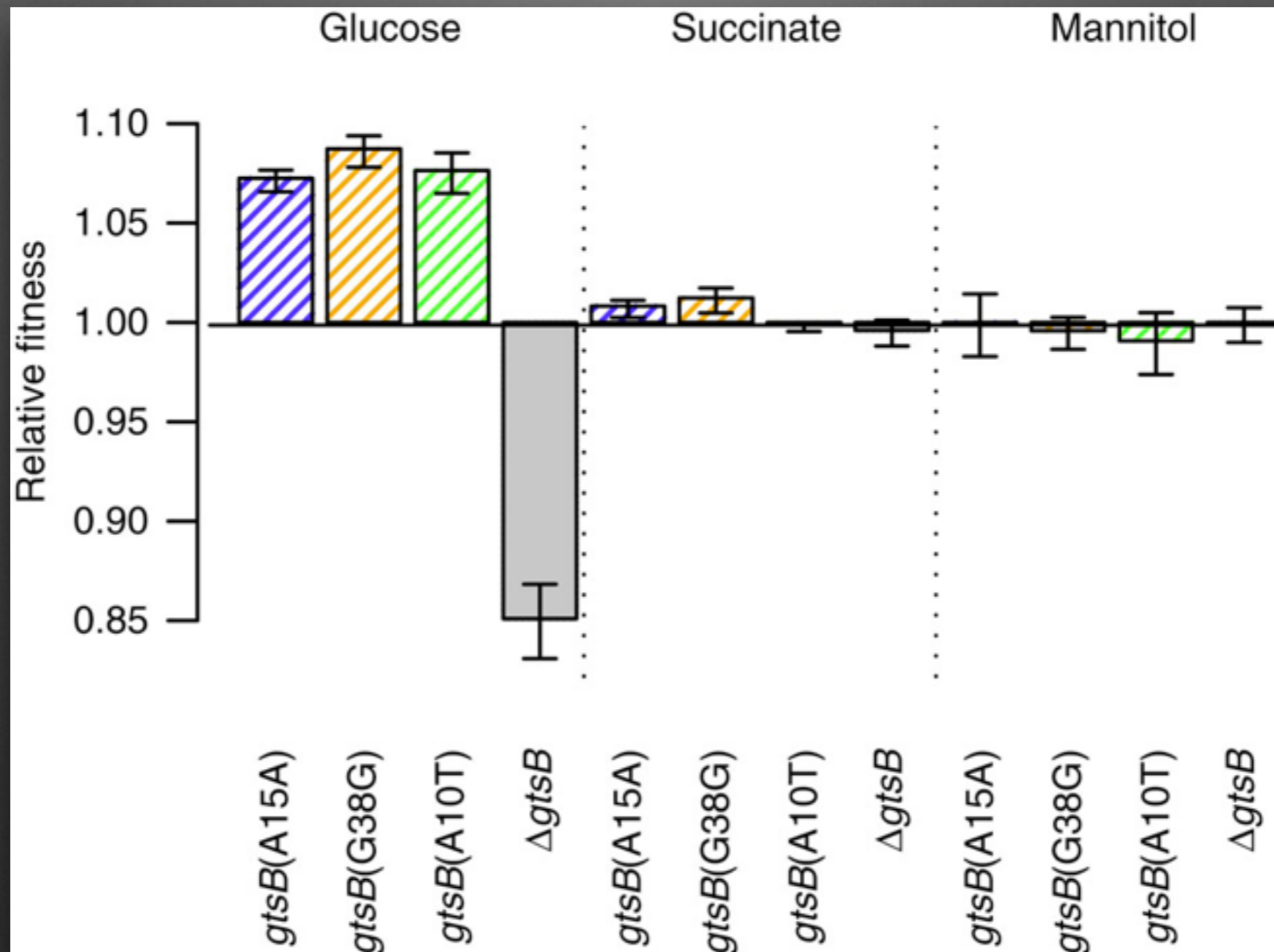


Where are the mutations?



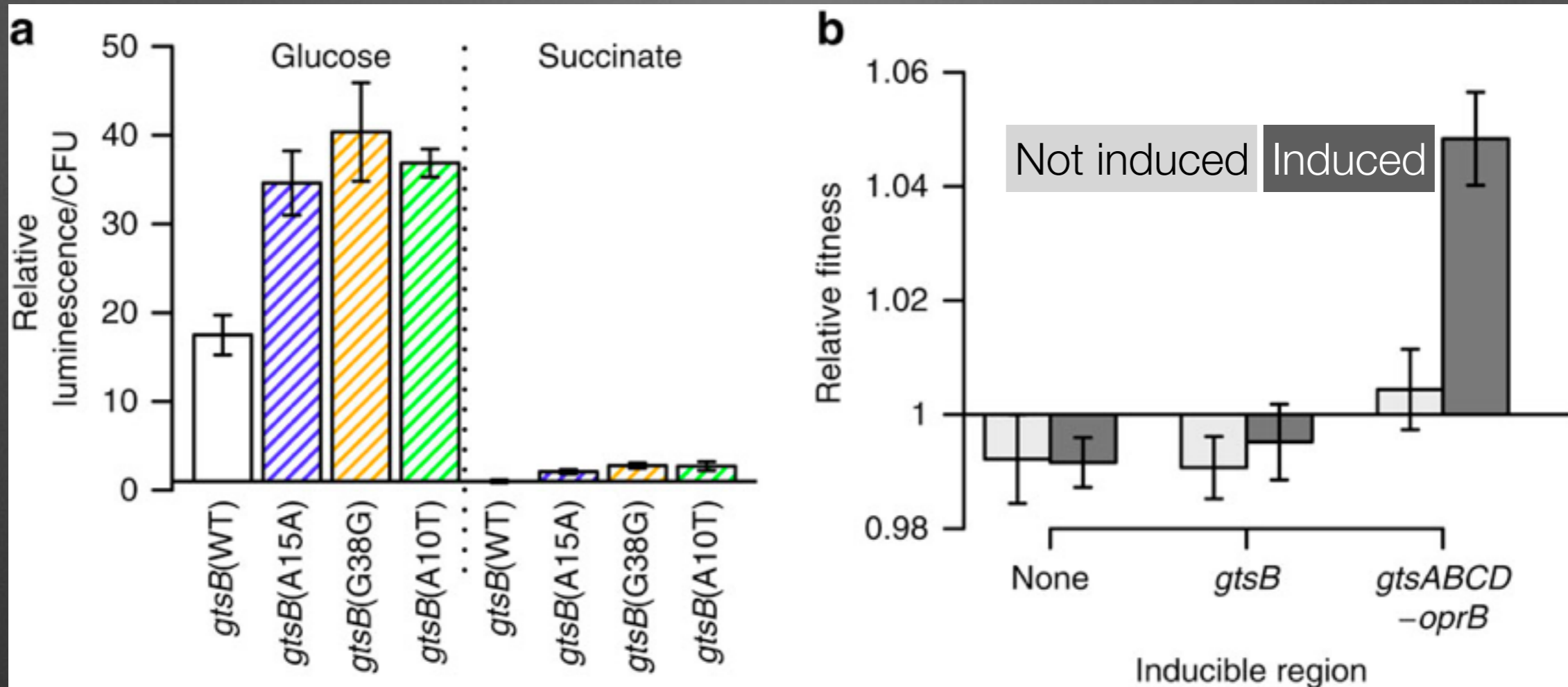
Near the start of *gtsB*, which is part of an ATP-binding cassette (ABC) transporter specific to glucose

gtsB is a target of selection...



...and fitness advantages are specific to glucose

Mechanism involves increased gene expression

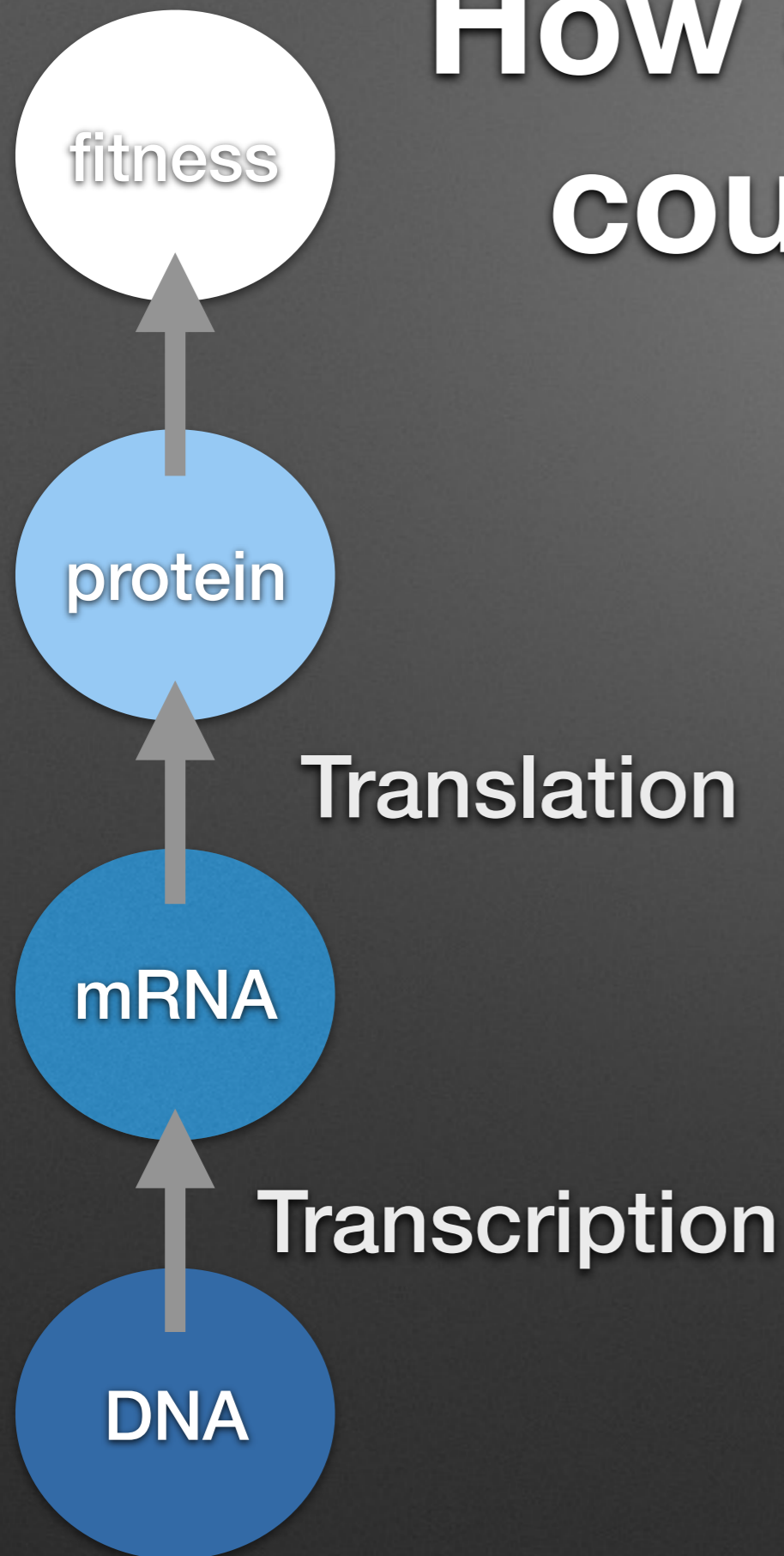


Transcript abundance obtained via luciferase reporter (*gtsB* fusion with *luxA-E*)

