The Evolutionary Logic of Antimicrobial Resistance

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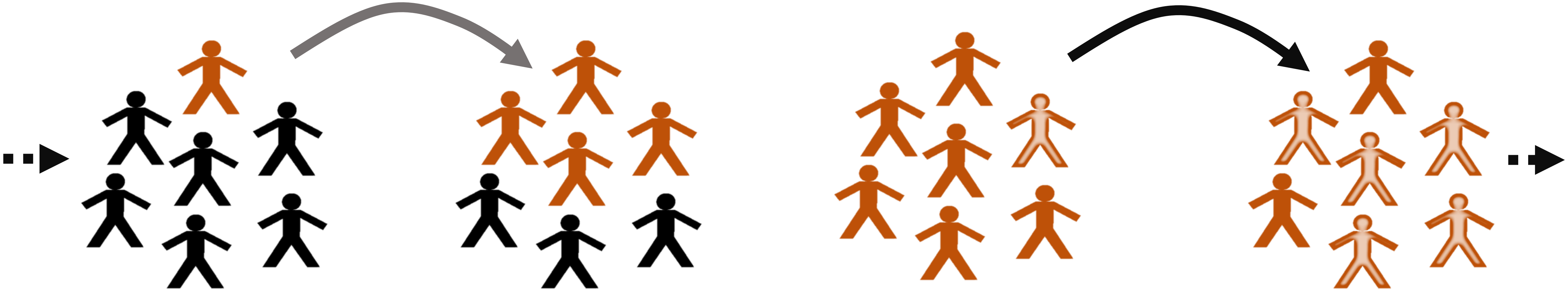
Mutation, mechanism, selection and evolution

Natural selection 101

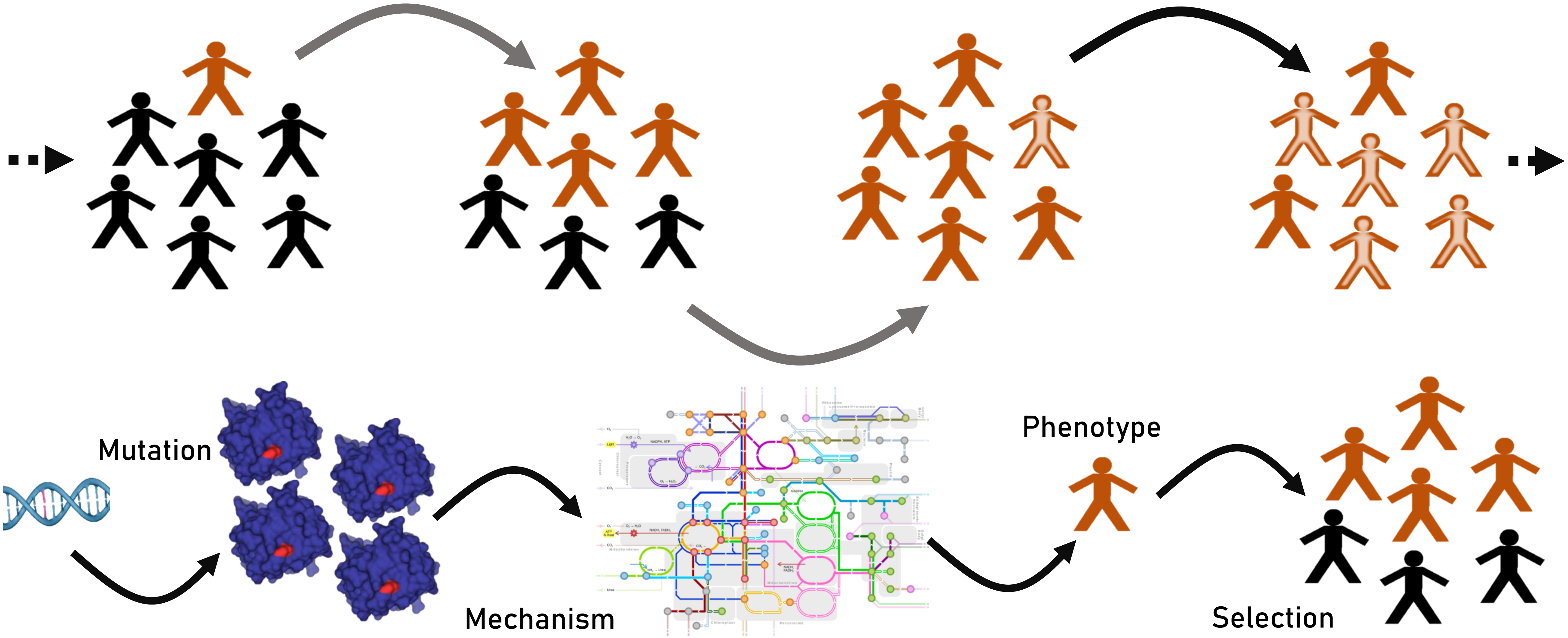


Pre-existing variation

Differential
reproductive success

Phenotypes differences
are heritable

Mutation, mechanism, selection and evolution



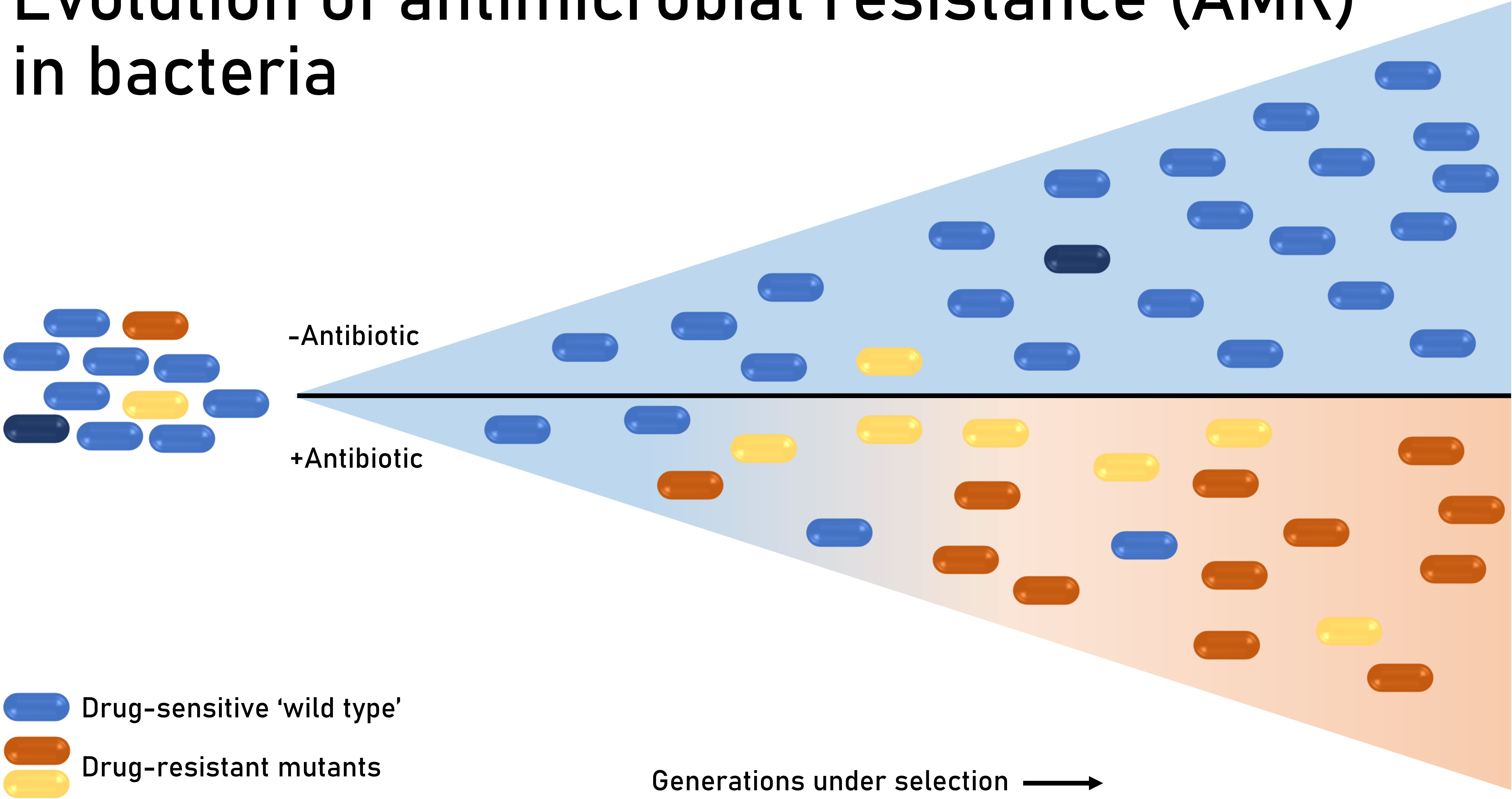
Mutation

Mechanism

Phenotype

Selection

Evolution of antimicrobial resistance (AMR) in bacteria



-Antibiotic

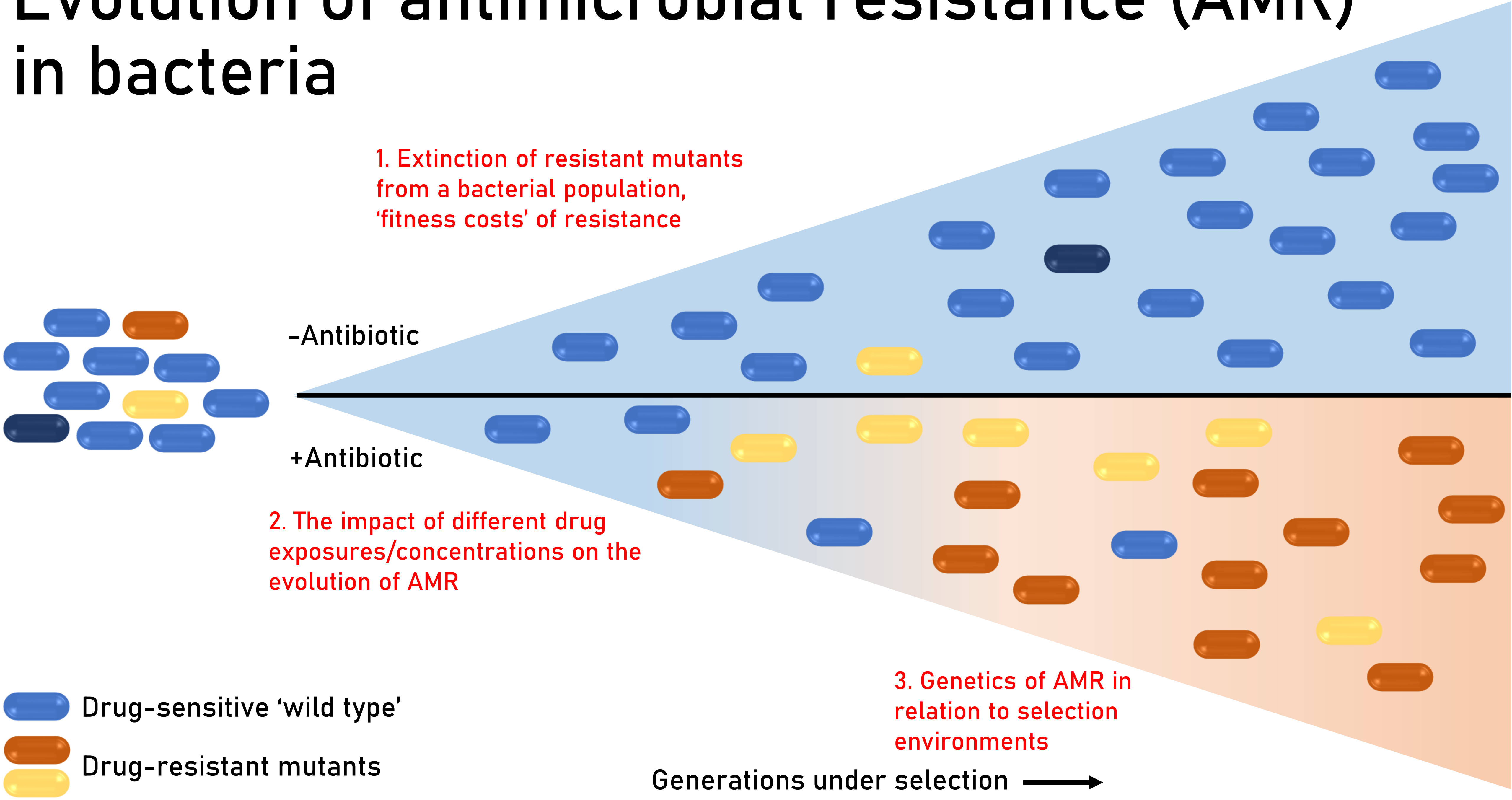
+Antibiotic

🟦 Drug-sensitive ‘wild type’

