How ecological interactions shape microbial mutation rates to antimicrobial resistance

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Science and Engineering. School of Natural Sciences, Department of Earth and Environment

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Abbreviations

r - Growth rate as doubling time

CFU – Colony Forming Units

D – Final Population density - The estimated number of cells per ml at the end of the culture cycle

DAMP – Density-associated mutation-rate plasticity

m – Number of mutational events

MMR – Methyl-directed DNA mismatch repair

MRP – Mutation Rate Plasticity

N_0 – The initial population size of cells.

N_t – The population size at the end of the culture period

N_e – The effective population size

SIM – Stress-Induced Mutagenesis

Abstract

Mutagenesis is responsive to many environmental factors. Evolution therefore depends on the environment not only for selection but also in determining the variation available in a population. One such environmental dependency is the inverse relationship between mutation rates and population density in many microbial species. Here we determine the mechanism responsible for this mutation rate plasticity. Using dynamical computational modelling and in vivo mutation rate estimation we show that the negative relationship between mutation rate and population density arises from the collective ability of microbial populations to control concentrations of hydrogen peroxide. We demonstrate a loss of this density-associated mutation rate plasticity when Escherichia coli populations are deficient in the degradation of hydrogen peroxide. We further show that the reduction in mutation rate in denser populations is restored in peroxide degradation-deficient cells by the presence of wild-type cells in a mixed population. Together, these model-guided experiments provide a mechanistic explanation for density-associated mutation rate plasticity, applicable across all domains of life, and frames mutation rate as a dynamic trait shaped by microbial community composition.

Declaration

Data collected by HW and CB and submitted for MSc Medical Microbiology is included in chapter 1 and detailed in appendix 1. No other portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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Acknowledgements

Thanking MERMan etc

Simplified Abstract

A simplified abstract about mutations etc

1 Introduction

Uncovering the mechanisms behind environmentally responsive mutagenesis informs our understanding of evolution, notably antimicrobial resistance, where mutation supply can be critical (Gifford et al. 2023; Ragheb et al. 2019). Microbial mutation rates are responsive to a wide variety of environmental factors including population density (Krašovec et al. 2017), temperature (Chu et al. 2018), growth rate (Ram P. Maharjan and Ferenci 2018; Liu and Zhang 2019), stress (MacLean, Torres-Barceló, and Moxon 2013; Foster 2007), growth phase (Loewe, Textor, and Scherer 2003) and nutritional state (Ram P. Maharjan and Ferenci 2017). Such mutation rate plasticity inspires the idea of "anti-evolution drugs", able to slow the evolution of antimicrobial resistance during the treatment of an infection (Ragheb et al. 2019; Cirz et al. 2005; Domenech et al. 2020; Alam et al. 2016). Even small reductions in the mutation rate (2-5-fold) can have dramatic effects on the capacity of bacterial populations to adapt to antibiotic treatment, particularly when evolution is limited by mutation supply, as is the case for small pathogen populations (Ragheb et al. 2019).

Microbial mutation rates have an inverse association with population density across all domains of life, we have previously shown that 93% of otherwise unexplained variation in published mutation rate estimates is explained by the final population density (Krašovec et al. 2017). This density-associated mutation rate plasticity (DAMP) is a distinct phenotype from stress-induced mutagenesis, which acts via independent genetic mechanisms (Krašovec et al. 2018). Population density alters not only the rate but also the spectrum of mutations, with

significantly higher rates of AT>GC transitions seen in low density populations (Gifford et al. 2023). Density effects are likely relevant to natural populations given that population sizes and densities vary greatly, for example, *Escherichia coli* populations in host faeces can range in density by 5 orders of magnitude (**16**), and infections can be established by populations as small as 6×10^3 cells (**17**). We therefore aim to mechanistically describe the widespread phenotype of DAMP.

In order to test potential mechanisms generating DAMP, we developed and systematically assessed a computational model connecting metabolism and mutagenesis in a growing *E. coli* population. This model generates the hypothesis that the key determinants of DAMP are the production and degradation rates of reactive oxygen species (ROS). Though molecular oxygen is relatively stable it can be reduced to superoxide ($^{\bullet}O_{2-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($HO^{\bullet}$). These "reactive oxygen species" are strong oxidants able to damage multiple biological molecules including nucleotides and DNA (**18**). We tested the role of ROS in controlling DAMP by estimating mutation rate plasticity under different conditions of environmental oxygen and with genetic manipulations known to alter ROS dynamics. We find that the reduction in mutation rate at increased population density results from the population's increased ability to degrade H_2O_2, resulting in reduced ROS-associated mutagenesis. We show that this density effect is also experienced by cells deficient in H_2O_2 degradation when cocultured with wild-type cells able to detoxify the environment. Mutation rates therefore depend not only on the genotype of the individual but also on the community's capacity to degrade H_2O_2.

