The evolution of mutation rates in DNA polymerases

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Question: How did mutation rates get so low?

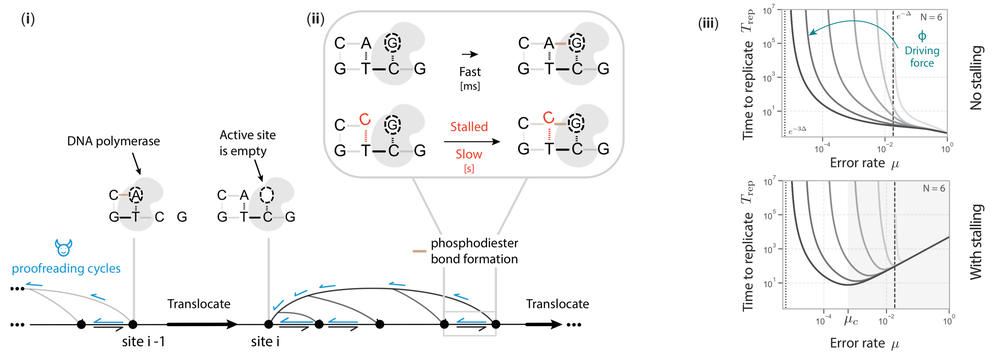
	Mammal	Yeast	Bacteria	Viruses		Parasitic plasmids	
	H. sapiens	*S. cerevisiae*	*E. coli*	HSV-1	T2	pGKL-1	pGKL-2
Mutation rate (subst. per bp)	10^{-10}	10^{-9}	10^{-9}	10^{-8}	10^{-7}	10^{-9}	10^{-9}
Genome size (bp)	10^{9}	10^{7}	10^{6}	10^{5}	10^{5}	10^{4}	10^{4}

How? Kinetic proofreading: non-equilibrium error correction

Why? Traditional explanation: selection against deleterious load

Today, an alternative: low mutation rates as consequence of selecting for ***speed***

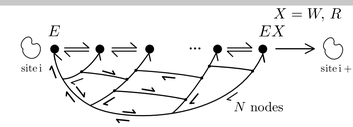
Theory: Stalling of replication at mismatches inverts speed-accuracy trade-offs

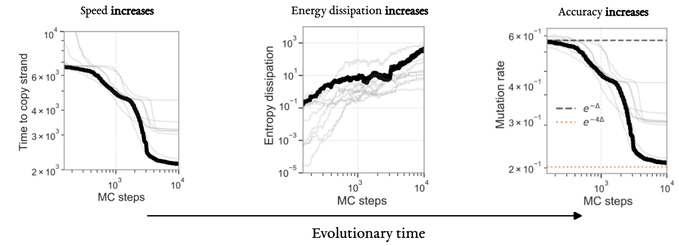


Theory: selection for speed alone lowers mutation rates

Initialise a random Markov network at equilibrium (i.e. detailed balance)

Evolve to minimise the time to copy a strand.





Abstract

The high-fidelity of DNA replication is ensured by kinetic proofreading, a baroque non-equilibrium error-correction scheme implemented by DNA polymerases across the tree of life. Proofreading is typically assumed to have evolved via selection against deleterious load, over associated costs in energy and time.

In contrast, here we test the **counterintuitive idea** that fidelity can evolve simply due to selection for faster replication, without any selection against deleterious load.

Using methods from non-equilibrium statistical mechanics, we study correlations between the fidelity and processivity of DNA replication. Counterintuitively, we find that highly mutagenic polymerases are slow and unprocessive, while fast polymerases are highly accurate. Consequently, evolutionary selection for processivity alone could give rise to the high fidelity of DNA replication.

To test this hypothesis, we are developing a high-throughput Luria-Delbruck assay to measure the mutation rate and processivity of thousands of polymerase variants in a single experiment. We will select variants for speed alone, and measure the resultant evolution of mutation rates. Our work highlights the importance of molecular pleiotropy and biophysical constraints on the evolution of mutation rates, and sheds light on the origins of the high fidelity of biological processes.