Transcription induction

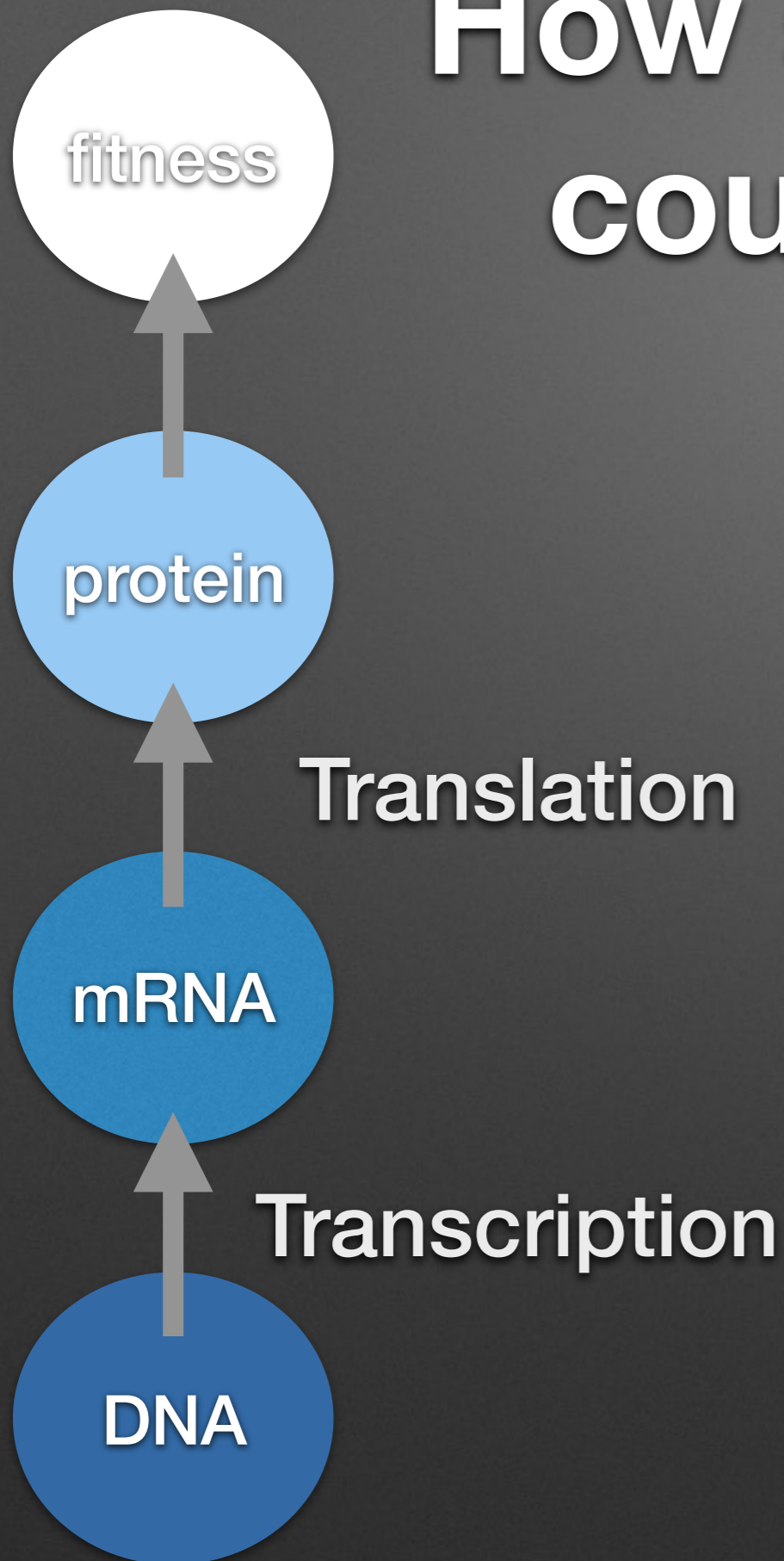
(and increased glucose transport...analysis pending)

How synonymous SNPs could impact fitness



- Codon bias
 - Common codon -> increase translation speed
 - Rare codon -> more efficient/accurate translation
- mRNA stability
 - Decrease means easier access to tRNAs
 - Increase leads to slower decay rates
- Post-transcriptional regulation
 - Catabolite repression
 - sRNA inhibition
- Protein secondary structure

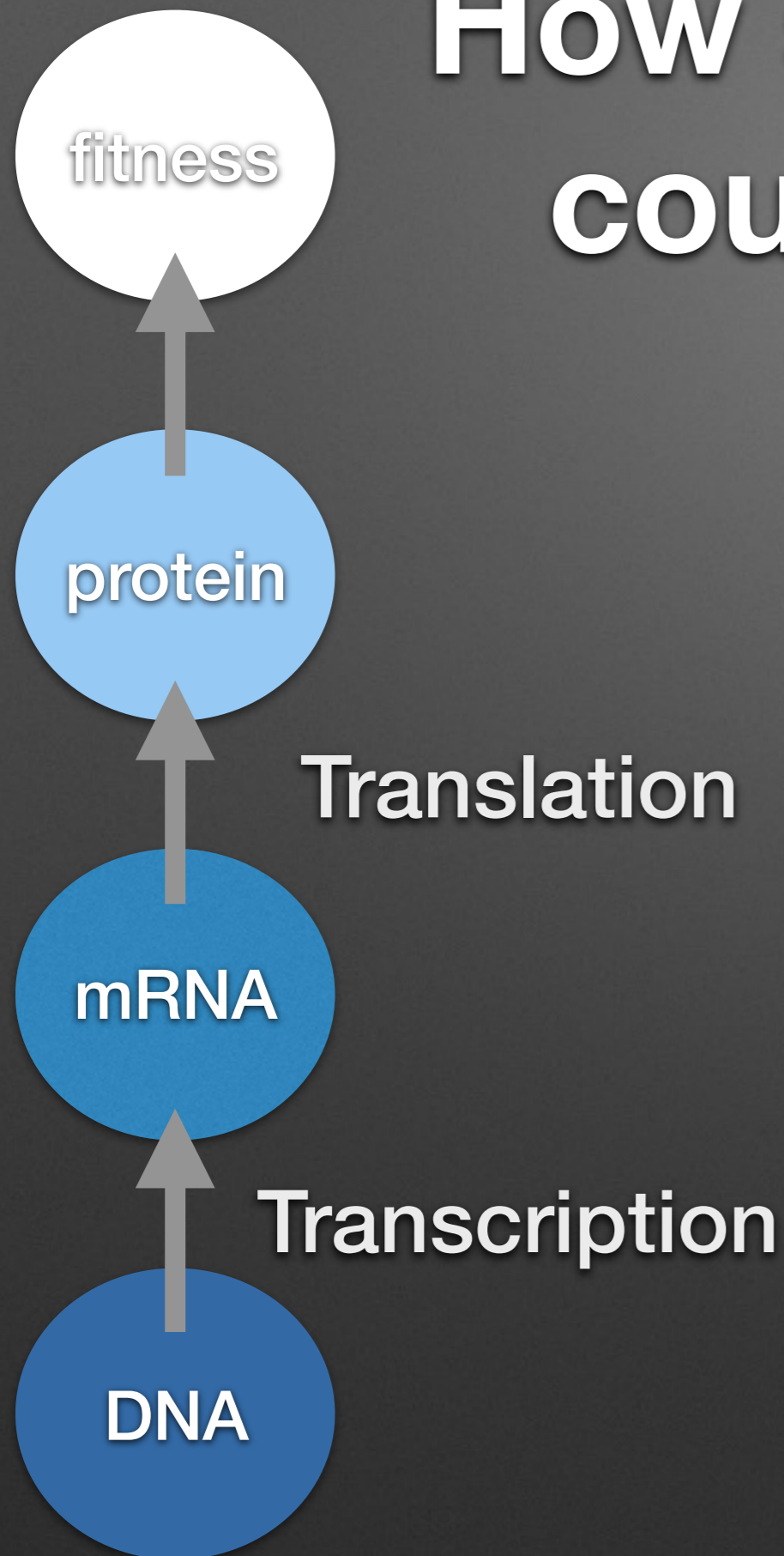
How synonymous SNPs could impact fitness



- Codon bias
 - Nope - A15A & G38G mutate to rarer codons, A10T to a more common codon translation