2 Working together to control mutation: how collective peroxide detoxification determines microbial mutation rate plasticity

2.1 Introduction

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2.2 Results

2.2.1 Initial computational model of nucleotide metabolism in a growing microbial population fails to reproduce mutation rate plasticity

To generate hypotheses for the mechanisms of density-associated mutation rate plasticity we constructed a system of ordinary differential equations (ODEs) that recapitulates the dynamics of metabolism, growth and mutagenesis in a 1mL batch culture of *E. coli* Figure 2.1. The enzyme MutT, responsible for degrading mutagenic oxidised GTP (Maki and Sekiguchi 1992), is essential in DAMP (Krašovec et al. 2017); the ODE model is therefore focussed on guanine bases. In the model external glucose (***eGlc***) is taken up by a small initial *E. coli* population (***wtCell***). Internal glucose (***iGlc***) is then metabolised to produce ***ROS***, ***dGTP*** and, largely, 'other' molecules ('Sink' in Figure 2.1). ***dGTP*** is then either integrated into a newly synthesised DNA molecule (***DNA***) or it reacts with ***ROS*** to produce 8-oxo-2'-deoxyguanosine triphosphate (***odGTP***). In this model, non-oxidised ***dGTP*** always pairs correctly with cytosine, producing non-mutant DNA (***DNA***). In a second round of DNA replication the guanine base is now on the template strand, cytosine is correctly inserted opposite producing new chromosomes (***wtCell***). ***odGTP***, if it is not dephosphorylated by MutT into *dGMP* (Sink), can either pair correctly with cytosine (becoming ***DNA***) or mis-pair with adenine (becoming ***mDNA***). When ***odGTP*** is inserted opposite adenine into DNA (***mDNA***) it may be repaired by the MutS or MutY proteins, converting the ***mDNA*** back to ***DNA***. The key output of interest is the mutation rate, which is defined as the number of mutant base pairs (***mCell***) divided by

the number of non-mutant base pairs (***wtCell***). The model comprises 10 ordinary differential equations (ODEs), one for each substance variable in Figure 2.1 (excluding 'Sink'), plus ***cytVol***, the total population cytoplasmic volume within which all the reactions occur (**Table 1**, **Eq. 1**-**10**, Methods). These equations require 14 parameters (some of them composite, **Table 2**); the structure and parameter values are largely taken from the existing literature (for details see Methods). Un-measurable parameters (notably the rate of ***dGTP*** oxidation to ***odGTP*** by ***ROS***, '**O2'**) were set to give the observed mutation rate (2×10^{-10} mutations per base pair per generation, (**20**)) at a final population density of 3×10^{8} CFU ml^{-1}, typical of 250 mg L^{-1} glucose in minimal media. As with most experiments demonstrating density-associated mutation rate plasticity (**3**, **21**), final population density is controlled by varying initial external glucose. We initiated 28h simulations of 1ml cultures with 2175 cells (a small number, typical of fluctuation assays estimating mutation rate, **Fig. S10**), no internal metabolites and external glucose concentrations relevant to wet-lab experiments – across a log scale from 55 to 1100 mg L^{-1} (**Table 1**). The dynamics of external glucose, population size and mutation rate for these simulations are shown in **Fig.1B-D**.

