

Announcements:

① Advance copy of notes on canvas

② Final project groups & topics due this Friday (March 7)

(final writeups due 11:59 pm Friday March 21)

⇒ if looking for group, check out google doc
(link in Canvas announcement)

⇒ will save a few mins @ end of class today.

Last time: molecular errors & kinetic proofreading

Simple example:

① Irreversible step (e.g. ATP)



$$\frac{1}{k_{off}} = k_{on} \cdot e^{\frac{\Delta G_A}{kT}}$$

② "off ramp"

$$\Rightarrow \frac{\text{rate of error product}}{\text{rate of correct product}} = \frac{[B]}{[A]} \left(e^{\frac{\Delta G_A - \Delta G_B}{kT}} \right)^2$$

irreversible step buys time to "test" k_{off} twice!

Tradeoffs:

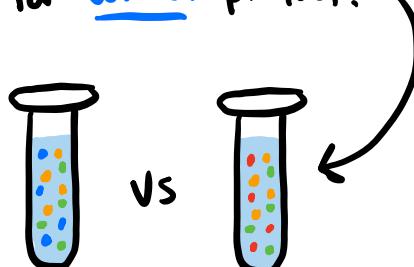
① "wastes" energy

② slower / lower throughput

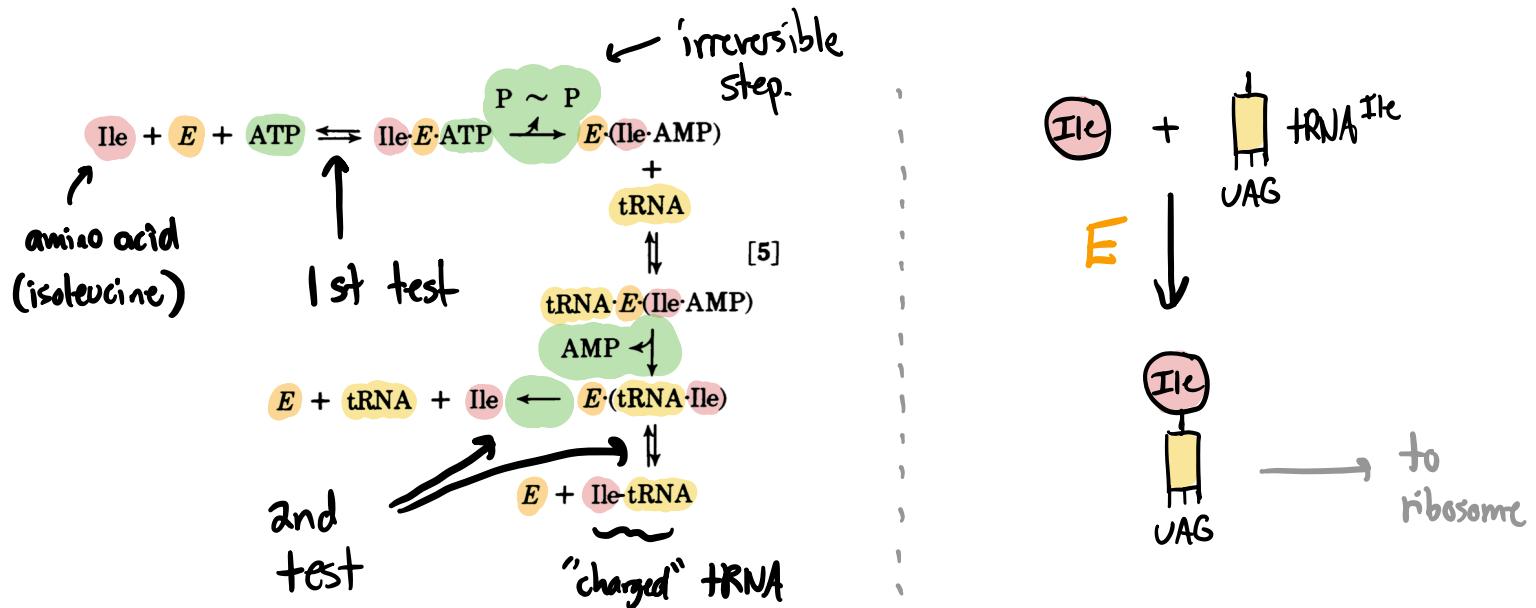
\Rightarrow Hopfield et al '76: test for kinetic proofreading

$$\frac{\text{rate of energy use}}{\text{rate of product}} = 1 + \frac{k_{off}}{k_{cat}}$$

→ should be larger for errors than for correct product!