🟧 🟨 Drug-resistant mutants

Generations under selection →

Evolution of antimicrobial resistance (AMR) in bacteria



1. Extinction of resistant mutants from a bacterial population, ‘fitness costs’ of resistance

-Antibiotic

+Antibiotic

2. The impact of different drug exposures/concentrations on the evolution of AMR

3. Genetics of AMR in relation to selection environments

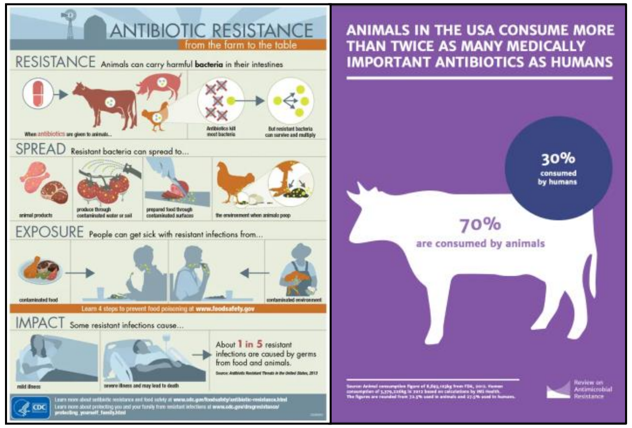
Generations under selection →

Drug-sensitive ‘wild type’

Drug-resistant mutants

Sub-lethal drug environments: Facilitators of AMR evolution

Source: Trafalgar Scientific



Source: CDC, USA

Journal of Clinical Periodontology

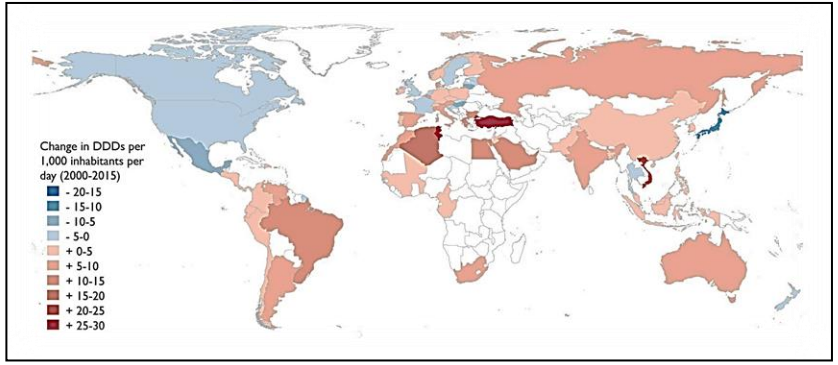
EFP

Subantimicrobial dose doxycycline as an adjunct to scaling and root planing: post-treatment effects

THE HINDU

GROUND ZERO | COMMENT

The superbugs of Hyderabad



Kleina et al. PNAS 2018

Evolutionary trajectories to resistance differ at low and high drug pressures

nature COMMUNICATIONS

ARTICLE

DOI: 10.1038/s41467-018-04059-1 OPEN

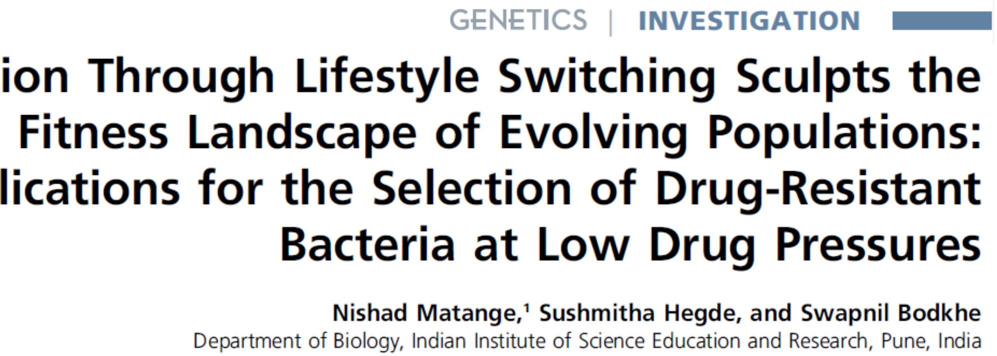
Evolution of high-level resistance during low-level antibiotic exposure

Erik Wistrand-Yuen[1], Michael Knopp[1], Karin Hjort[1], Sanna Koskiniemi[2], Otto G. Berg[2] & Dan I. Andersson[1]

MBE

Strength of Selection Pressure Is an Important Parameter Contributing to the Complexity of Antibiotic Resistance Evolution

Tugce Oz,[†,1] Aysegul Guvenek,[†,1] Sadik Yildiz,[†,1] Enes Karaboga,[1] Yusuf Talha Tamer,[1] Nirva Mumcuyan,[1] Vedat Burak Ozan,[1] Gizem Hazal Senturk,[1] Murat Cokol,[1] Pamela Yeh,[2] and Erdal Toprak*[,‡,1]



GENETICS | INVESTIGATION

Adaptation Through Lifestyle Switching Sculpts the Fitness Landscape of Evolving Populations: Implications for the Selection of Drug-Resistant Bacteria at Low Drug Pressures

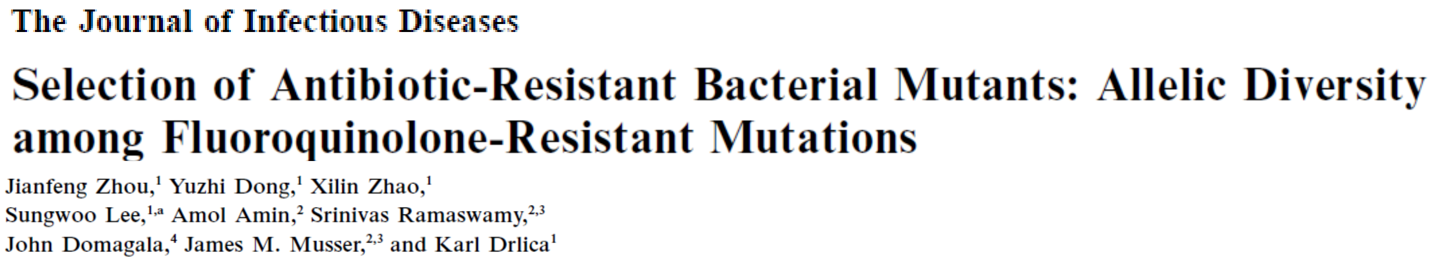
Nishad Matange,[1] Sushmitha Hegde, and Swapnil Bodkhe
Department of Biology, Indian Institute of Science Education and Research, Pune, India

Drug Resistance Updates

journal homepage: www.elsevier.com/locate/drup

Evolution of antibiotic resistance at non-lethal drug concentrations

Dan I. Andersson*, Diarmaid Hughes
Department of Medical Biochemistry and Microbiology, Box 582, SE-75123 Uppsala, Sweden



The Journal of Infectious Diseases

Selection of Antibiotic-Resistant Bacterial Mutants: Allelic Diversity among Fluoroquinolone-Resistant Mutations

Jianfeng Zhou,[1] Yuzhi Dong,[1] Xilin Zhao,[1]
Sungwoo Lee,[1,a] Amol Amin,[2] Srinivas Ramaswamy,[2,3]
John Domagala,[4] James M. Musser,[2,3] and Karl Drlica[1]

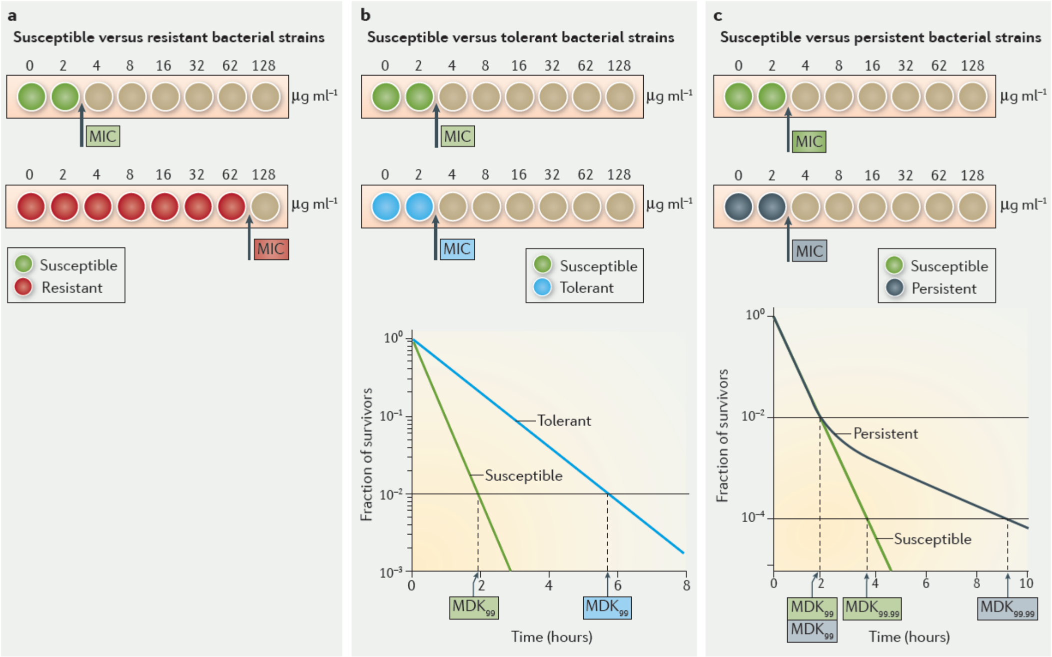
Many flavours of adaptation to antibiotics

Antibiotic-induced phenotypic changes

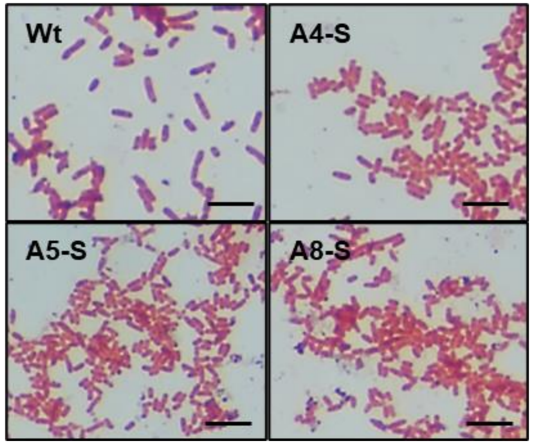
Resistance

Tolerance

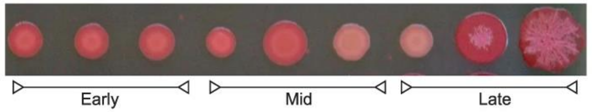
Persistence



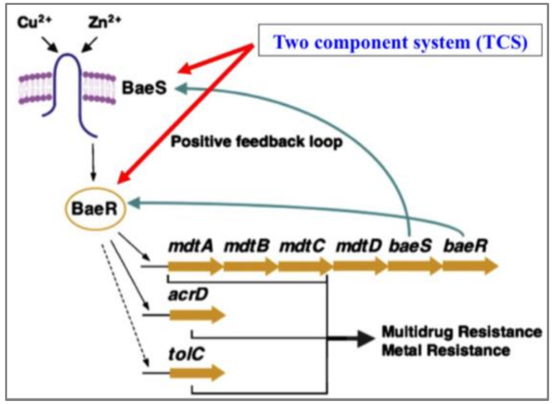
Brauner et al., Nat. Rev. Microbiol. (2016)