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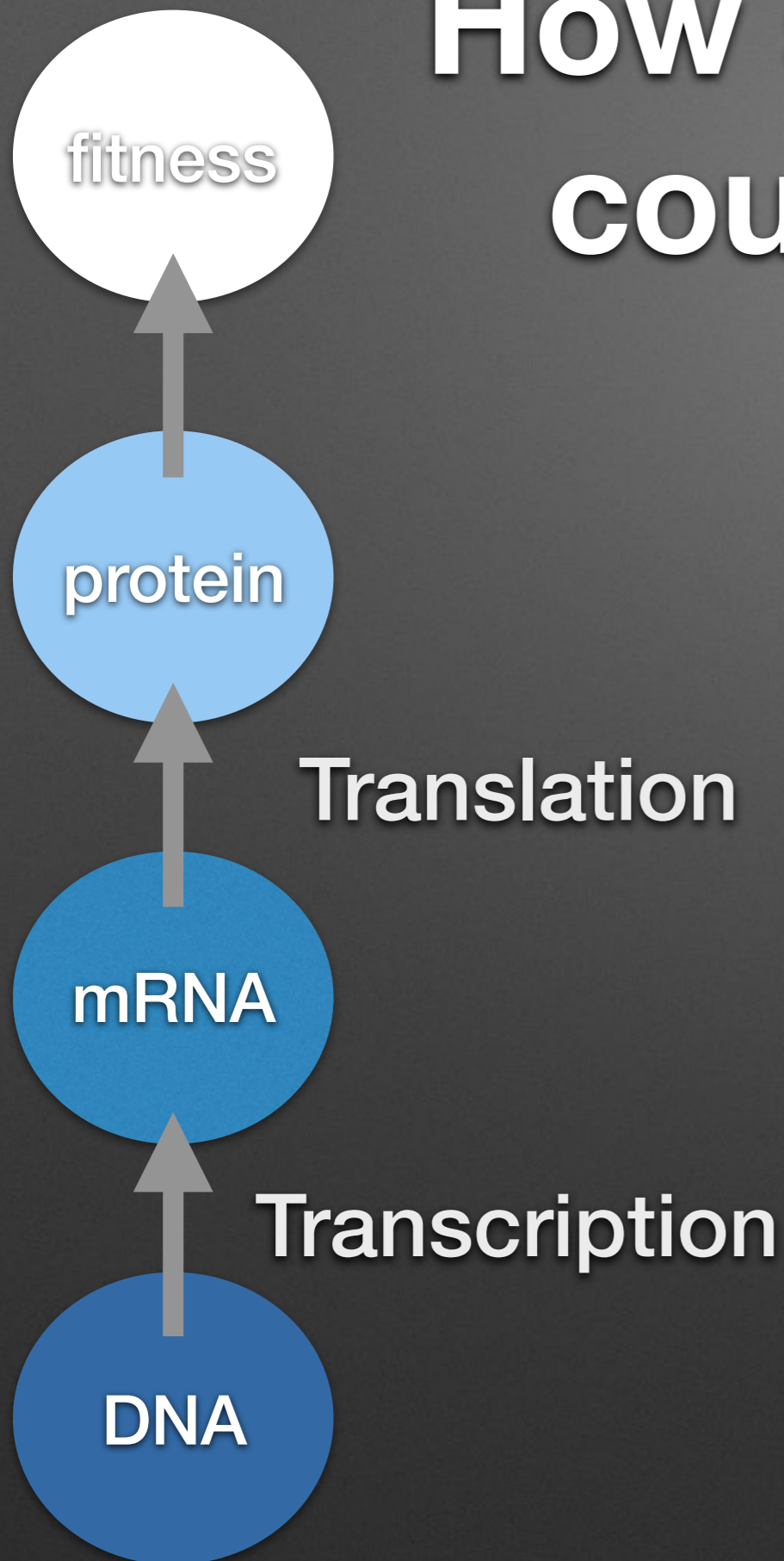


- Codon bias
 - Nope - A15A & G38G mutate to rarer codons, A10T to a more common codon translation

- mRNA stability
 - Nope - A10T & G38G are more stable, A10T is less stable (according to mfold)

- Post-transcriptional regulation
 - Catabolite repression
 - sRNA inhibition
- Protein secondary structure

How synonymous SNPs could impact fitness



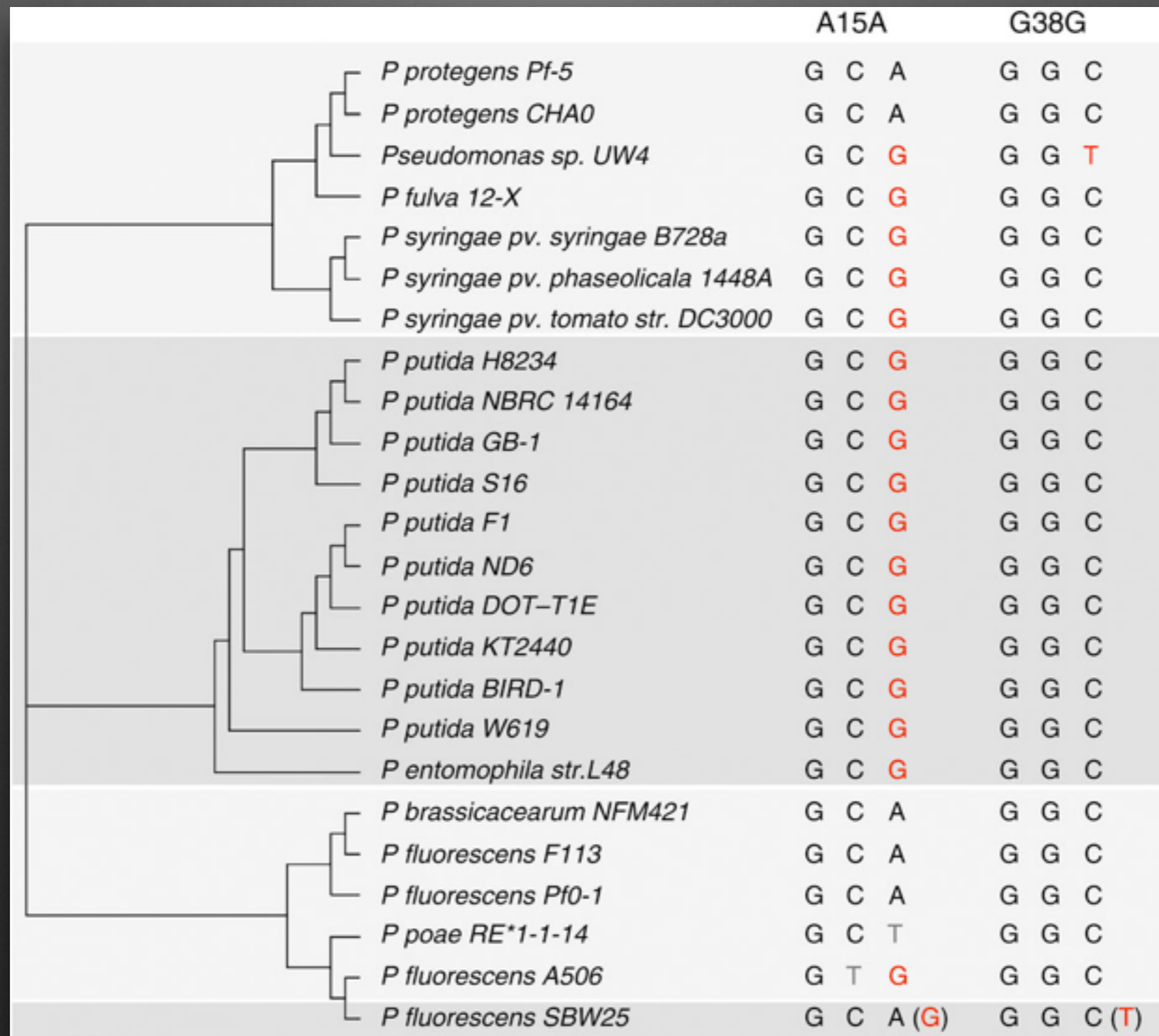
- Codon bias
Nope - A15A & G38G mutate to rarer codons, A10T to a more common codon translation

- mRNA stability
Nope - A10T & G38G are more stable, A10T is less stable

- Post-transcriptional regulation
Nope - genetic KOs provide no evidence for either mechanism (ask me later for details if interested)

- Protein secondary structure

How common are these mutations?