Hoogfield et al '76: applications to tRNA charging



In vitro measurements: Ile (correct) : ~1.5 ATP per product
 Val (error!) : ~270 ATP "

\Rightarrow error is $\sim 270 \times$ more likely to dissociate than form product
 (vs $1.5 \times$ for correct amino acid \Rightarrow $\sim 30\%$ "wasted")

\Rightarrow decreases error rate by extra factor of $\frac{270}{1.5} = 180$

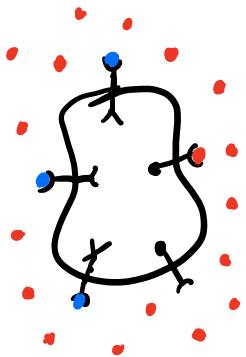
\Rightarrow initial error rate ($\text{Ile} + E + \text{ATP} \rightleftharpoons \text{Ile}\cdot E\cdot \text{ATP}$) $\sim 1/100$

\Rightarrow total error rate $= \frac{1}{100} \times \frac{1}{180} = 5 \times 10^{-5} \text{ } \ast$

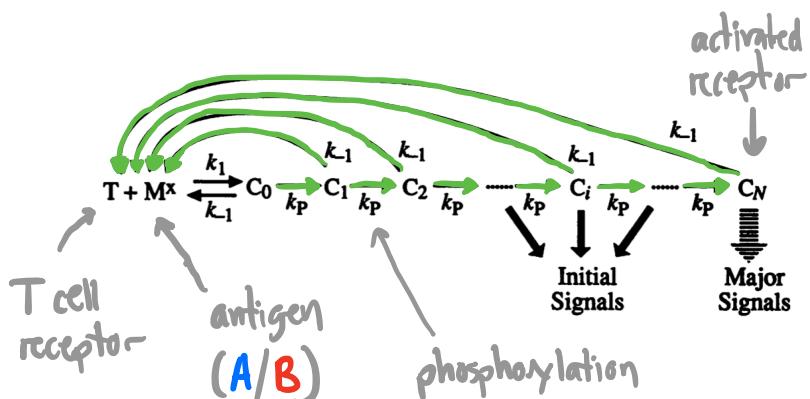
⇒ general idea can be extended to many other contexts

E.g. T-cell receptor signaling in immune system

Problem: how to detect low conc. of **foreign antigens** in sea of weakly binding **self-antigens**?



⇒ McKeithan '95: proofreading via multiple phosphorylation steps?

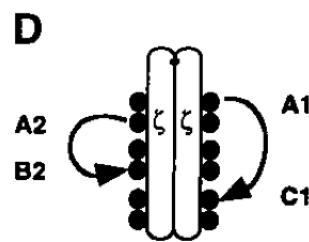


$$\frac{C_N^B}{C_N^A} = \frac{[B]}{[A]} \left(\frac{k_p + K_i^A}{k_p + K_{-1}^B} \right)^{N+1}$$

Fidelity of T Cell Activation Through Multistep T Cell Receptor ζ Phosphorylation

Ellen Neumeister Kersh, Andrey S. Shaw, Paul M. Allen*

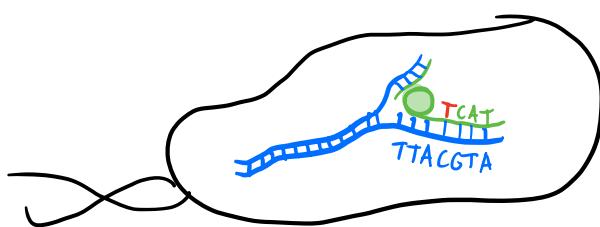
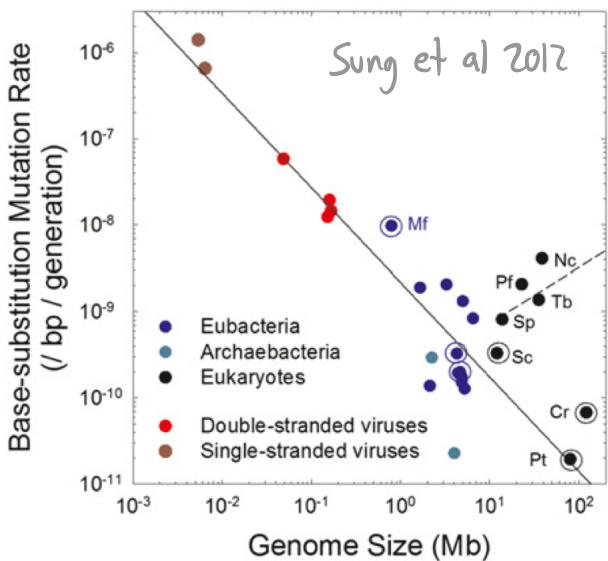
The T cell receptor (TCR) $\alpha\beta$ heterodimer interacts with its ligands with high specificity, but surprisingly low affinity. The role of the ζ component of the murine TCR in contributing to the fidelity of antigen recognition was examined. With sequence-specific phosphotyrosine antibodies, it was found that ζ undergoes a series of ordered phosphorylation events upon TCR engagement. Completion of phosphorylation steps is dependent on the nature of the TCR ligand. Thus, the phosphorylation steps establish thresholds for T cell activation.