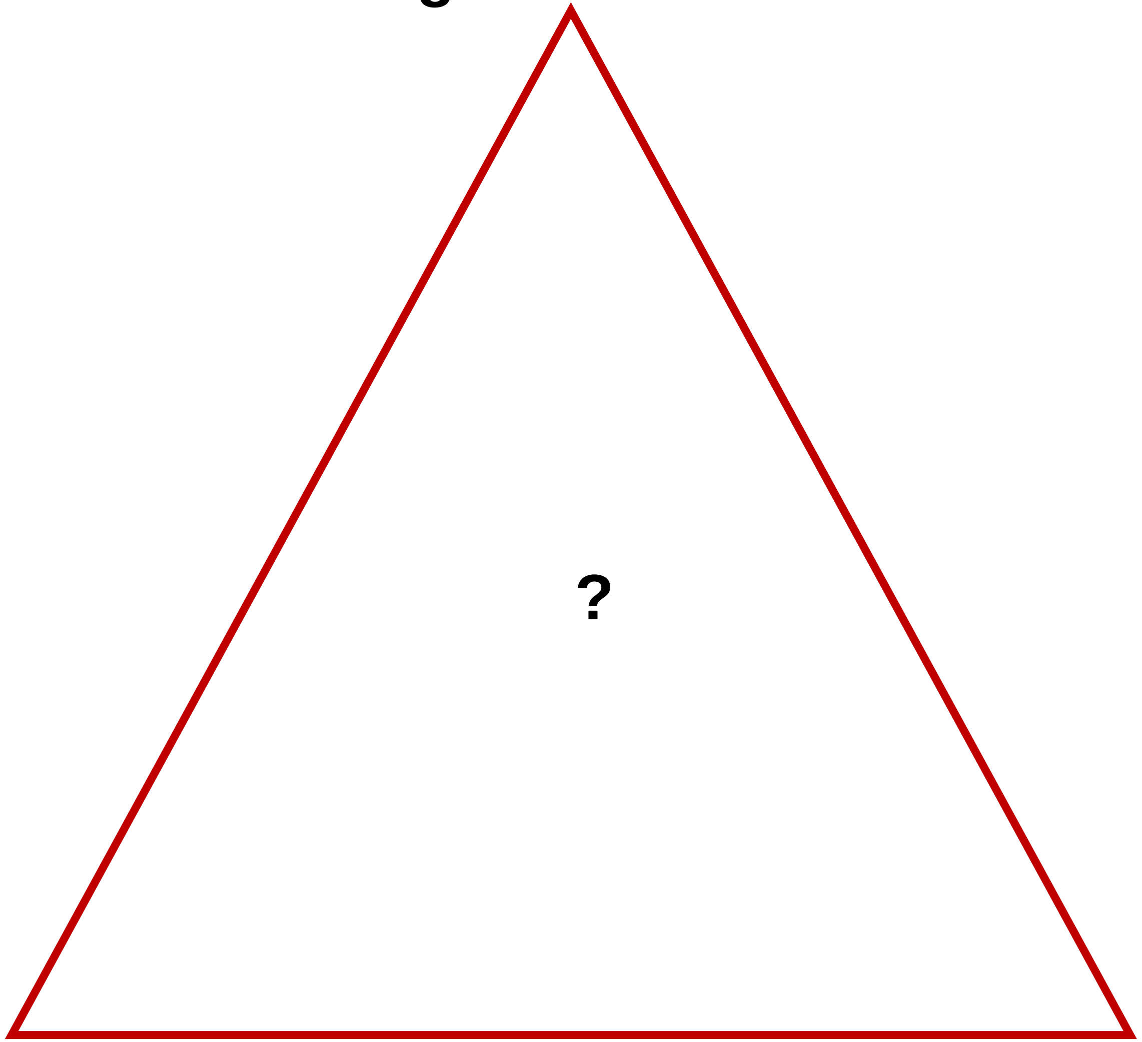
Matange et al., Genetics (2019)



Trampari et al., Biofilms and Microbiomes (2021)



Xu and Yan, Antibiotics (2015)



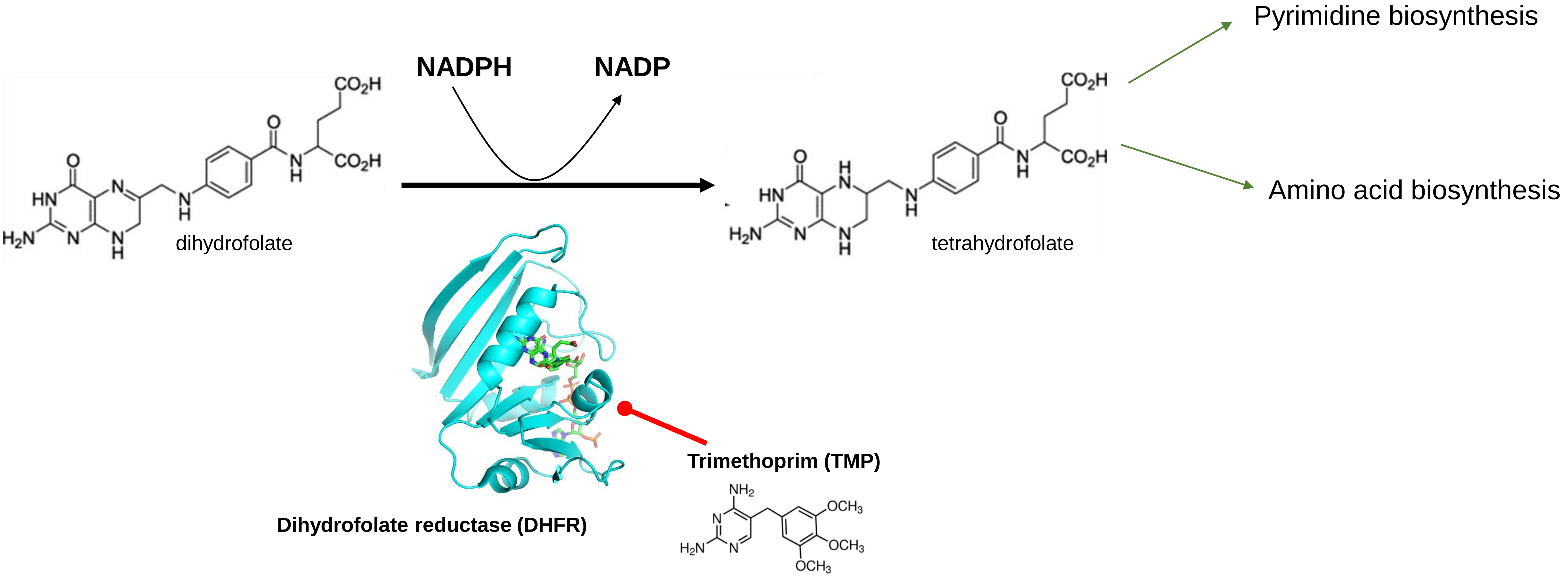
Strength of selection

?

Molecular mechanisms

Modes of adaptation

Our model: Trimethoprim resistance in *Escherichia coli*



Pyrimidine biosynthesis

NADPH **NADP**

dihydrofolate

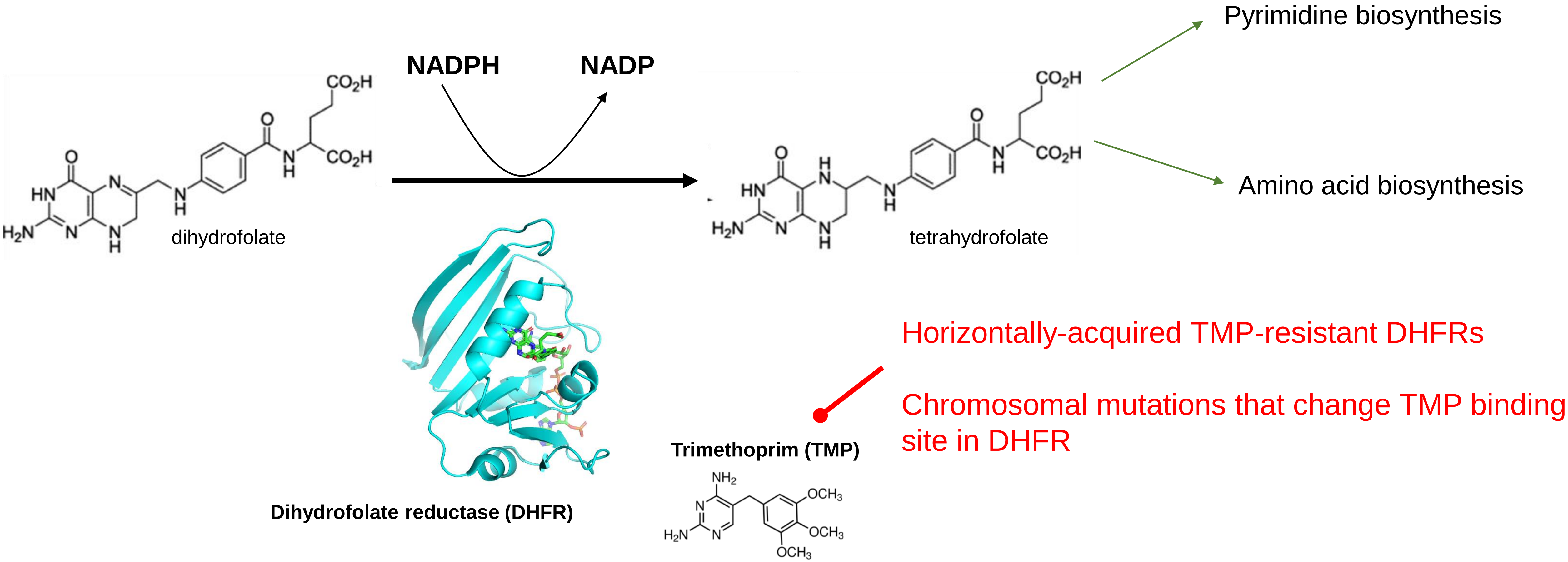
tetrahydrofolate

Amino acid biosynthesis

Trimethoprim (TMP)

Dihydrofolate reductase (DHFR)

Our model: Trimethoprim resistance in *Escherichia coli*



Pyrimidine biosynthesis

Amino acid biosynthesis

Dihydrofolate reductase (DHFR)

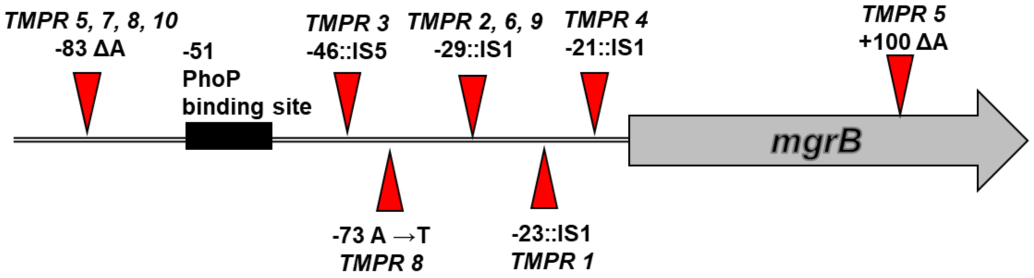
Trimethoprim (TMP)

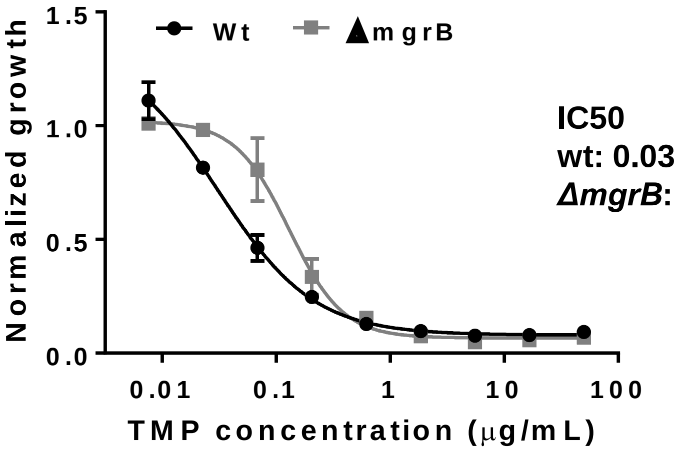
Horizontally-acquired TMP-resistant DHFRs