We are studying the evolution of error correction in DNA replication. Surprisingly, we find that ***faster*** polymerases are also more ***accurate***, suggesting that low mutation rates can evolve simply by selecting for speed.

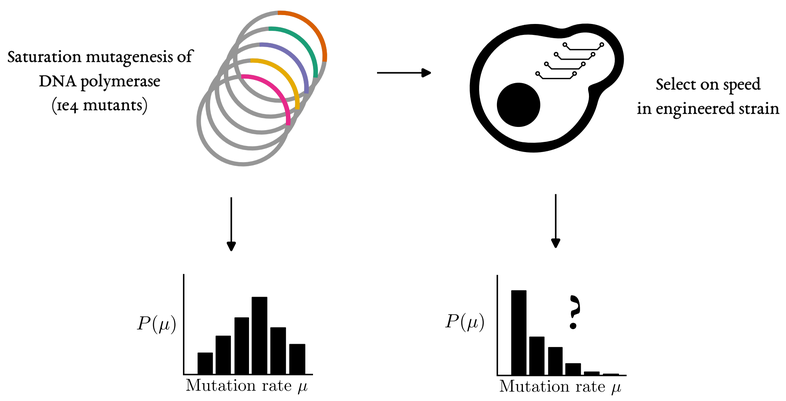
To test this, we have developed a system to orthogonally select on the speed and fidelity of DNA replication. At the same time, we have innovated on the classic Luria and Delbruck experiment to build an assay to measure thousands of mutation rates in a single experiment.

We are now building a saturation mutagenesis library of a DNA polymerase to test whether selection for replication speed alone can maintain the high fidelity of DNA replication.

Experiment: *OrthoRep*, an orthogonal replication system in yeast

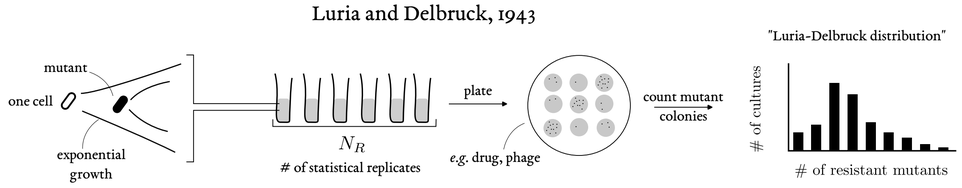
p1: a small virus inside yeast
replicated by a dedicated DNA polymerase

Experiment: Saturation mutagenesis of a DNA polymerase

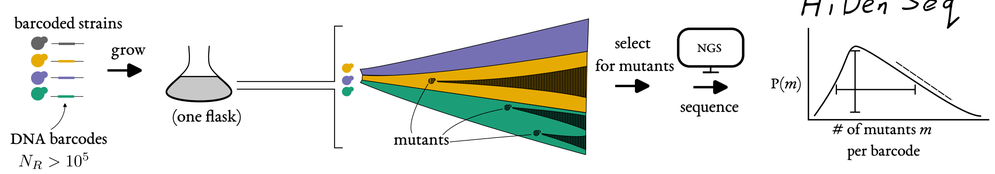


Experiment: Measuring thousands of mutation rates with a barcoded Luria-Delbruck assay

Luria and Delbruck, 1943



Us, 2020s



Preliminary data:
resolving the Luria-Delbruck distribution on log scales

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Acknowledgements

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Preprints:

(more to come!)

A minimal scenario for the origin of non-equilibrium order
R Ravasio, K Husain*, CG Evans, R Phillips, M Ribezzi, JW Szostak, A Murugan*

(Theory)

Theory

Direct and indirect selection in a proofreading polymerase
K Husain, V Sachdeva*, R Ravasio, M Peruzzo, W Liu, B Good, A Murugan*

(Experimental system)

Experiment