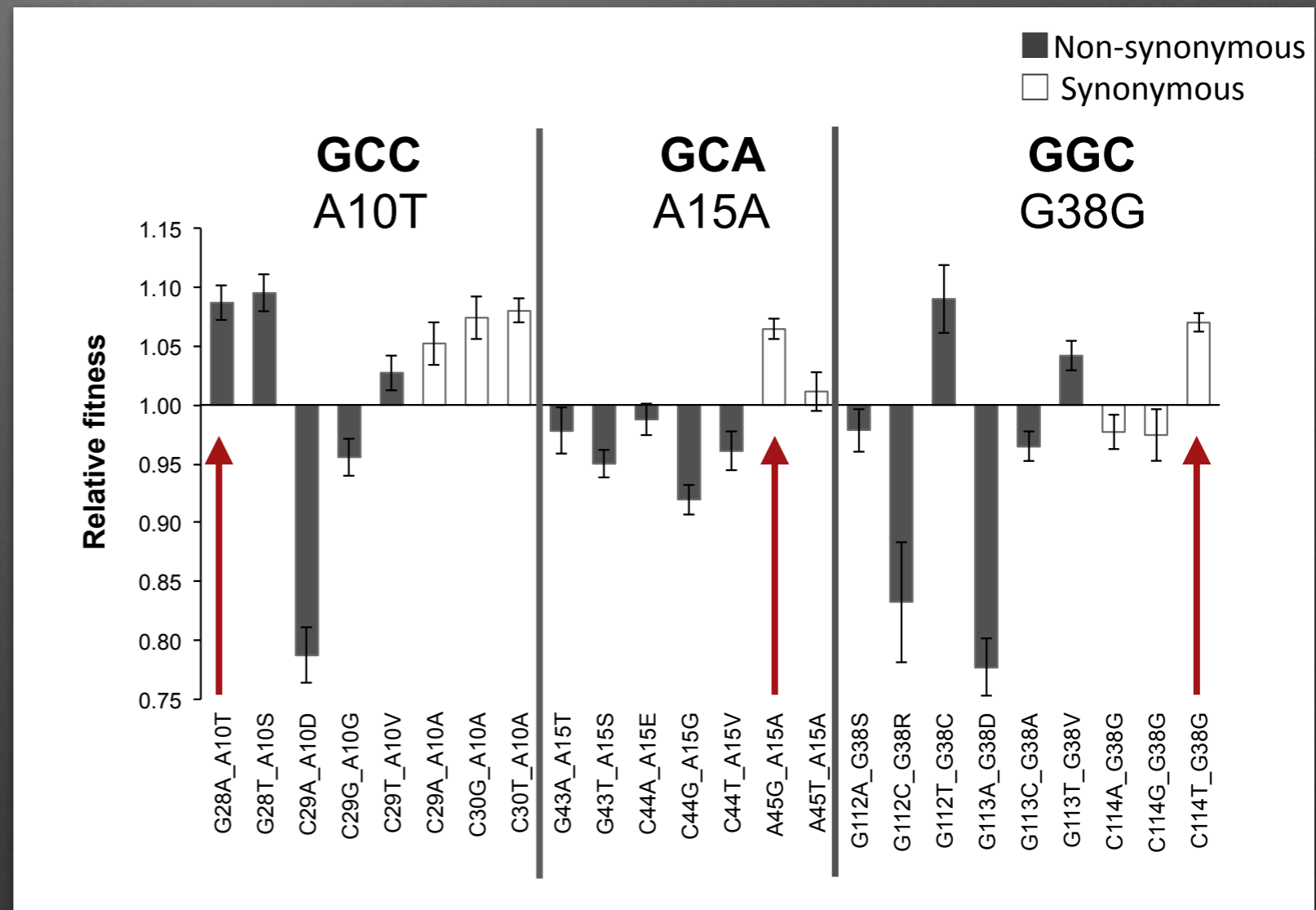


Adaptive synonymous mutations

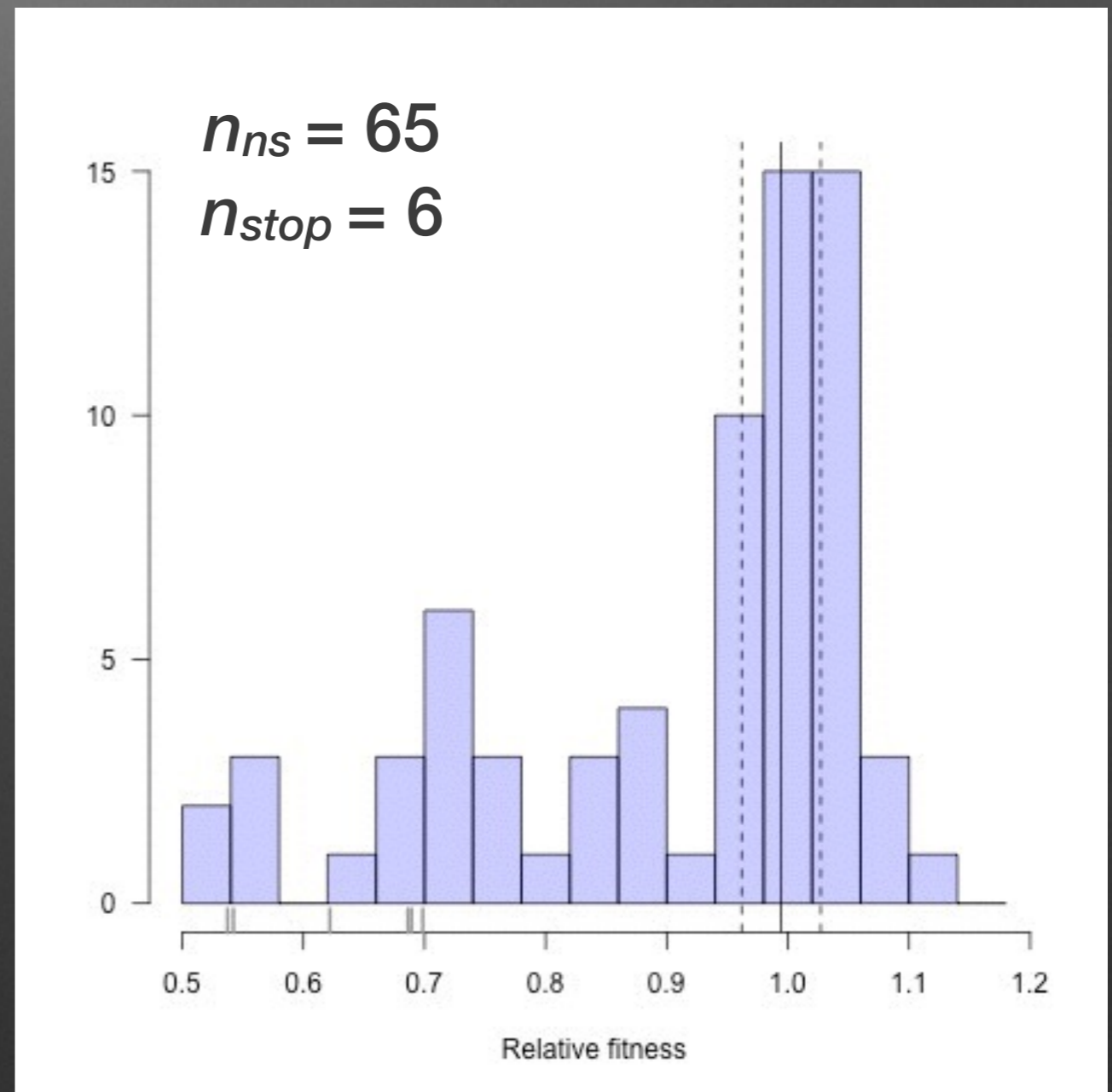
- Yes, they can happen.
- They are not unusual in terms of their effect on fitness
- The mechanism involves increased gene expression and (probably) increased transport rates for glucose into the cell
- Unlikely to be idiosyncratic, since they are present in natural isolates of *Pseudomonas*

How often do synonymous mutations have non-neutral fitness effects?

- ‘Medium through-put’ site-directed mutagenesis using ‘Golden Gate’ assembly
- Aim to saturate every 10th AA with all single-nucleotide mutations
- Measure fitness in head-to-head competitions with wt