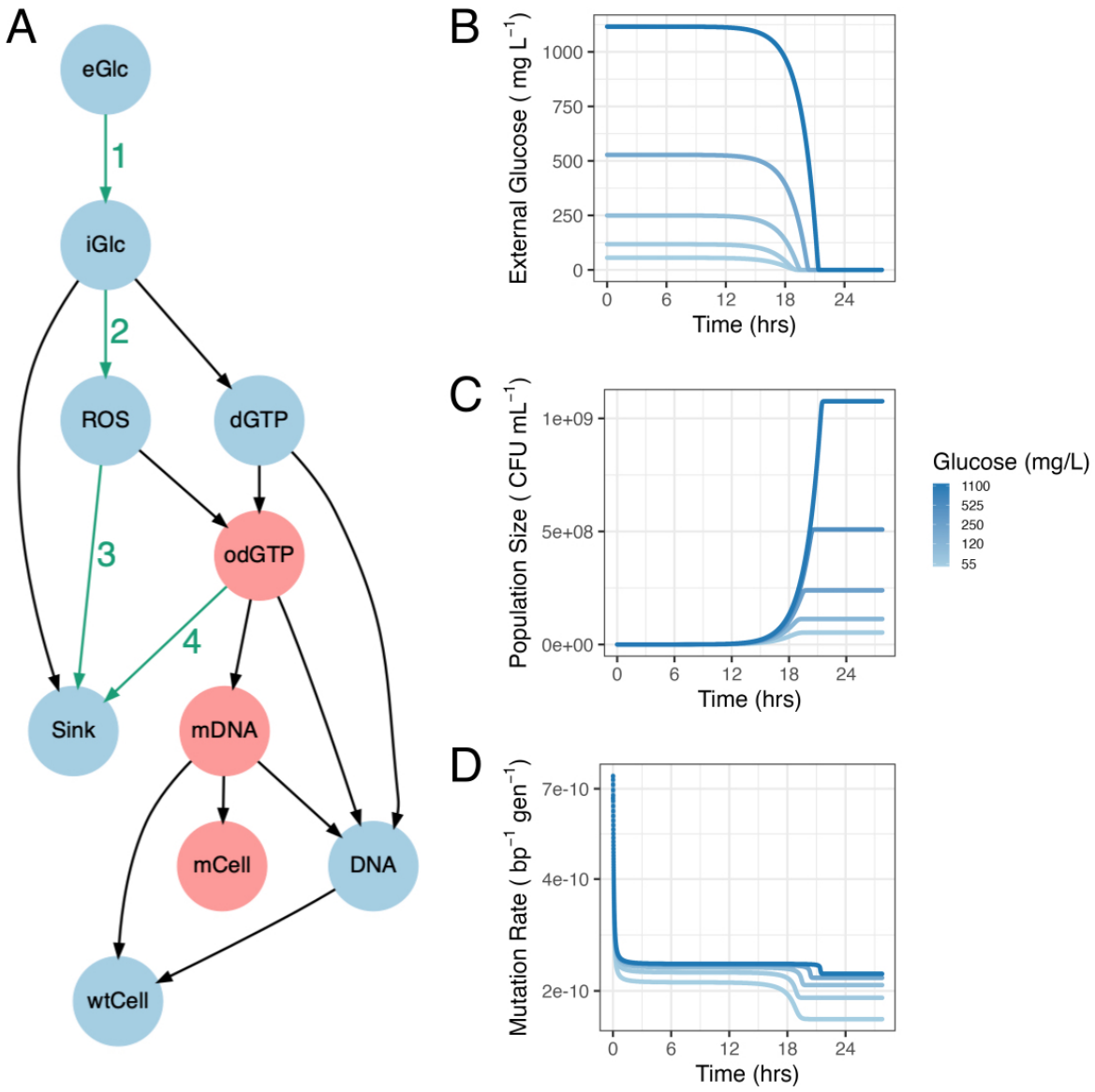

Figure 2.1: **Dynamical computational model of growth, metabolism and mutagenesis in E. coli.** A: Model structure connecting variables. Red variables indicate the pathway to mutagenesis; green numbered arrows indicate pathways targeted by model variants. This structure was represented in ODEs, parameterised from the literature (Methods), and simulated to give output shown in B-D. B: Kinetics of external glucose concentration (eGlc), C: population size (wtCell divided by G nucleotides in the E. coli genome) and D: mutation rate (mCell/wtCell). Note log scale on y-axis in panel D. Panels B-D are plotted for 5 initial glucose concentrations (range 55 – 1100 mg L-1 as shown in legend), initial glucose concentration indicated by line colour.

3 Discussion

The discussion discusses mutations.

4 Summary

In summary, this book has no content whatsoever.

The slope of the below graph is 0.5

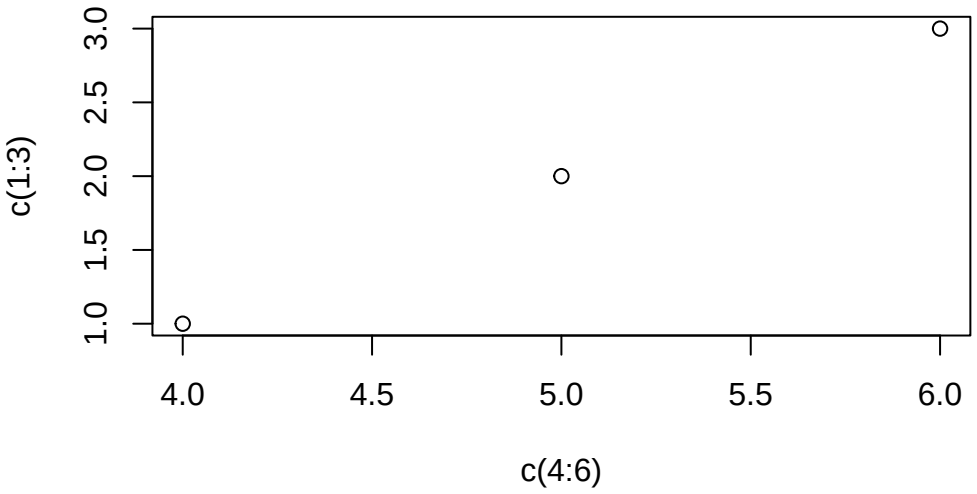

Figure 4.1: Plot of numbers

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