Phosphorylation sites
on ζ component of TCR

(n = 6)

⇒ Started w/ discussion of errors during DNA replication...



⇒ what do real cells do?

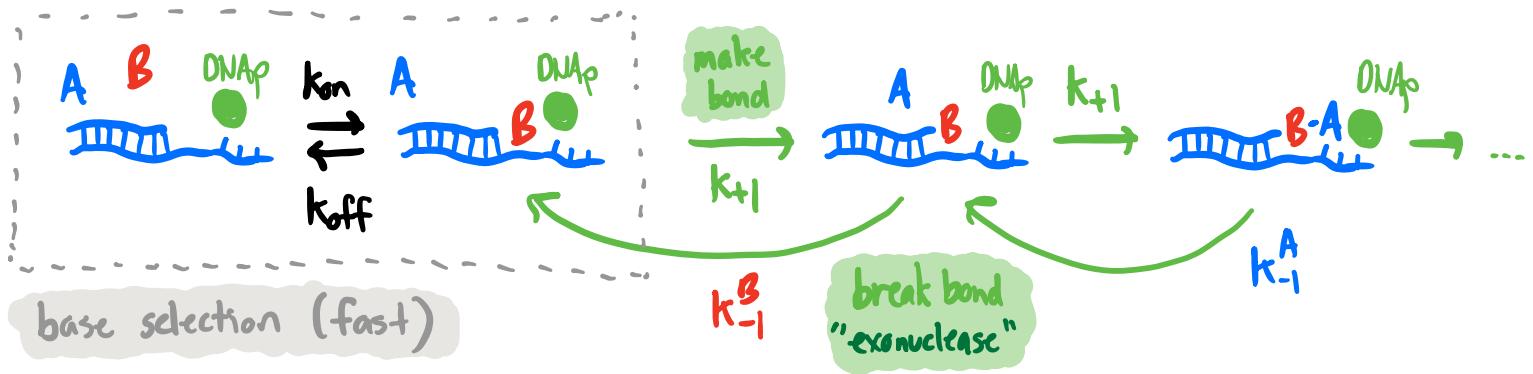
⇒ a little more complicated than above...

⇒ some key factors:

① DNAP can modify ΔG relative to value in sol'n (e.g. exclude H₂O)

(see e.g. Petruska et al '86)

② Many DNAPs also use "exonuclease proofreading"



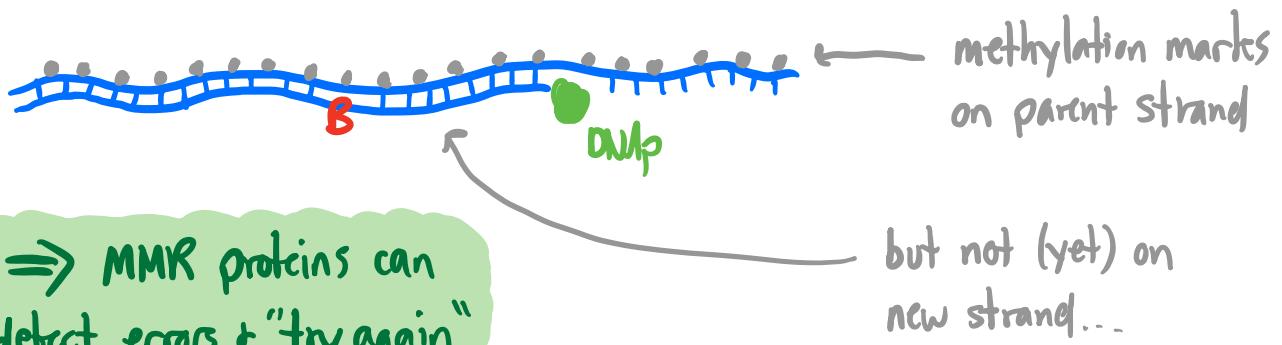
⇒ not technically kinetic proofreading, but similar idea:

(i) Irreversible step + "off ramp" can enhance specificity

(ii) Tradeoffs = "wasted" energy + reduced processivity

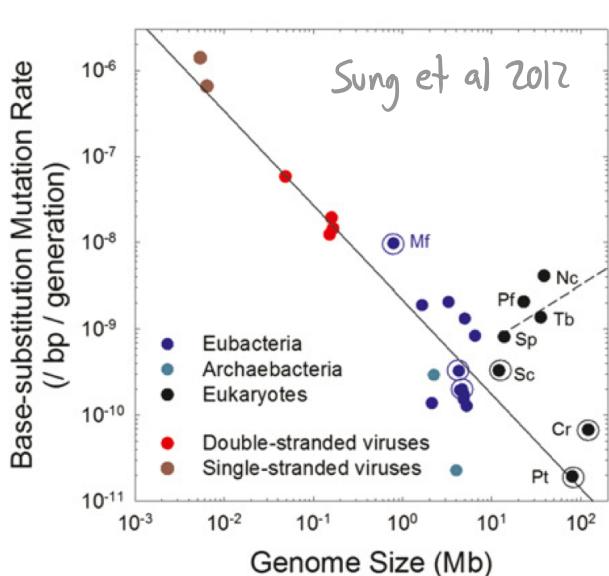
③ Additional error correction mechanisms beyond DNAP

E.g. mismatch repair (MMR) pathway



knocking out these genes can increase mutation rates ~10-1000x

Upshot: cells have evolved multiple layers of machinery to reduce mutation rates below $\exp(-\Delta G_0/kT)$



mut'n rate is under
genetic control &
can evolve over time!

Question: why these values? could evolution do "better"?

⇒ will see that physics (diffusion!) imposes fundamental limits on evolution as well...

⇒ systematic treatment in:

APPHYS 237/BIO 251:
"Quantitative evolutionary dynamics + genomics"

offered
⇒ next spring
(Spring 2026)

⇒ will outline basic flavor here...

Question: why would evolution select for lower mut'n rates?

⇒ one possibility: fewer lethal mutations

e.g. E. coli genome: 

↳ "essential" sites
(mutation = death)

⇒ probability of viable offspring = $1 - \mu L P_x$

μ = mutation rate / bp/division ($\sim 10^{-10}$ in E.coli)

L = length of genome ($\sim 4 \times 10^6$ in E.coli)

P_x = prob. that mut'n is "lethal" ($\sim 10^{-6} - 10^{-9}$ in E.coli)

"error catastrophe"

⇒ Upper bound on mut'n rates: $\mu < 1 / L P_x$

$$\sim \frac{1}{4 \times 10^6 \times 10^{-6}} \sim 2 \times 10^{-5}$$

⇒ but observed values much lower...

key insight: rare lethal mut'n's still impose weak fitness costs that can add up over time...

E.g. E.coli w/ "Super MMR" pathway ($\mu \rightarrow 0$)



$$\Pr[\text{viable offspring}] = 1$$



$$\Pr[\text{viable offspring}] = 1 - \underbrace{\mu L P_x}_{\approx 10^{-4}}$$

\Rightarrow after one division:

$$n_{SMMR} \rightarrow n_{SMMR} \times 2$$

$$n_{WT} \rightarrow n_{WT} \times 2(1 - \mu L P_x)$$

\Rightarrow after t divisions:

$$n_{SMMR} \rightarrow n_{SMMR} \times 2^t$$

$$n_{WT} \rightarrow n_{WT} \times [2(1 - \mu L P_x)]^t$$

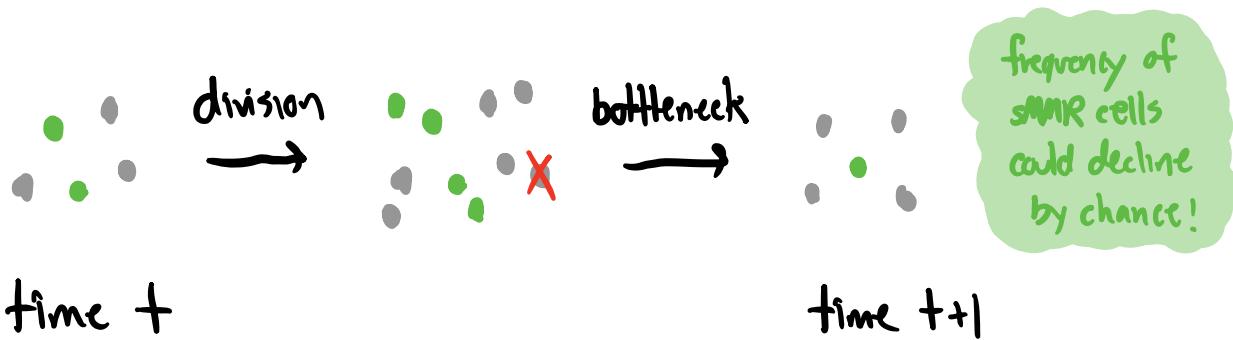
$$\Rightarrow \frac{n_{SMMR}(t)}{n_{WT}} \propto (1 - \mu L P_x)^{-t} \propto e^{\frac{10^{-4}}{\mu L P_x} t - \delta_s t}$$

i.e., the Super MMR strain has a small but steady advantage

\Rightarrow natural selection to lower mutation rates

One missing ingredient: population can't double forever...

⇒ e.g. finite resources / predation limits pop'n to size N.

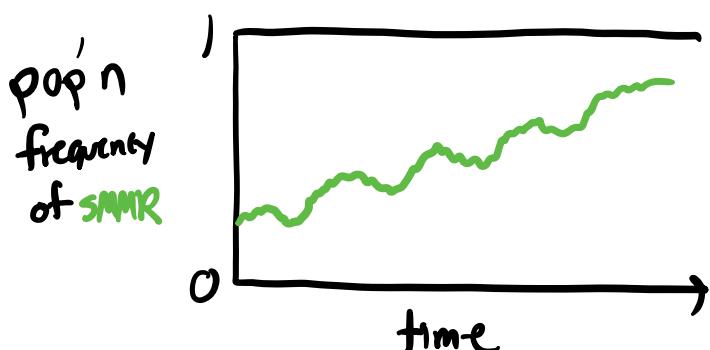


⇒ Simplest model (~"coin flipping")

$$n_{\text{SMNR}}(t+1) \sim \text{Binomial}\left(N, \frac{n_{\text{SMNR}}(t)}{n_{\text{SMNR}} + n_{\text{WT}}(1 - \mu L \rho_x)}\right)$$

⇒ or in terms of population frequency ($f \equiv n_{\text{SMNR}}/N$)

$$f(t+1) \equiv \frac{1}{N} \cdot \text{Binomial}\left(N, \frac{f(t)}{f + (1-f)(1 - \mu L \rho_x)}\right)$$



frequency of SMNR strain undergoes "random walk" / "Brownian motion"

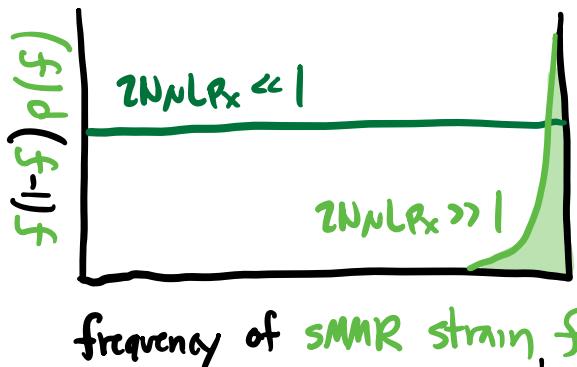
$$\Rightarrow \langle \Delta f \rangle \approx \mu L P_x f(1-f) \Leftrightarrow "F_f" \quad \text{e.g.-lectures 4&5...}$$

$$\text{Var}(\Delta f) \approx \frac{1}{N} f(1-f) \Leftrightarrow "2D"$$

Diffusion Equation: $\frac{\partial p(f,t)}{\partial t} = -\frac{\partial}{\partial f} \left[\underbrace{\mu L P_x f(1-f) p(f,t)}_{\text{natural selection ("}F_f\text{")}} + \frac{\partial^2}{\partial f^2} \left[\underbrace{\frac{f(1-f)}{2N} p(f,t)}_{\text{genetic drift ("}D=\frac{kT}{f}\text{")}} \right] \right]$