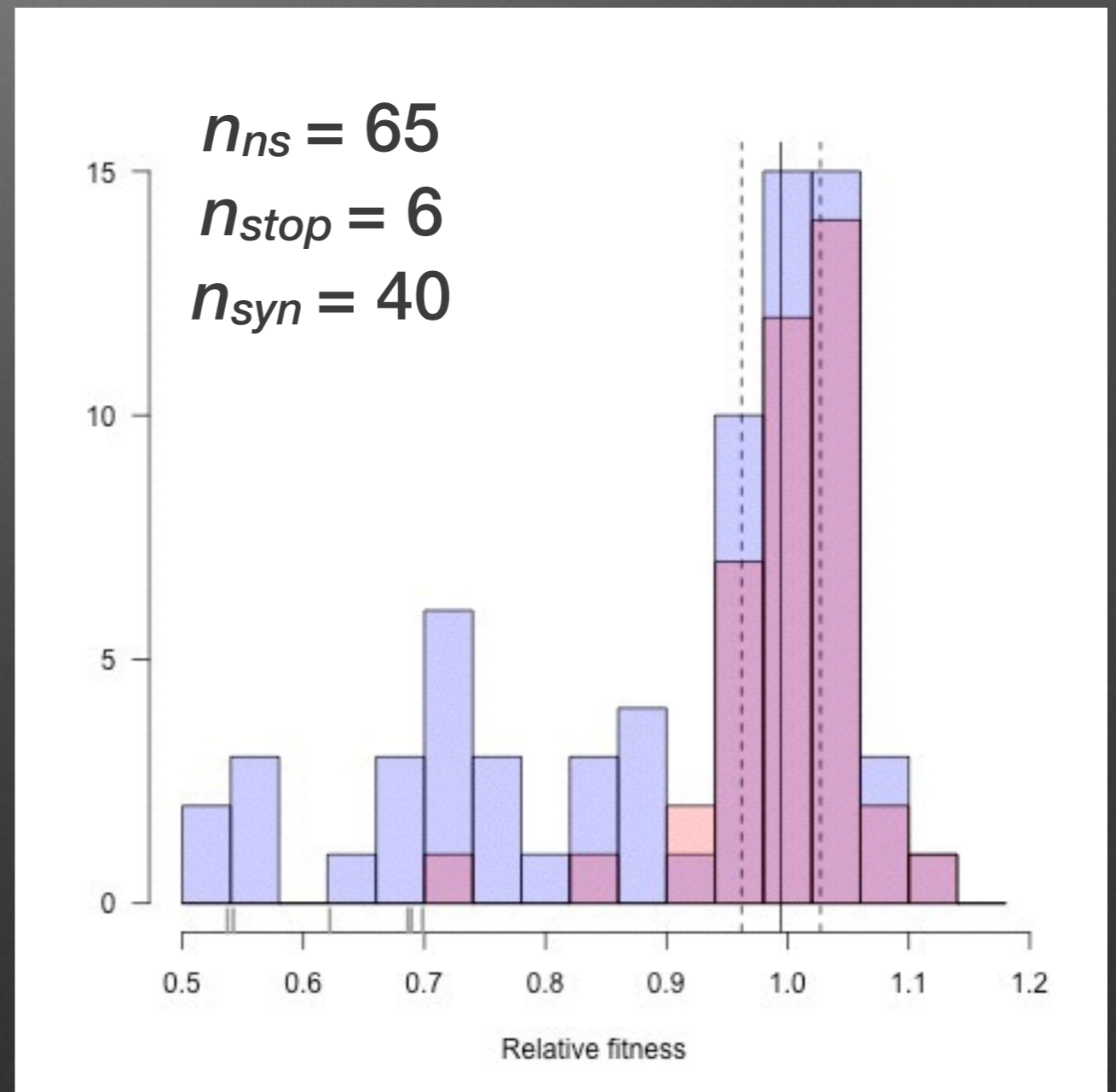


Distribution of fitness effects for NS and STOP mutations

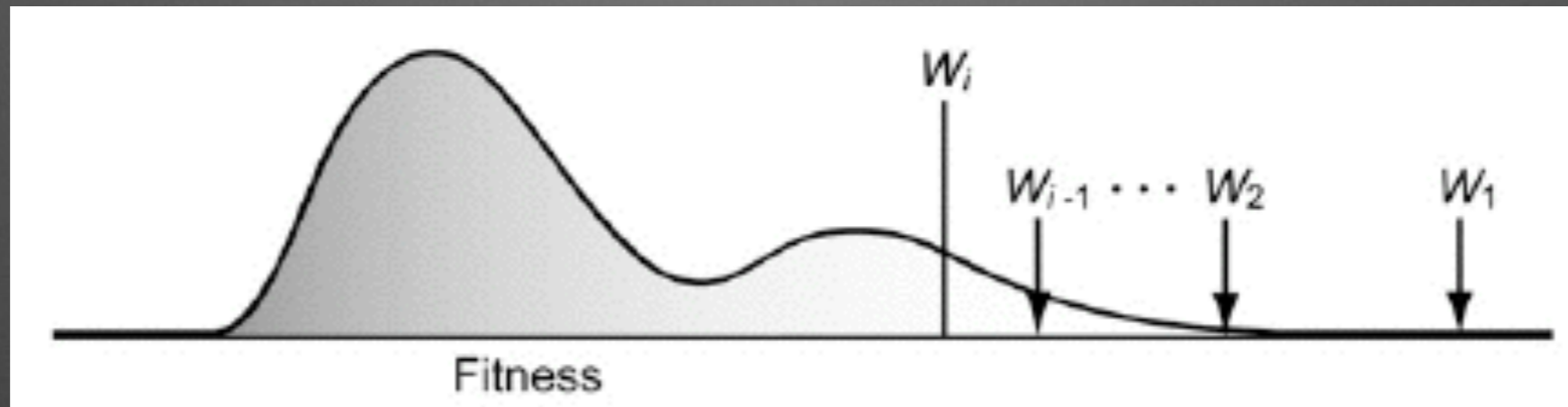


Distribution of fitness effects for synonymous mutations

- Permutation tests of Kolmogorov-Smirnov goodness-of-fit btwn NS & S:
 - All mutations: $P = 0.004$
 - Beneficial mutations only: $P = 0.702$

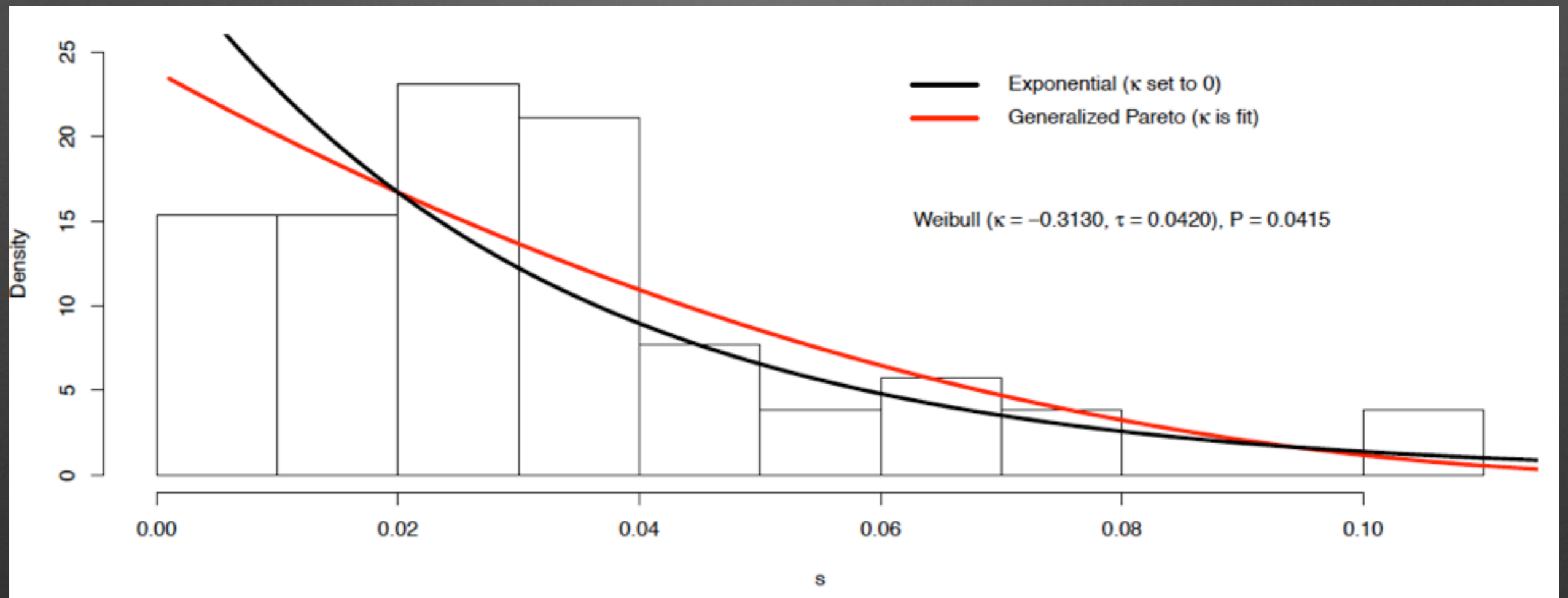


What does natural selection 'see' for beneficial mutations?



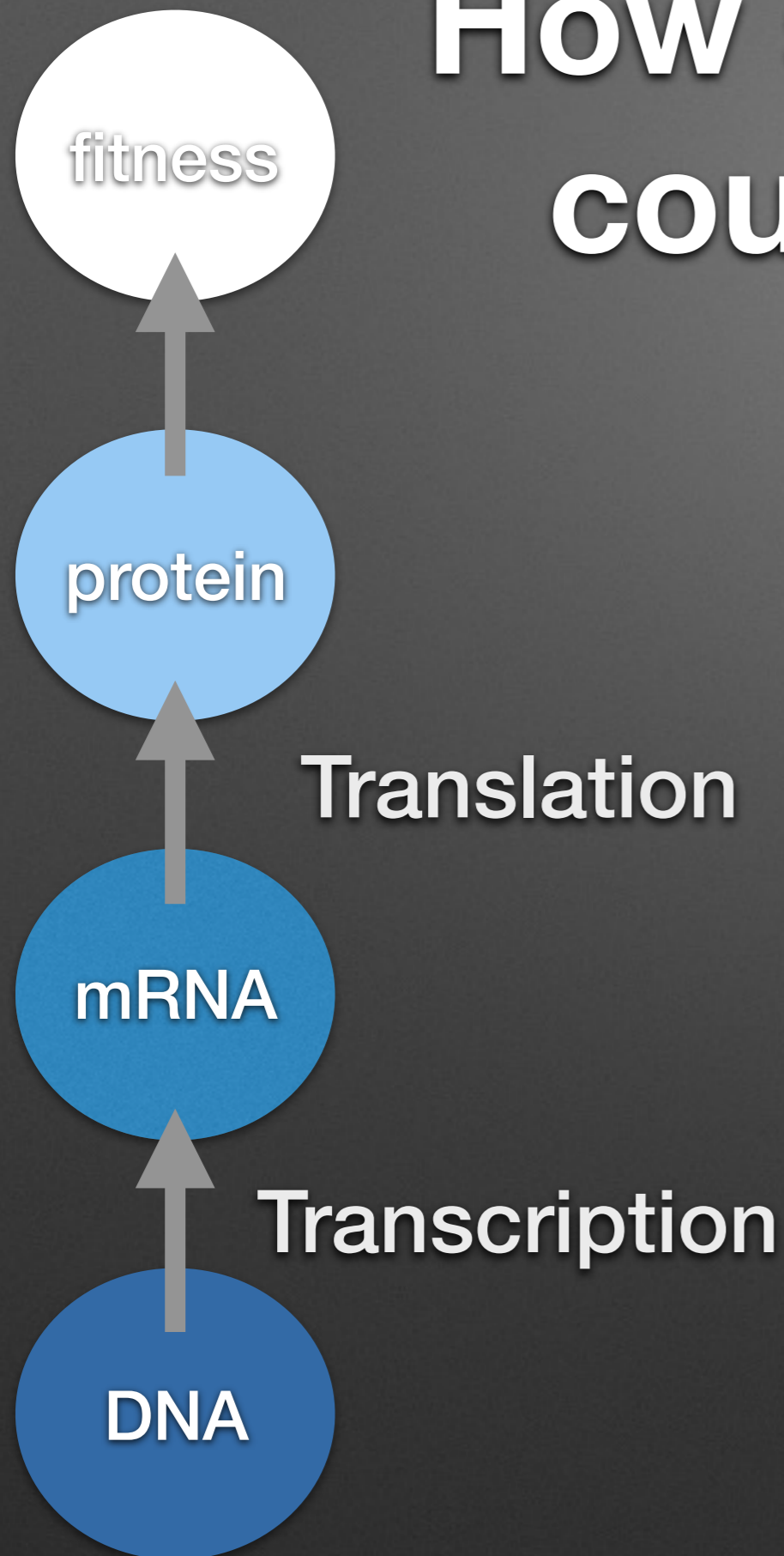
- Beneficial mutations can be considered draws from the right-hand tail of a distribution of fitness effects (DFE)
- ie - typically most mutations have small effects, few have large effects
- More technically: a generalized Pareto distribution with three possible domains of attraction: Gumbel (exponential); Weibull (truncated); Frechet (heavy-tailed)