Chromosomal mutations that change TMP binding site in DHFR

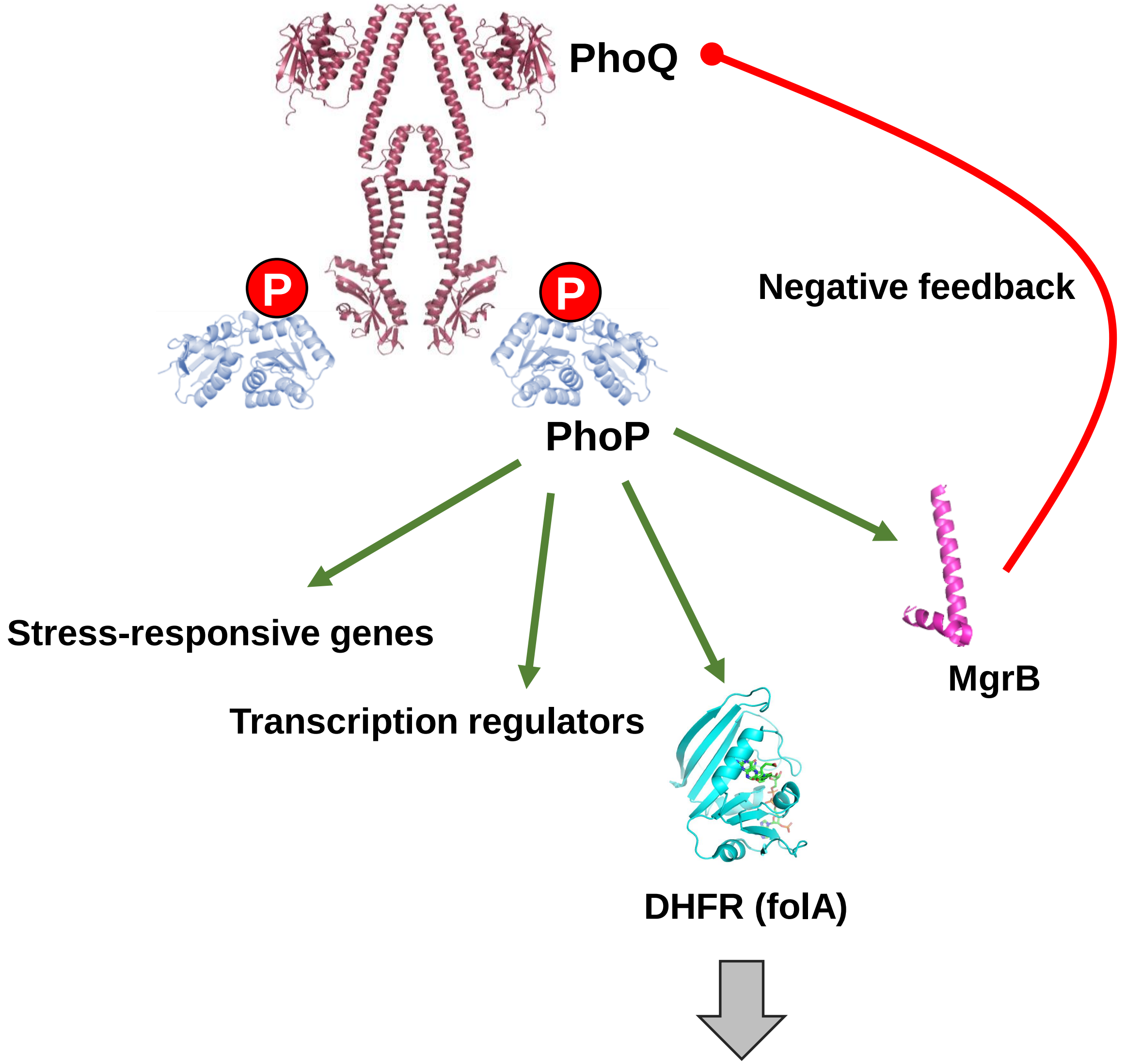
Trimethoprim-resistance in *E. coli* is preceded by trimethoprim tolerance

Loss-of-function mutations in the 'mgrB' gene or its promoter confer trimethoprim tolerance to *E. coli*





IC50
wt: 0.03 ± 0.01 μg/mL
***ΔmgrB*: 0.11 ± 0.02 μg/mL**


PhoQ

Negative feedback

P

P

PhoP

Stress-responsive genes

Transcription regulators

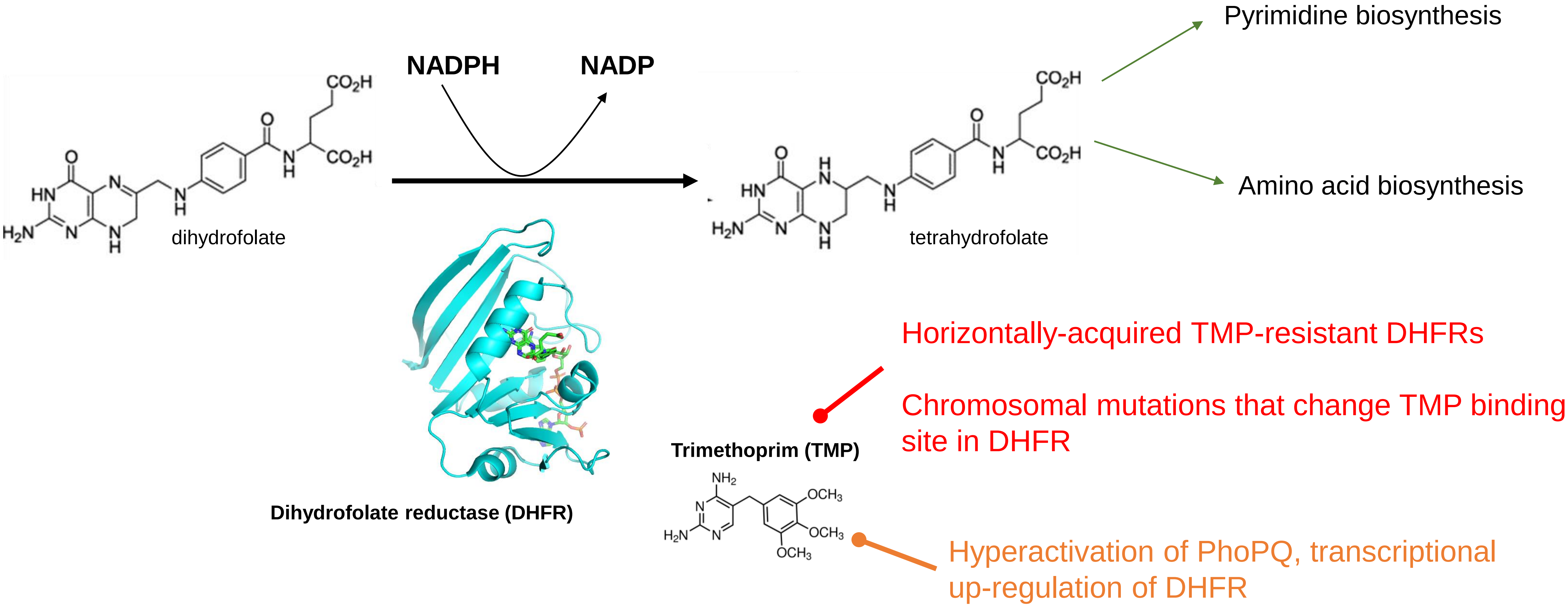
MgrB

DHFR (folA)

Transcriptional up-regulation, trimethoprim tolerance

Patel and Matange *eLife* (2021)

Our model: Trimethoprim resistance in *Escherichia coli*



Pyrimidine biosynthesis

Amino acid biosynthesis

Dihydrofolate reductase (DHFR)

Trimethoprim (TMP)

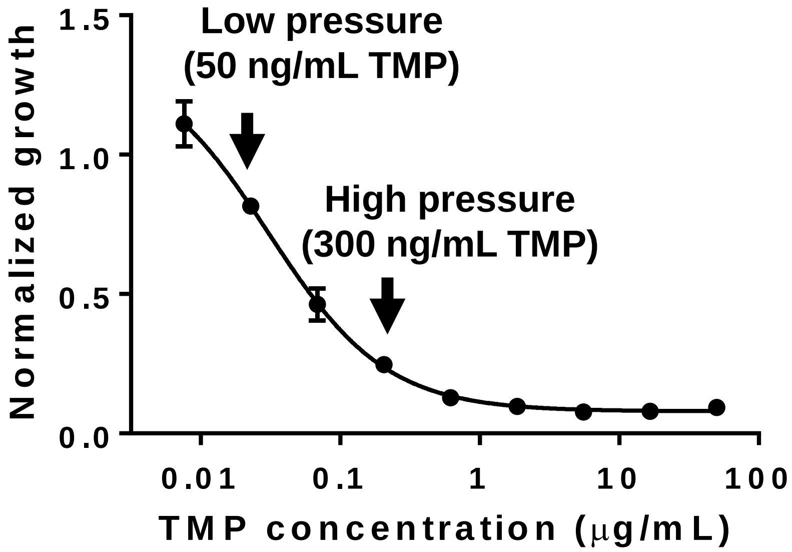
Horizontally-acquired TMP-resistant DHFRs

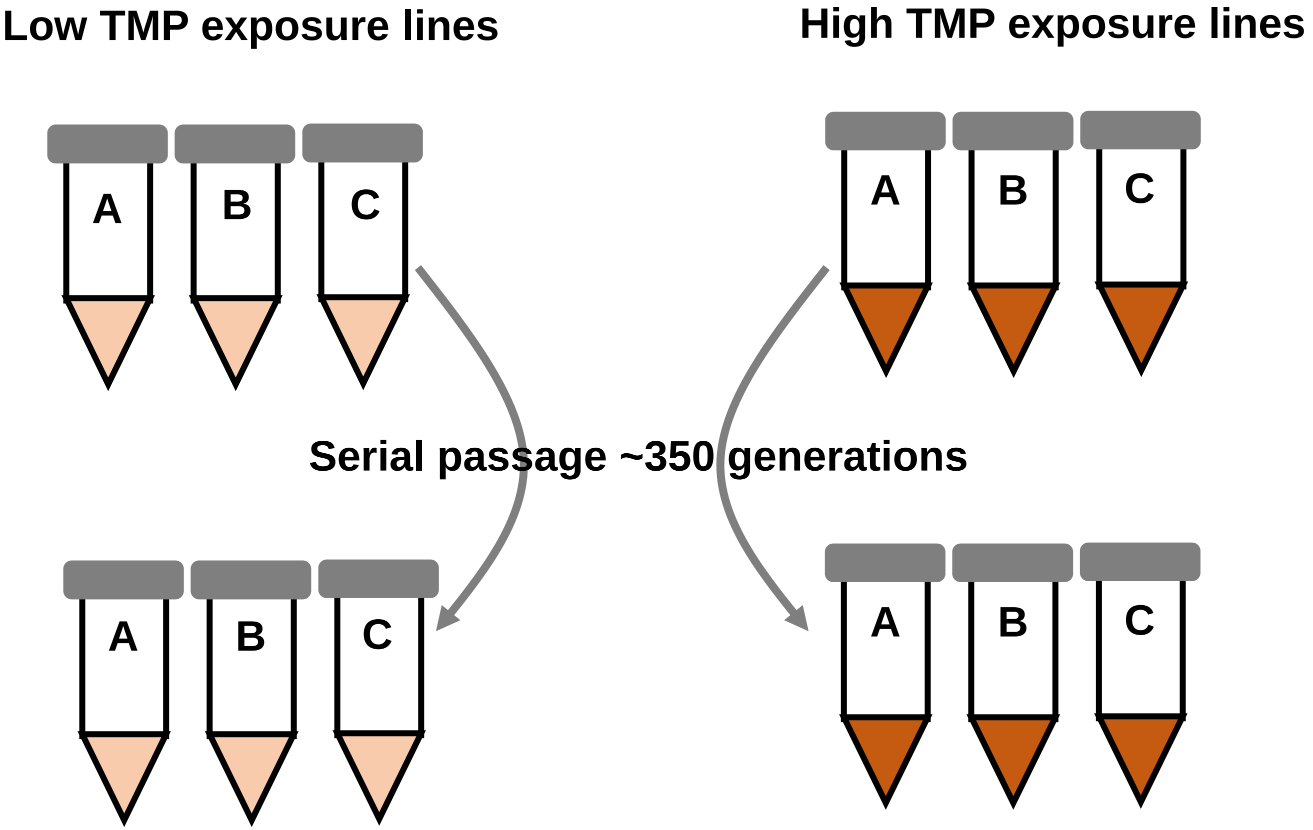
Chromosomal mutations that change TMP binding site in DHFR

Hyperactivation of PhoPQ, transcriptional up-regulation of DHFR

Do selection dynamics of resistant bacteria vary at different selection pressures?