New ingredient: diffusion const depends on f !

$$\Rightarrow @ \text{"equilibrium": } \overbrace{f(1-f)p(f)}^{c(f)} \propto e^{2N \cdot \mu L P_x \cdot f}$$



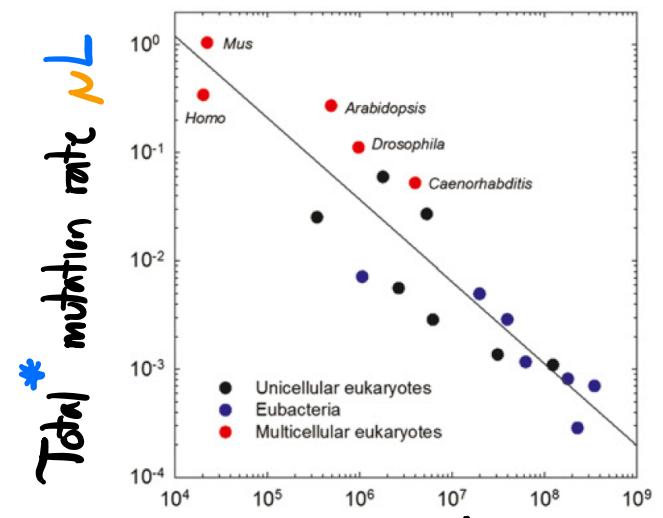
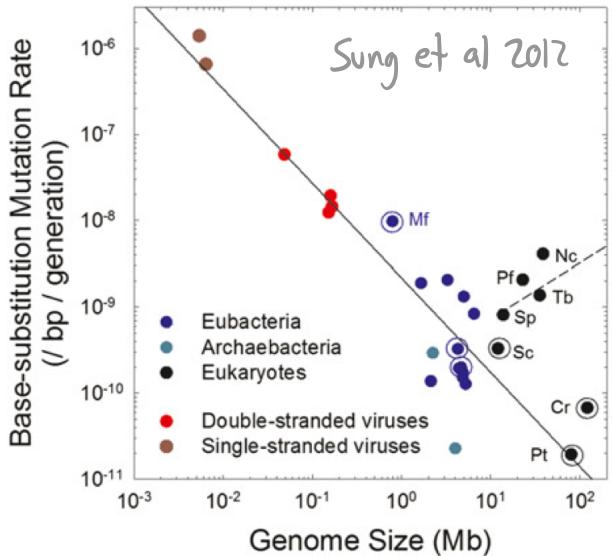
$-\mu L P_x f \leftrightarrow \text{"energy"}$
 $2N \leftrightarrow 1/kT$

analogue of
Boltzmann distribution

\Rightarrow natural selection is only effective if $N\mu L P_x \gg 1$

\Rightarrow fundamental limit to optimization
of mut'n rates $\mu L \gtrsim 1/N$ ("drift barrier")

\Rightarrow Do real organisms approach this limit?

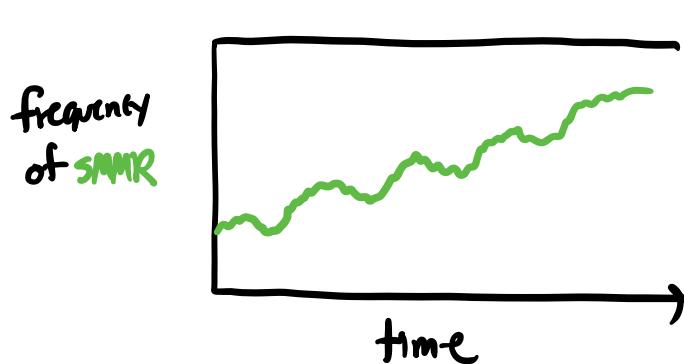


Estimated N

important caveats here...



Drift barrier = one hypothesis for explaining scaling of mutation rates across species...



Intimately connected
to physics of diffusion!

\Rightarrow for additional considerations, see Good & Desai (2016)
(on canvas)

↓
e.g. higher mut'n rates
provide benefits?