DFE for beneficial mutations (NS & S) is in the Weibull domain



Using the framework of Beisel et al. (2007) *Genetics* 176: 2441

How synonymous SNPs could impact fitness

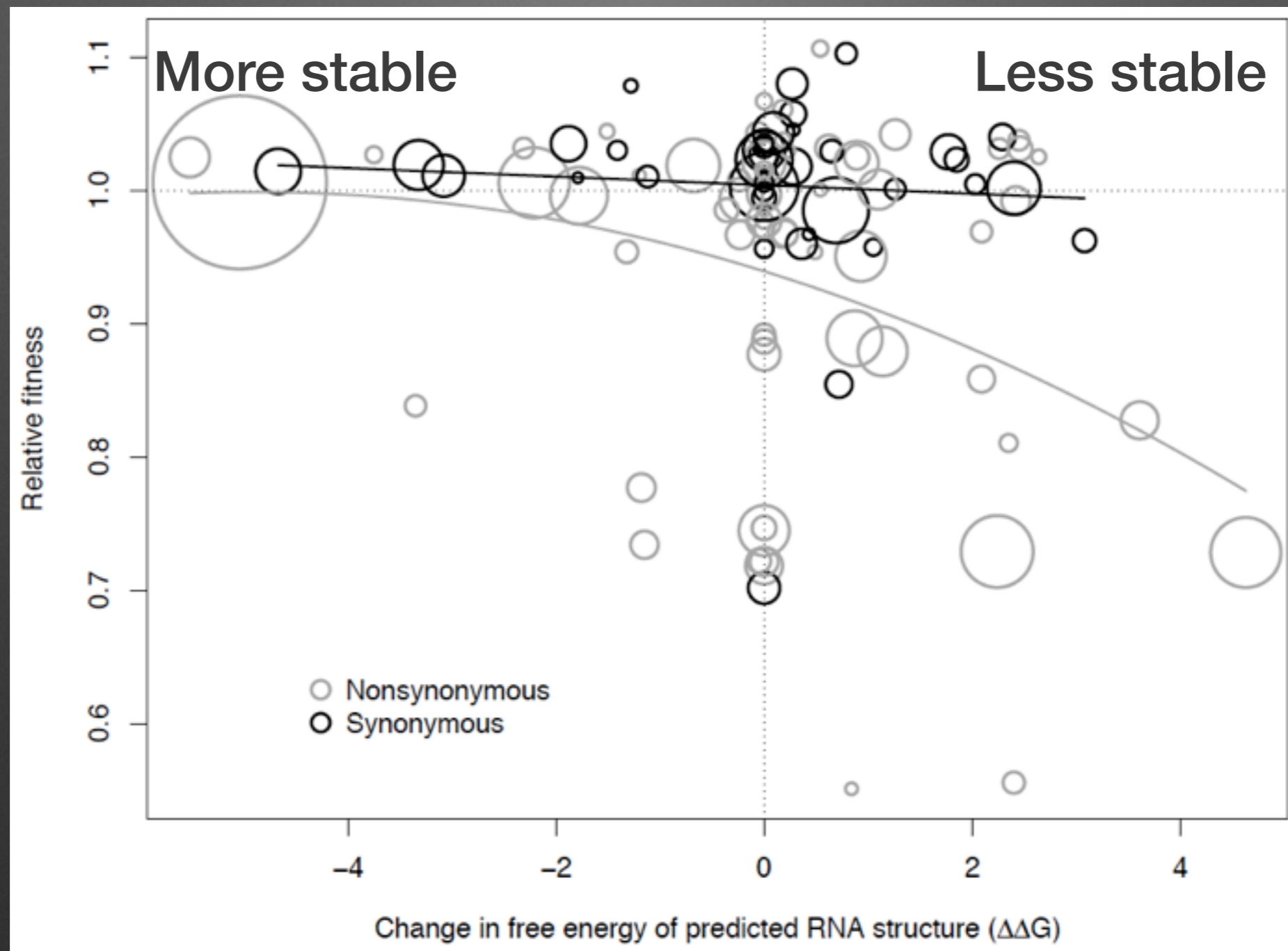


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mRNA stability emerges as the best of a bad lot, but...

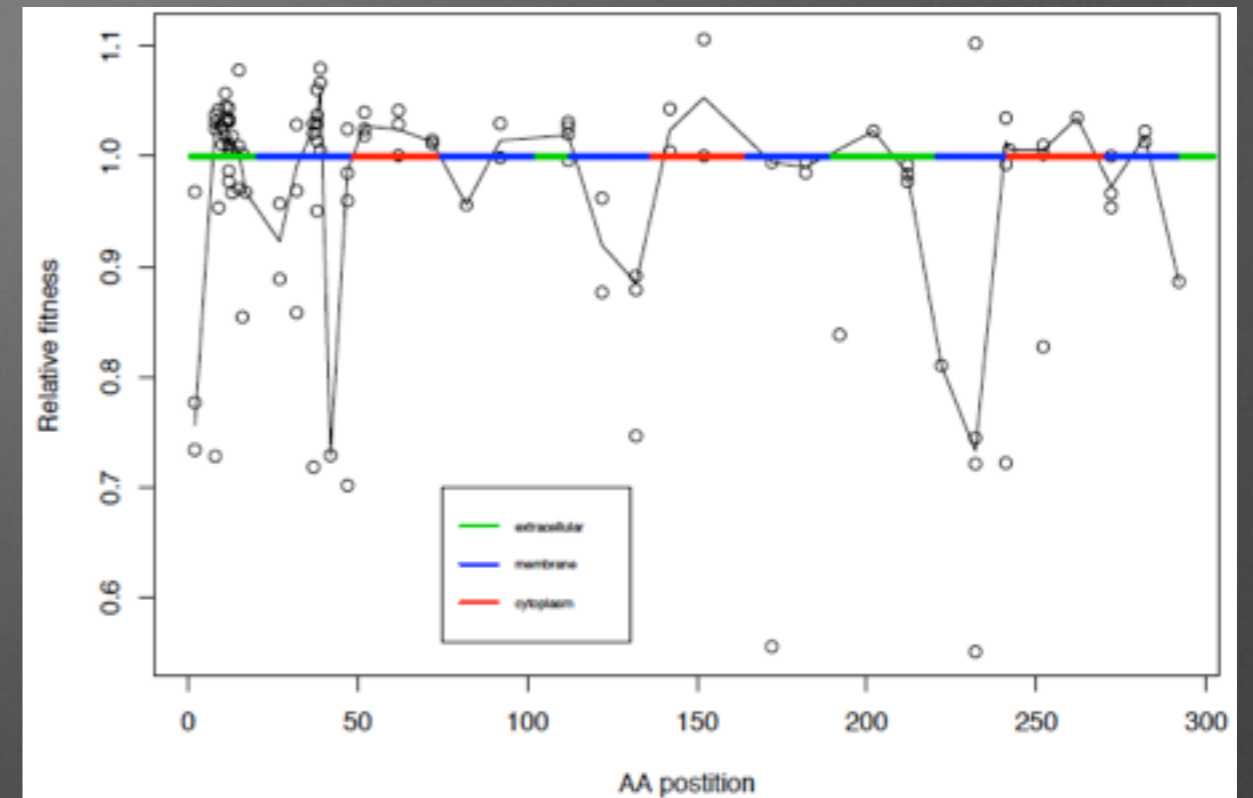
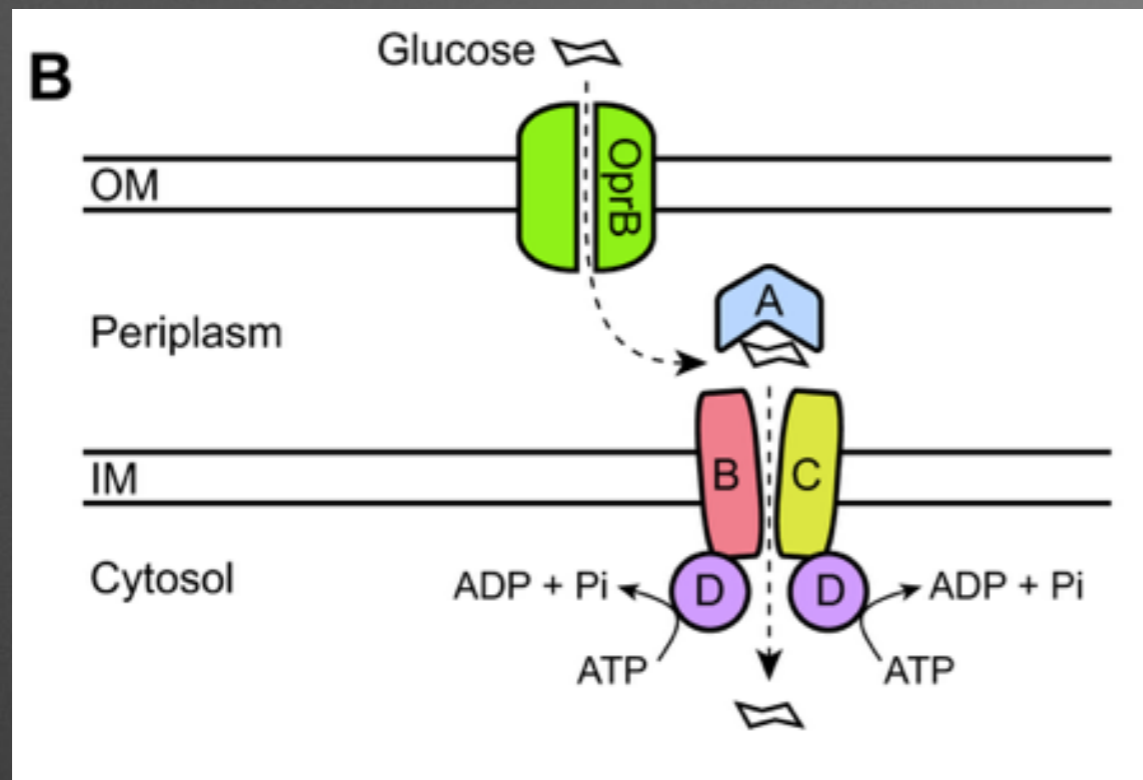
Effect	P-value	R ²
Mean <i>w</i> (Syn vs NS)	0.007	0.077
Transition - transversion	0.947	<0.0001
AA position	0.093	0.025
Change in codon bias	0.245	0.017
<i>mRNA stability</i>	0.004	0.106