Antibiotic dose vs growth for wild type *E. coli*



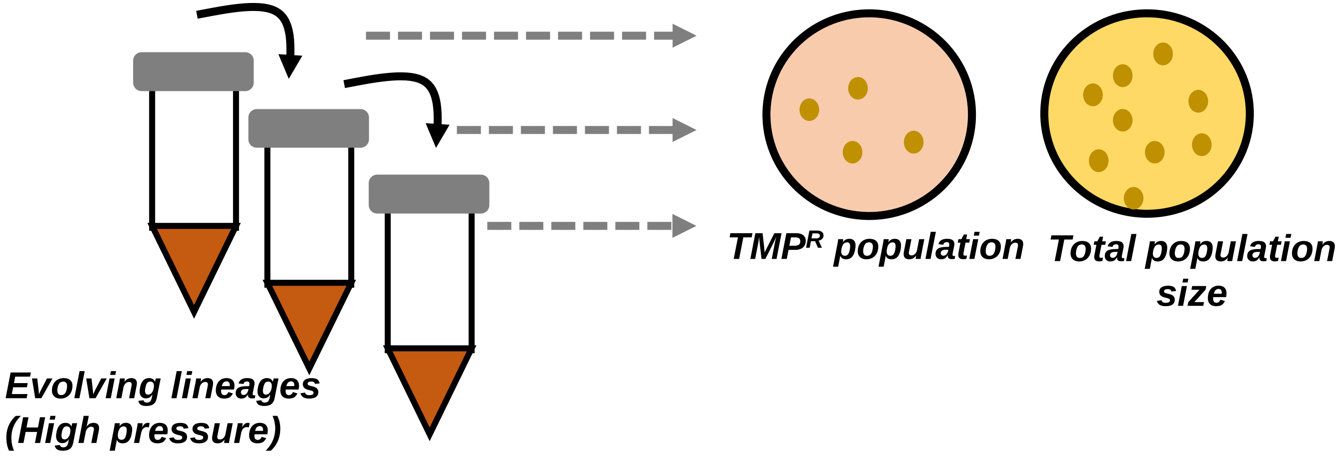

Low TMP exposure lines

High TMP exposure lines

Serial passage ~350 generations

Patel and Matange *eLife* (2021)

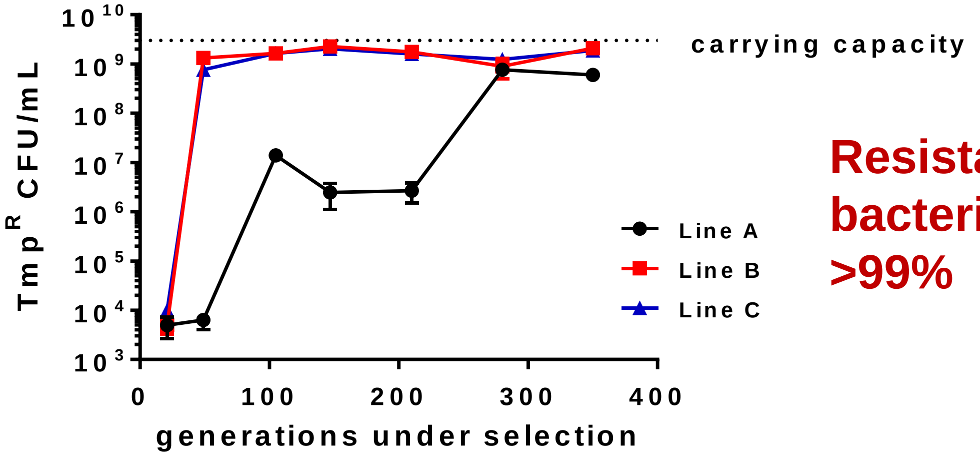
Do selection dynamics of resistant bacteria vary at different selection pressures?



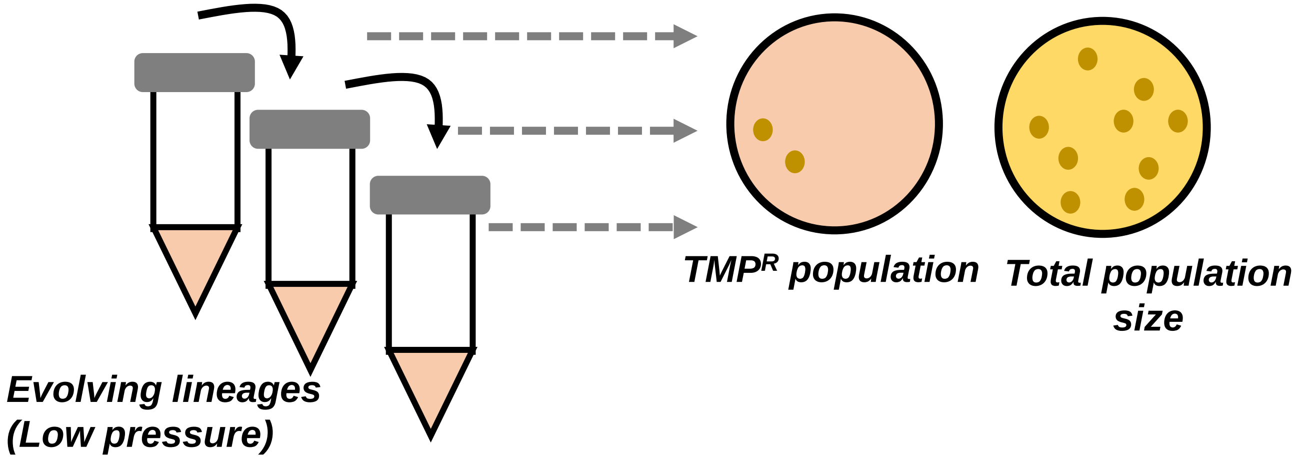
TMP^R population

Total population size

Evolving lineages (High pressure)



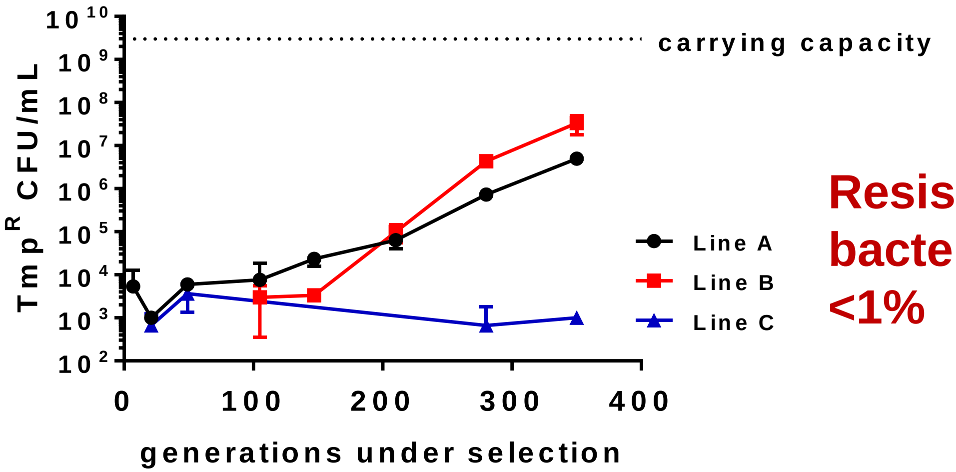
Resistant bacteria >99%



TMP^R population

Total population size

Evolving lineages (Low pressure)



Resistant bacteria <1%

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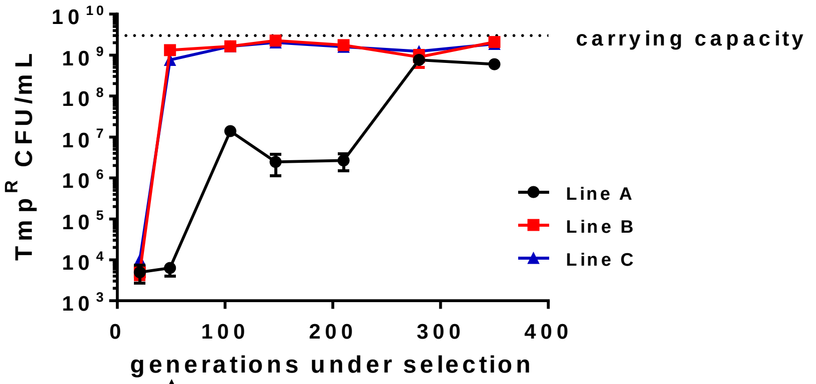
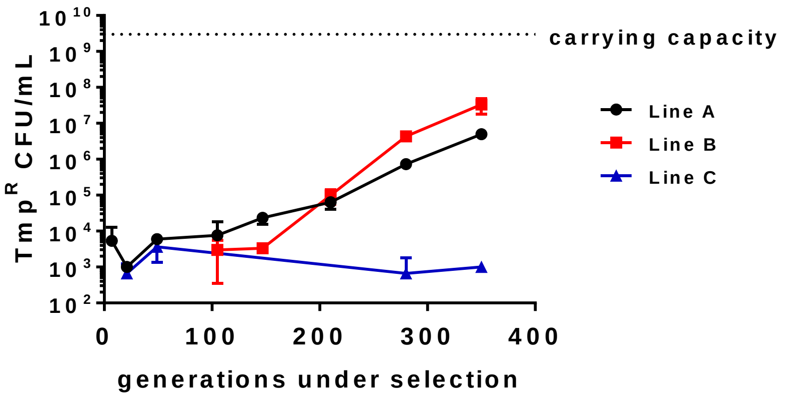
Drug-tolerant bacteria are fixed early in high and low pressure lineages





No mutations in DHFR detected

Genome sequence populations from ~20 generations of selection

Loss of mgrB ~70-80% of populations

Tolerance precedes resistance at both selection pressures

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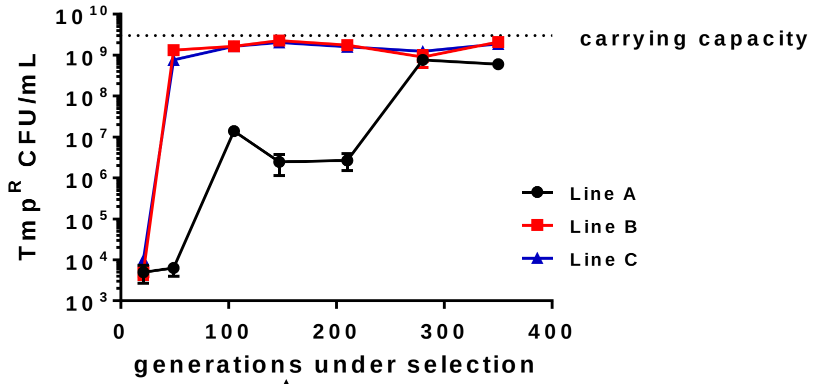
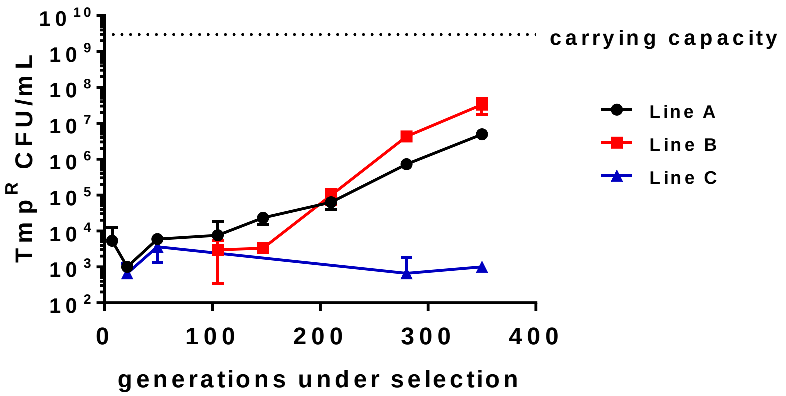
Drug-resistant bacteria are derived from drug-tolerant bacteria in high and low pressure lineages