...it cannot explain fitness variation among synonymous mutants

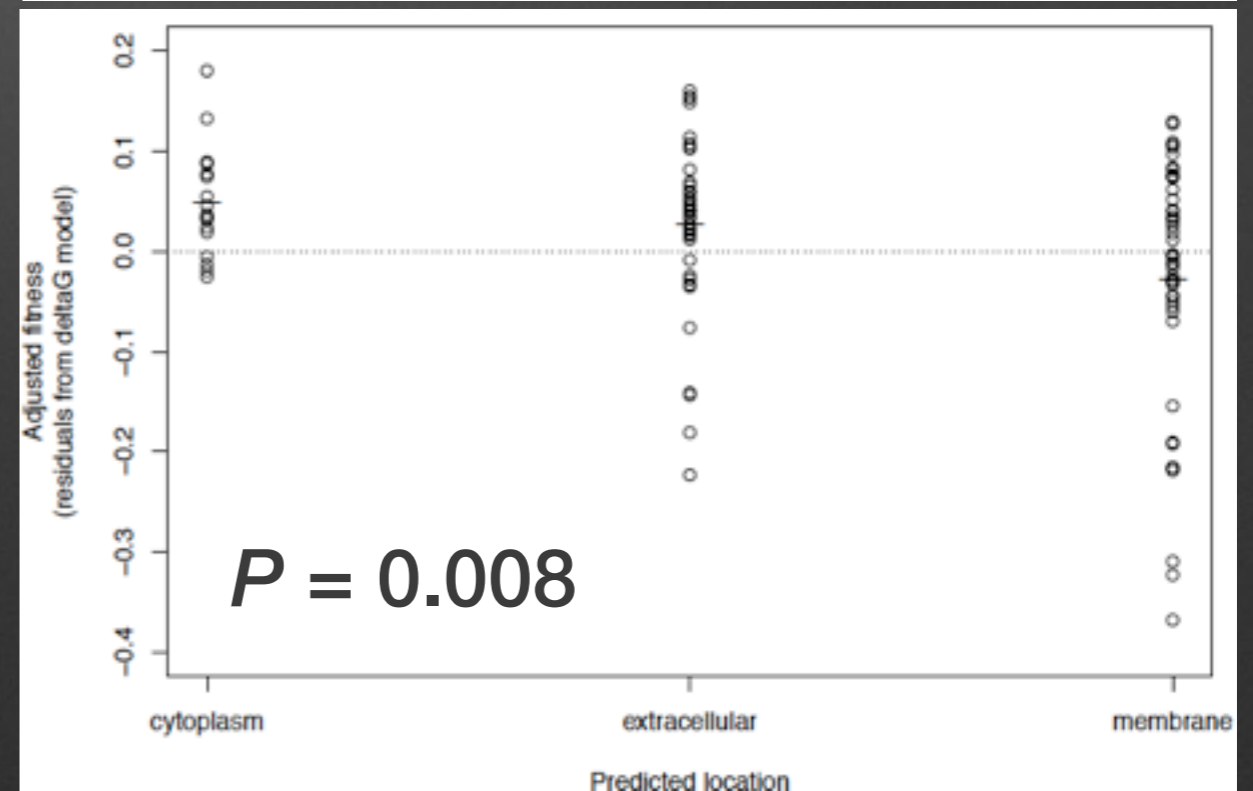


Size of circles are proportional to $1/se$

A structural interpretation?



- Predicted protein structure obtained from Phyre2
- No significant difference in secondary structure (alpha helix, beta-sheet, etc...)

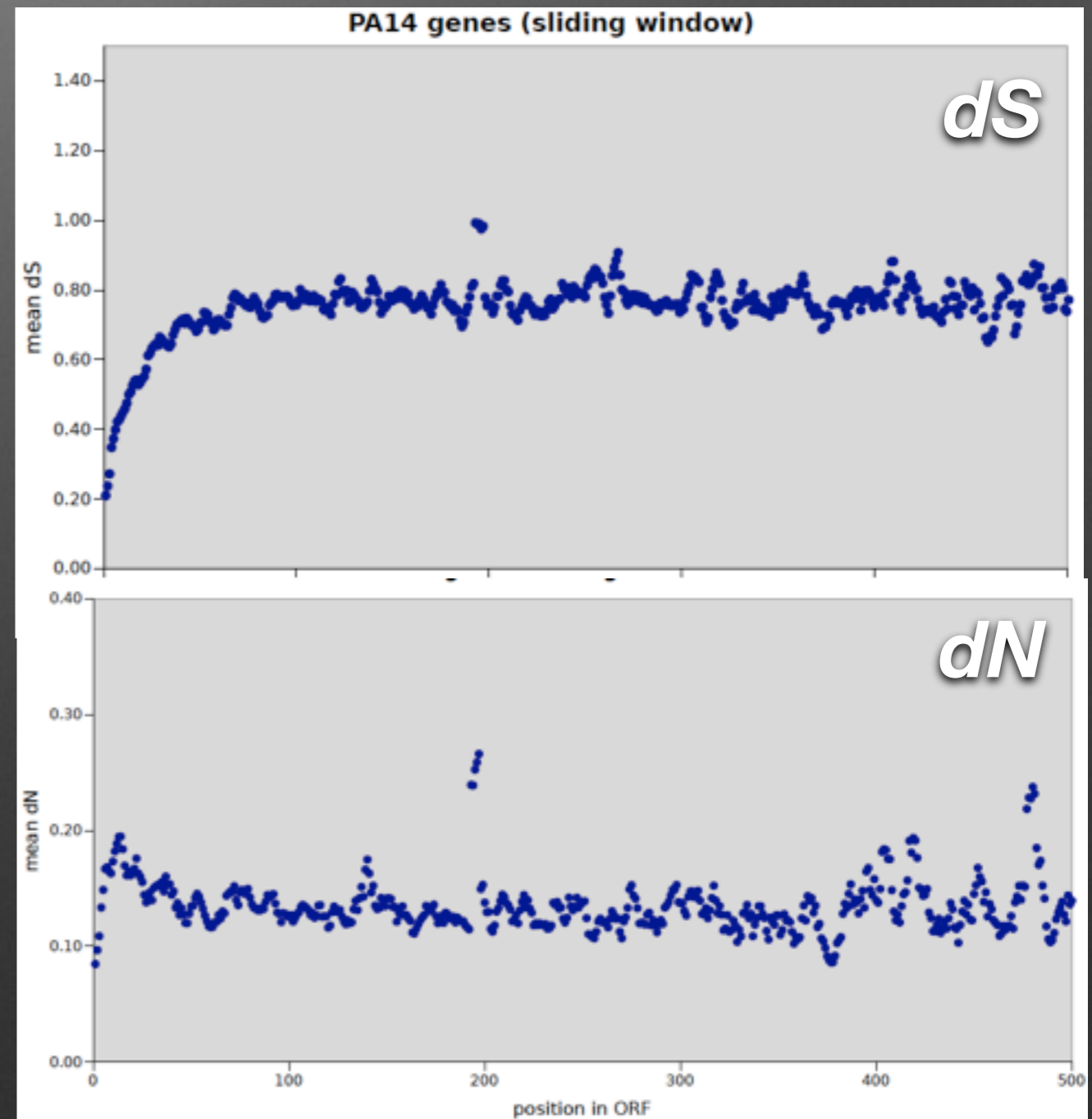


Variation in fitness effects of synonymous mutations

- Synonymous mutations have a DFE that, aside from the absence of stop codons, is indistinguishable from that of nonsynonymous mutations
- ‘Conventional’ mechanisms cannot explain the observed variation in fitness
- But protein structure and location seems to play a role

A final thought on the generality of synonymous mutations being under selection

- Codon-based estimates of dS & dN in reading frames of *P. aeruginosa*
- ~270 strains from chronic infections, acute infections, and the environment
- SLAC method in HYPHY
- Suggests strong purifying selection in the first ~50 codons of most genes



Sounds of silence

- Synonymous mutations can sometimes be beneficial and contribute to adaptation
- They can also be deleterious, though not as deleterious as some non-synonymous mutations
- Gene expression is key, though the underlying mechanisms remain unclear and probably diverse
- How common is selection on synonymous sites, and how often are they beneficial? We shall see...

Thanks to...