Genome sequence resistant isolates from ~150 generations of selection

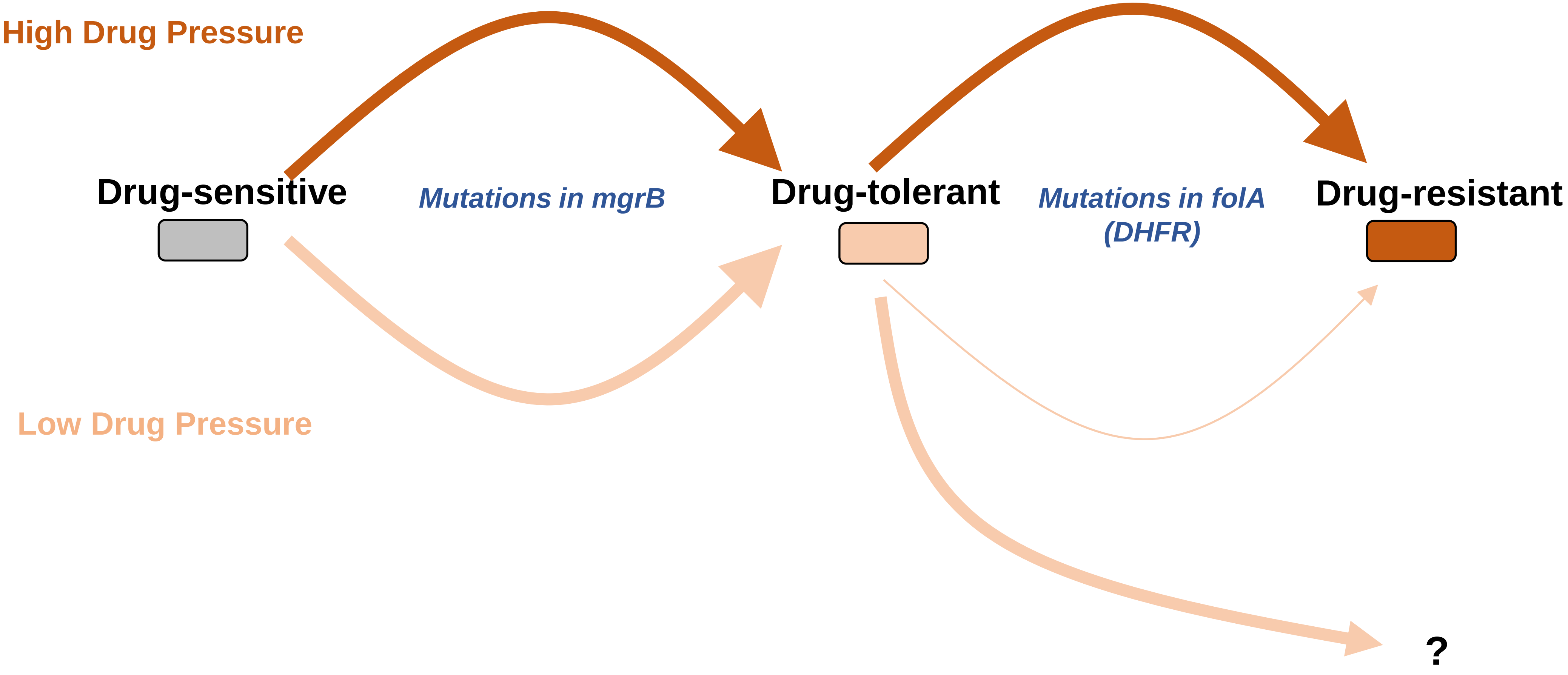
Resistant bacteria harbour mutations in DHFR in addition to loss-of-function mutations in mgrB

Few additional mutations in high selection lineages

Resistant bacteria are derived from tolerant bacteria in high and low pressure lineages

Patel and Matange *eLife* (2021)

Phenotypic transitions during selection of resistance: Dependency on selection pressure



High Drug Pressure

Drug-sensitive

Mutations in mgrB

Drug-tolerant

Mutations in folA (DHFR)

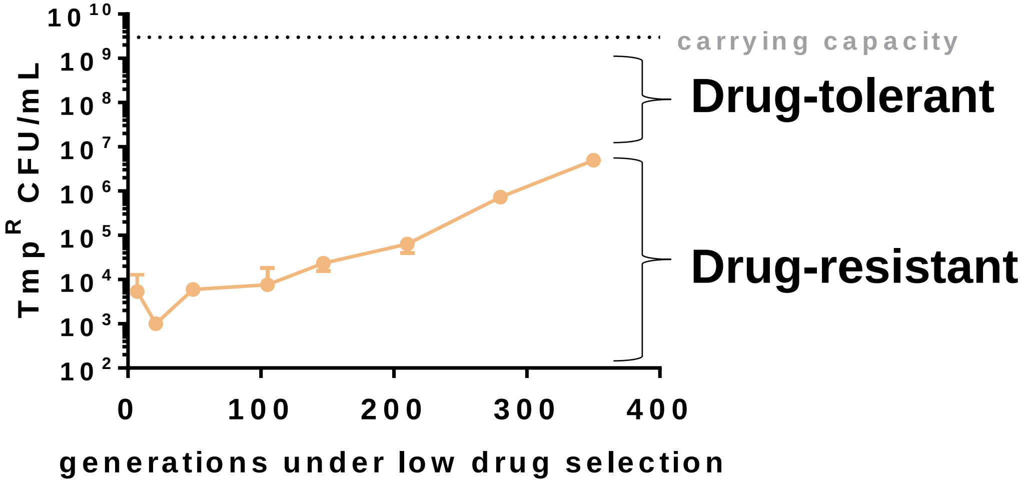
Drug-resistant

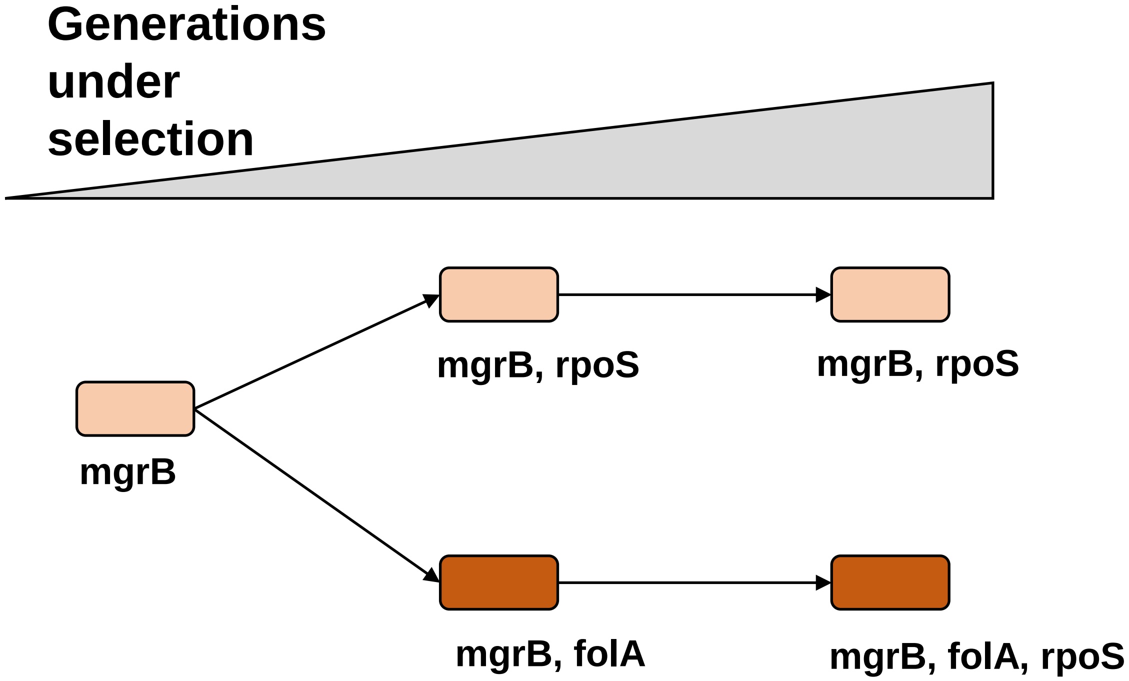
Low Drug Pressure

?

Patel and Matange *eLife* (2021)

Low pressure lineages show two different genetic trajectories of adaptation




Generations under selection

mgrB

mgrB, rpoS

mgrB, rpoS

mgrB, folA

mgrB, folA, rpoS

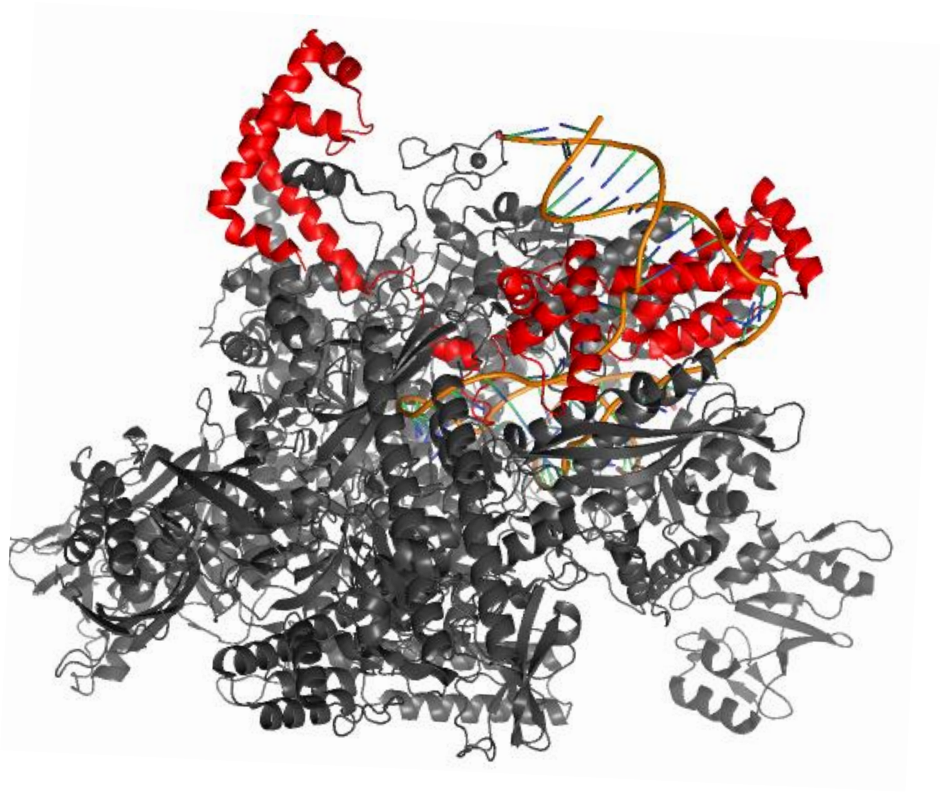
Loss of function mutations in RpoS

RpoS-deficiency does not enhance trimethoprim resistance

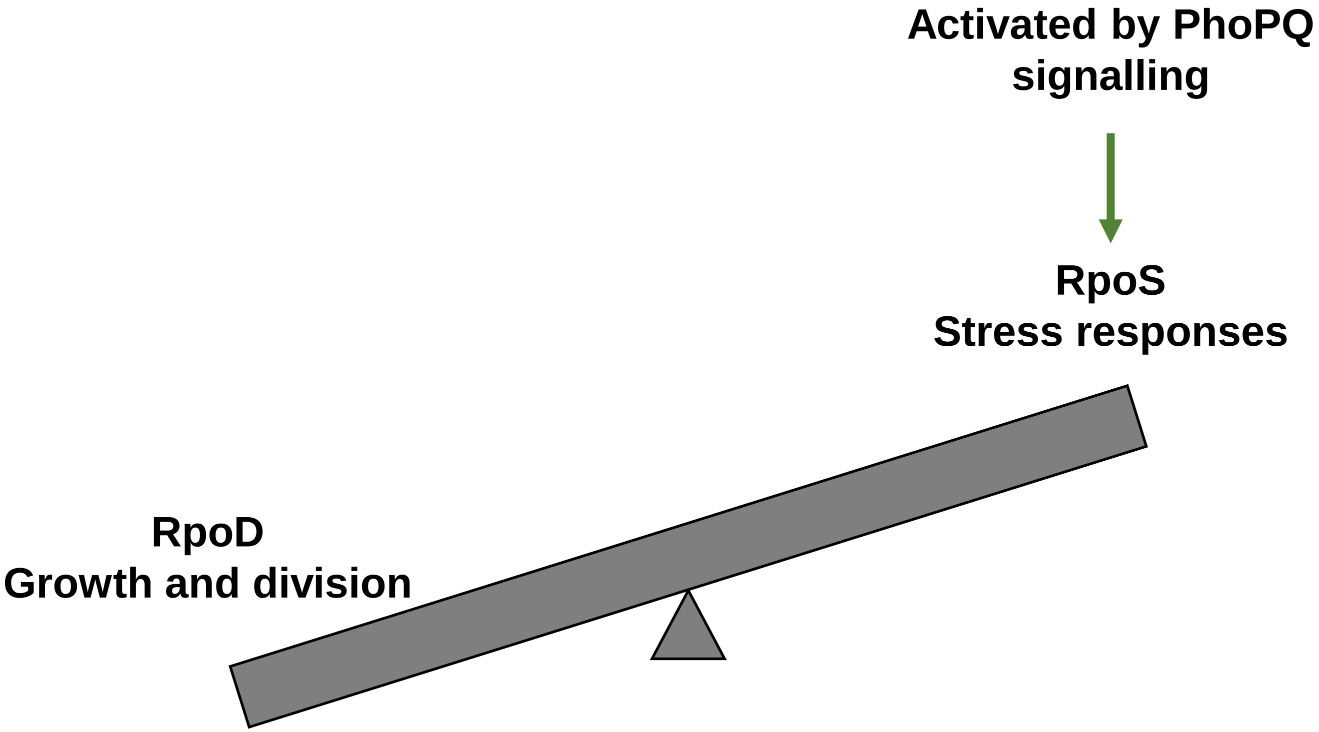
What drives the fixation of mutations in RpoS?