Susan Bailey
former PhD, now Assist
Prof, Clarkson U



Nick
McCloskey
MSc



Eleonore
Leboeuf-Taylor
MSc



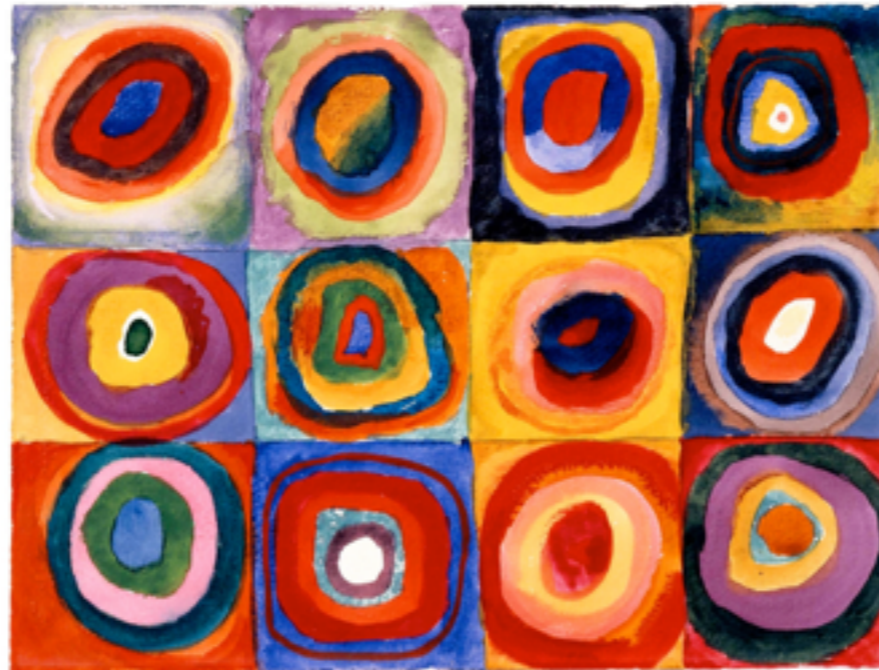
Aaron Hinz
PDF



Jeremy Dettman
Agriculture and Agri-food
Canada



EXPERIMENTAL
EVOLUTION and the
Nature of Biodiversity



Rees Kassen

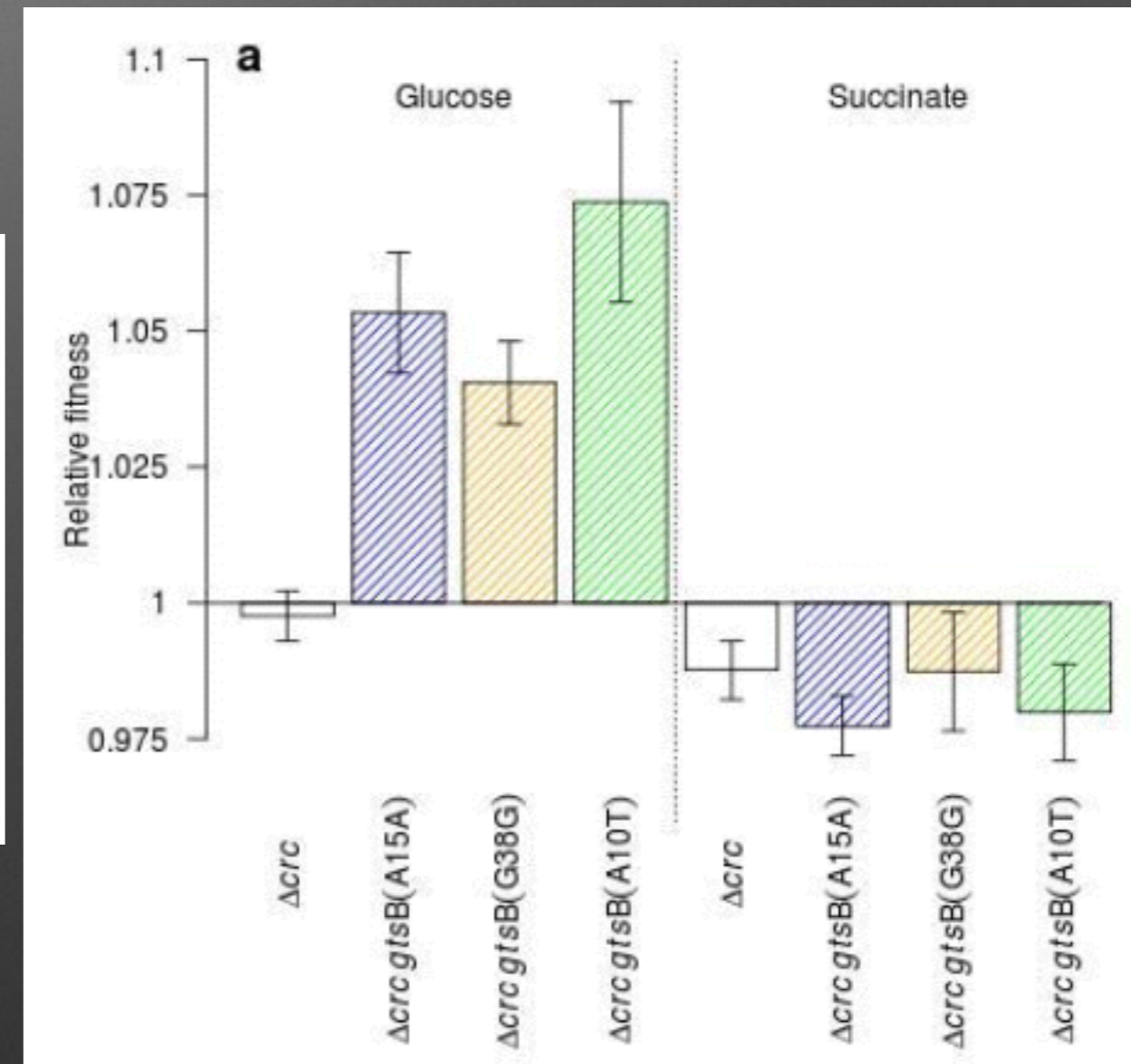
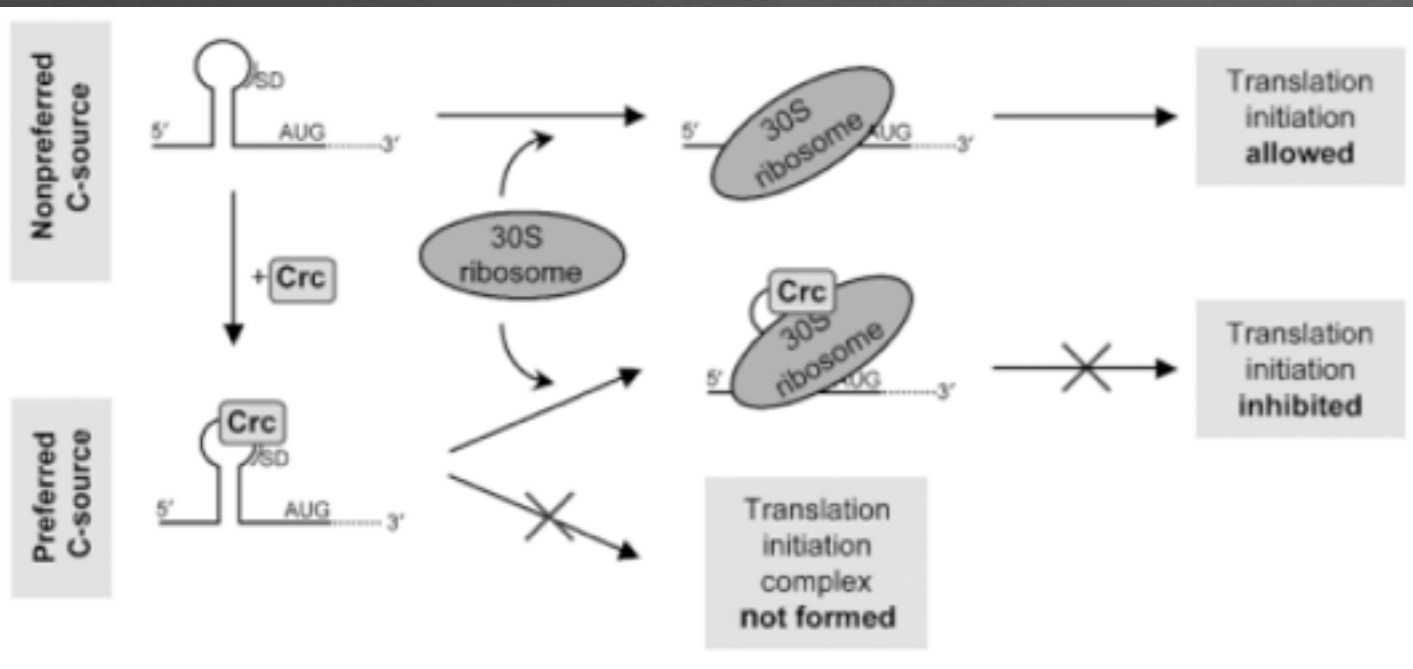
Using little things to answer big questions

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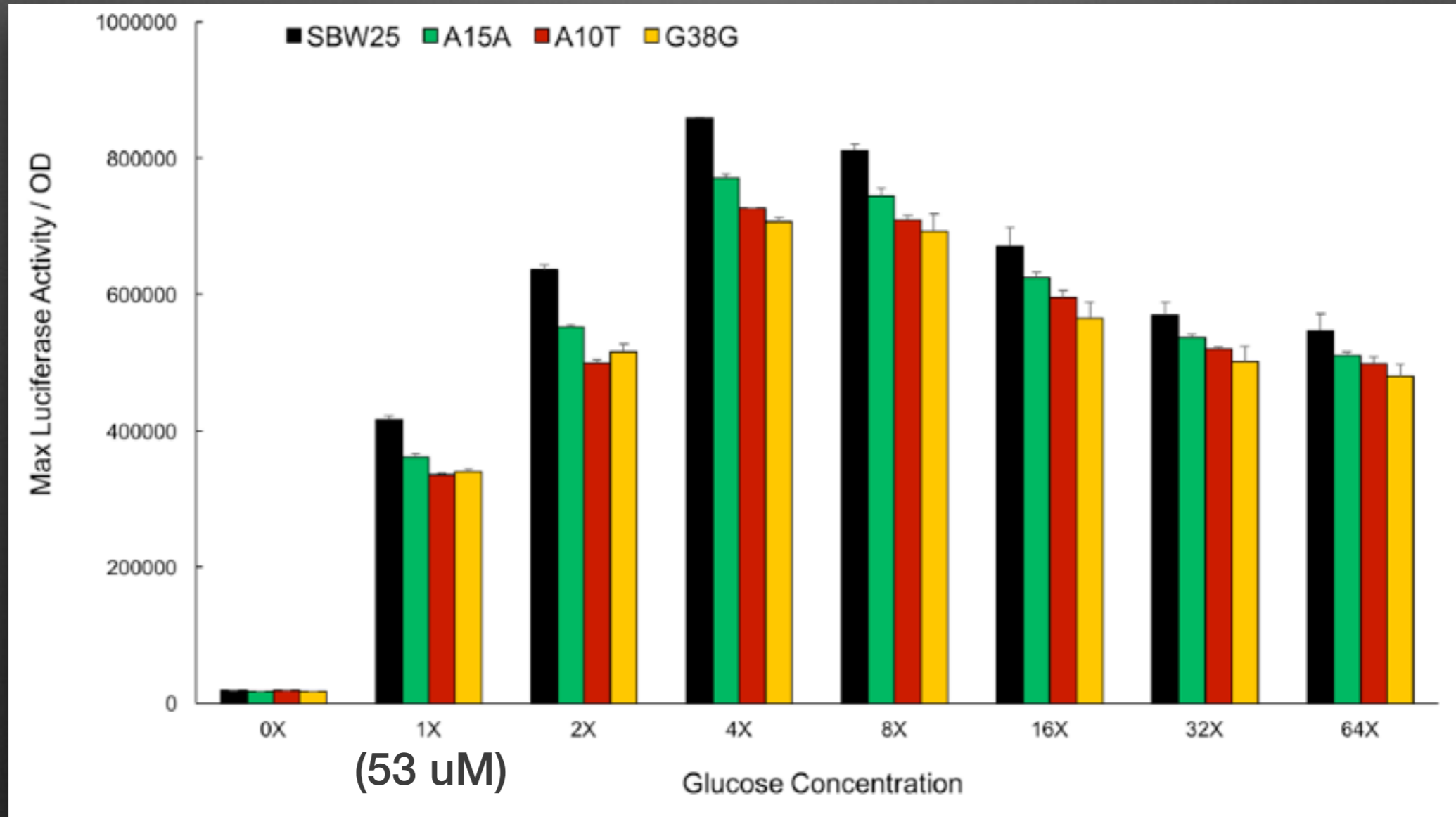
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(2014) Roberts and Co., Publishers

Catabolite repression: deleting *Crc* does not increase fitness of wt



Glucose is depleted faster in mutant strains



Measuring transcription rates using luciferase fusions

- *luxA-E* are co-transcribed with PFLU4845