Patel and Matange *eLife* (2021)

RpoS is an activator of bacterial stress responses and a target of PhoPQ signalling



RpoS (red) bound to DNA, part of RNA polymerase holoenzyme (PDB: 5IPL)



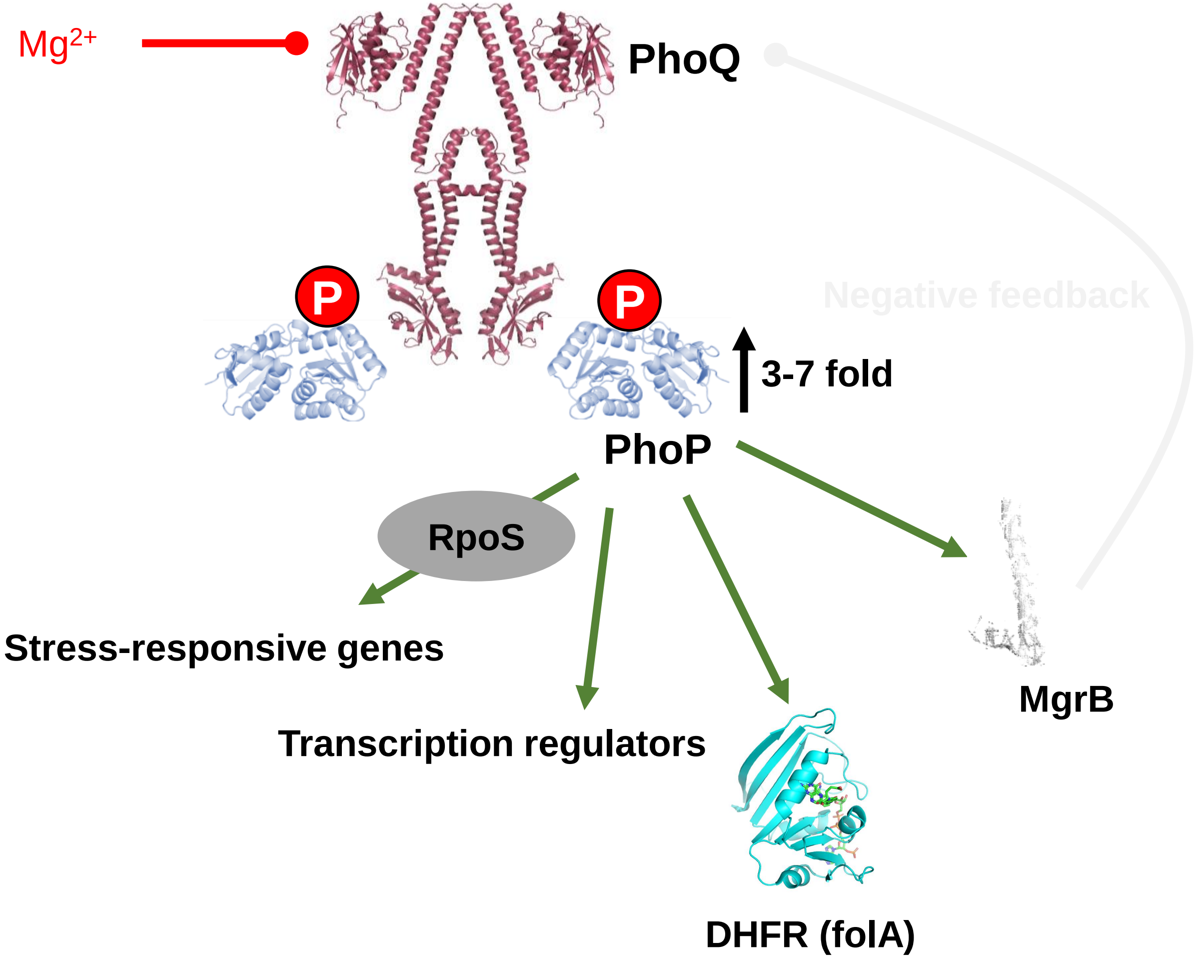
Activated by PhoPQ signalling

RpoS
Stress responses

RpoD
Growth and division

Could loss of RpoS alleviate the detrimental effects of hyperactive PhoPQ signalling in drug-tolerant bacteria?

Loss of mgrB, unwanted gene expression and fitness costs



Mg^{2+}

PhoQ

P

P

Negative feedback

3-7 fold

PhoP

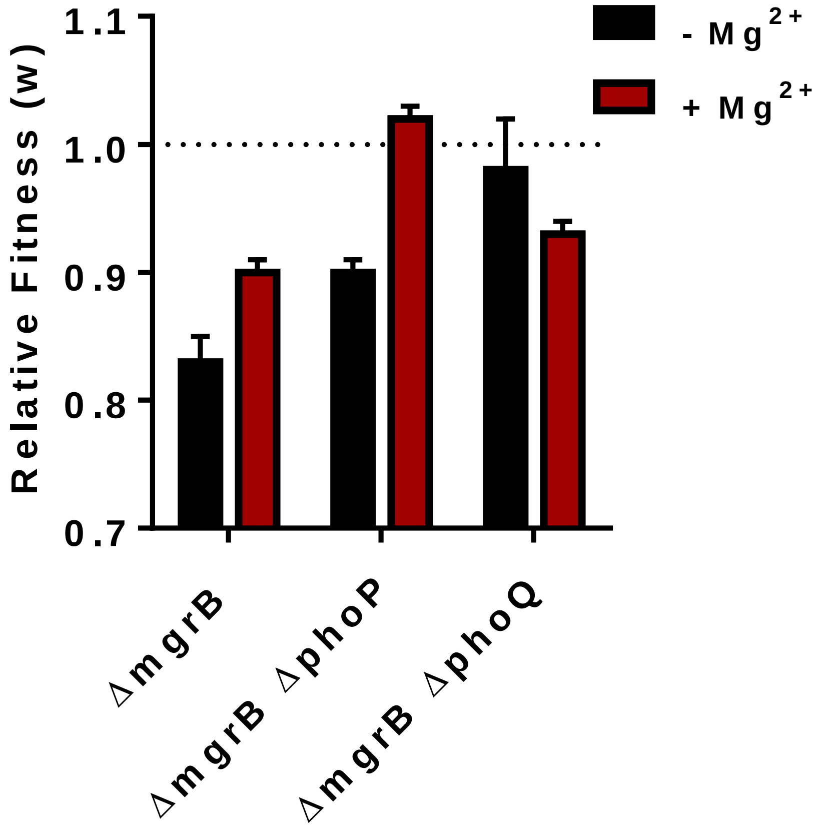
RpoS

Stress-responsive genes

Transcription regulators

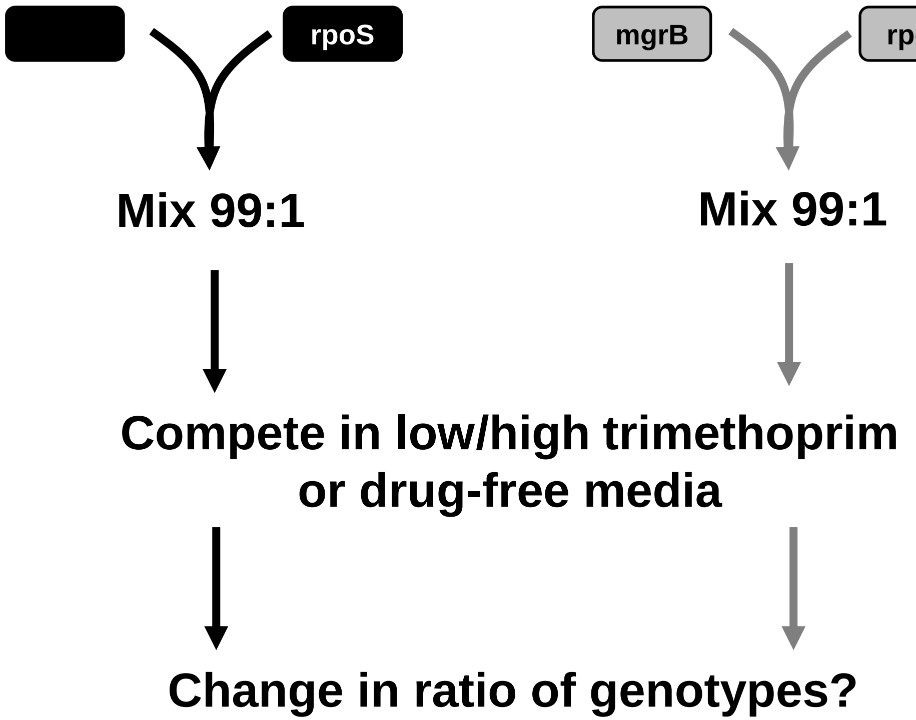
MgrB

DHFR (folA)



Chetna Yelpure and Rhea Vinchhi, unpublished

Inactivation of RpoS is beneficial in mgrB-deficient *E. coli* regardless of antibiotic

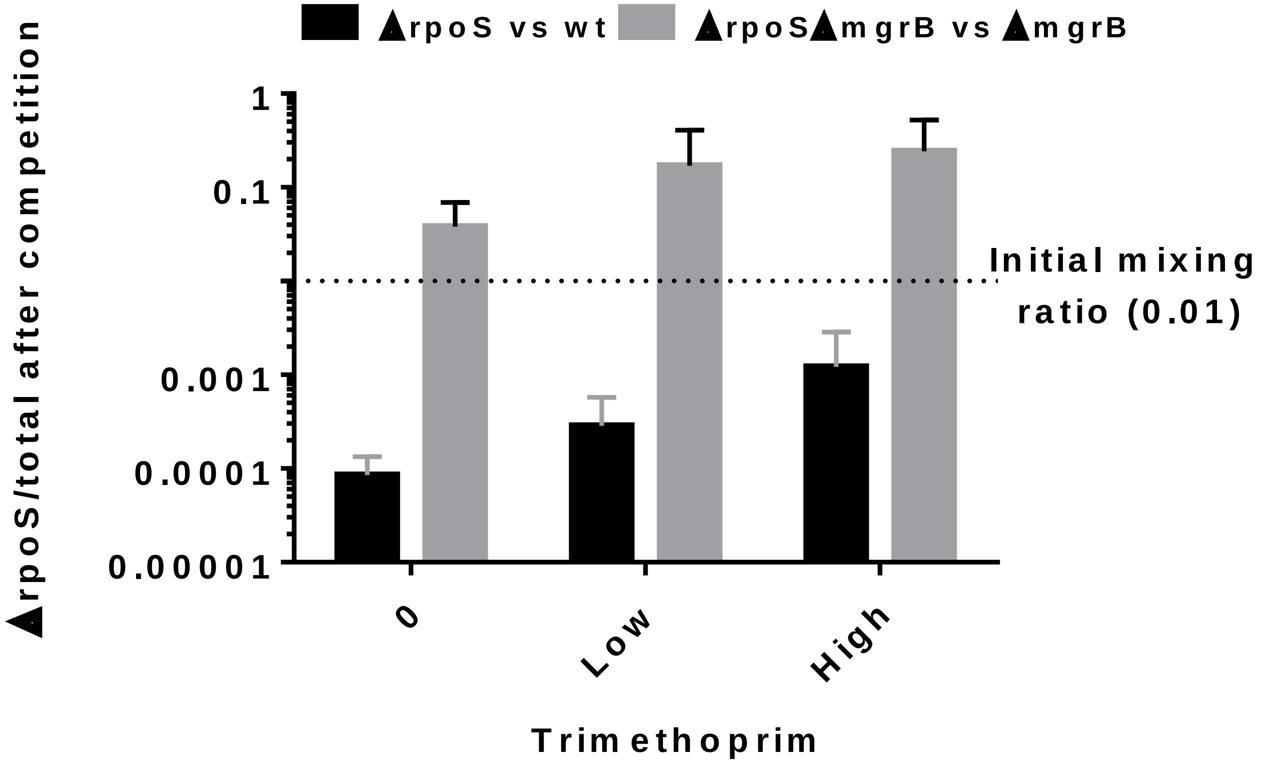


Mix 99:1

Mix 99:1

Compete in low/high trimethoprim or drug-free media

Change in ratio of genotypes?



Patel and Matange *eLife* (2021)

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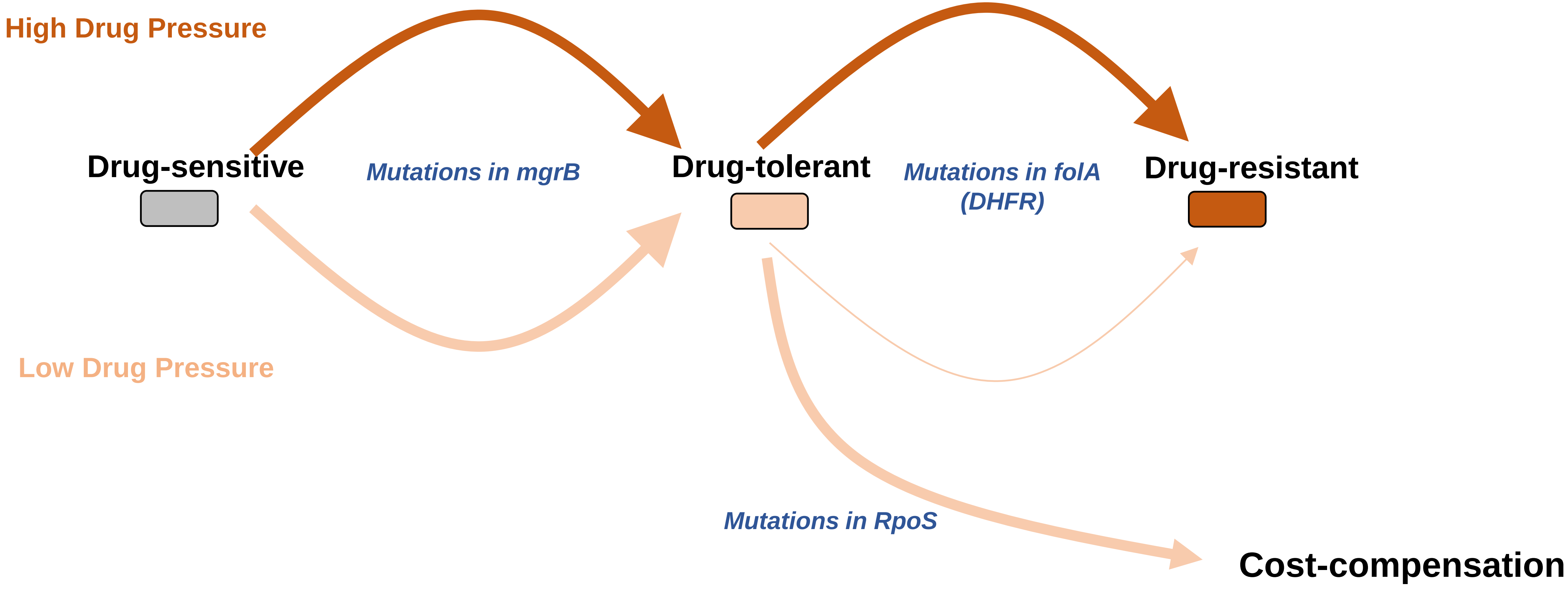

High Drug Pressure

Drug-sensitive

Mutations in mgrB

Drug-tolerant

Mutations in folA (DHFR)

Drug-resistant

Low Drug Pressure

Mutations in RpoS

Cost-compensation

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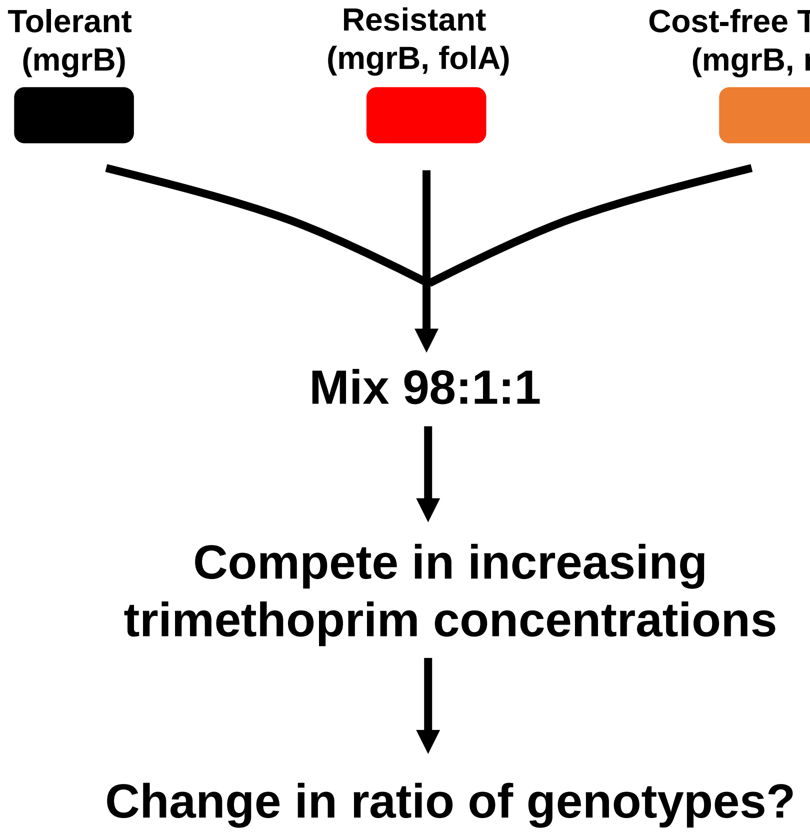
Strength of antibiotic pressure determines the most successful adaptive strategy



Tolerant (mgrB)

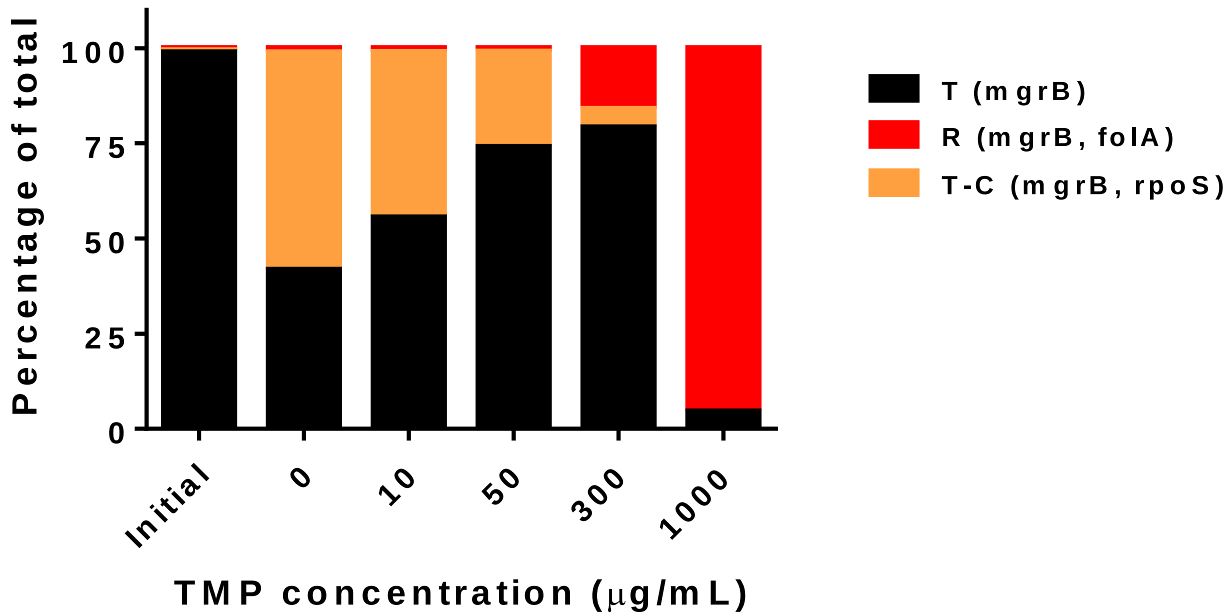
Resistant (mgrB, folA)

Cost-free Tolerant (mgrB, rpoS)

Mix 98:1:1

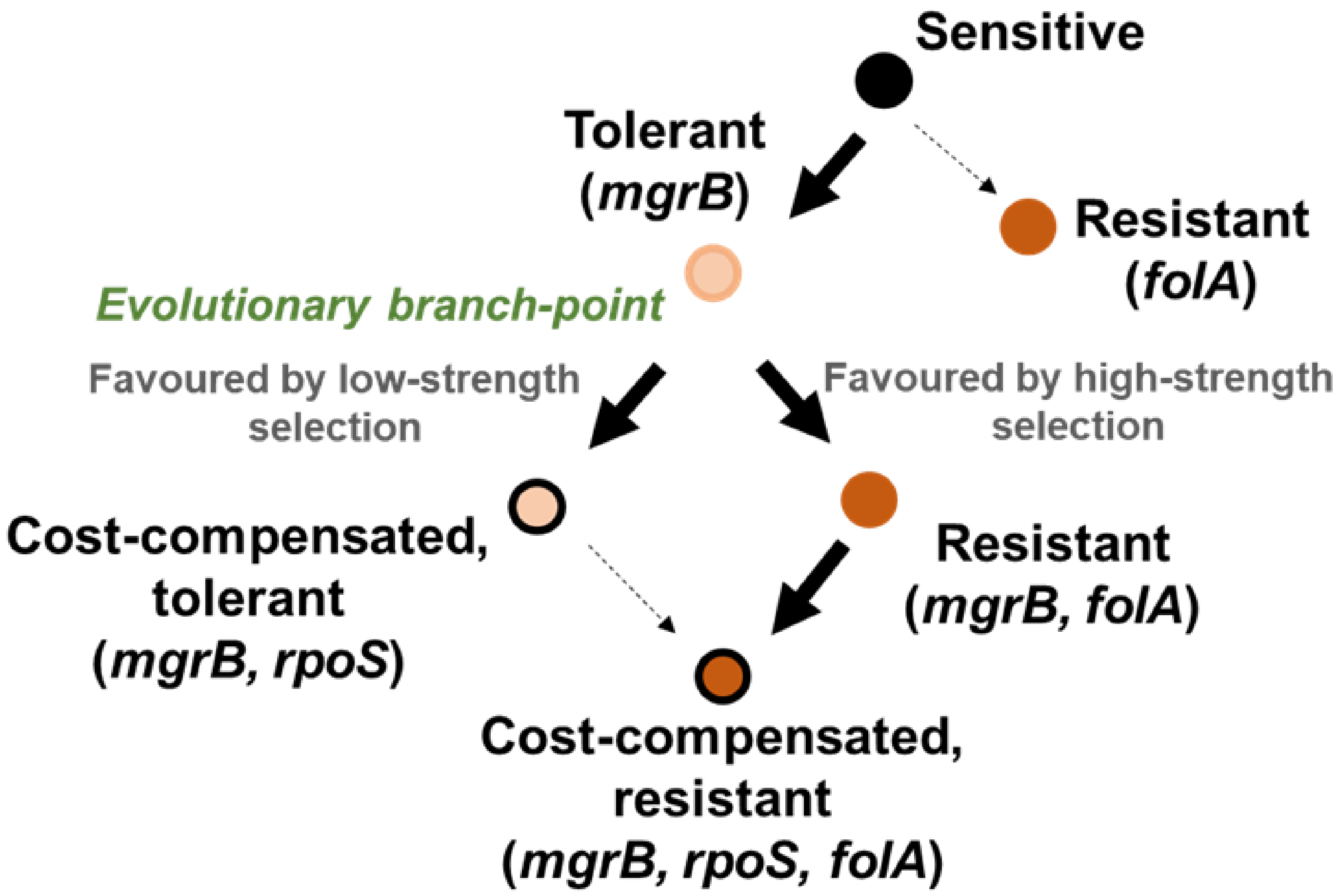
Compete in increasing trimethoprim concentrations

Change in ratio of genotypes?



Patel and Matange *eLife* (2021)

Phenotypic transitions during selection of resistance: Dependency on selection pressure



Sensitive

Tolerant (*mgrB*)

Resistant (*folA*)

Evolutionary branch-point

Favoured by low-strength selection

Favoured by high-strength selection

Cost-compensated, tolerant (*mgrB, rpoS*)

Resistant (*mgrB, folA*)

Cost-compensated, resistant (*mgrB, rpoS, folA*)

Patel and Matange *eLife* (2021)

Take home!

- **There are many ways to improve fitness in an environment**
- **Strength of selection determines which route will be preferred**
- **Adaptive strategies are not insulated from one another**
- **Genetic structures of adapting populations can be mechanistically explained**

Patel and Matange *eLife* (2021)