University of Pittsburgh School of Medicine

CENTER FOR EVOLUTIONARY BIOLOGY AND MEDICINE
CEBaM PITTSBURGH

Experimental evolution of antimicrobial resistance in *A. baumannii*

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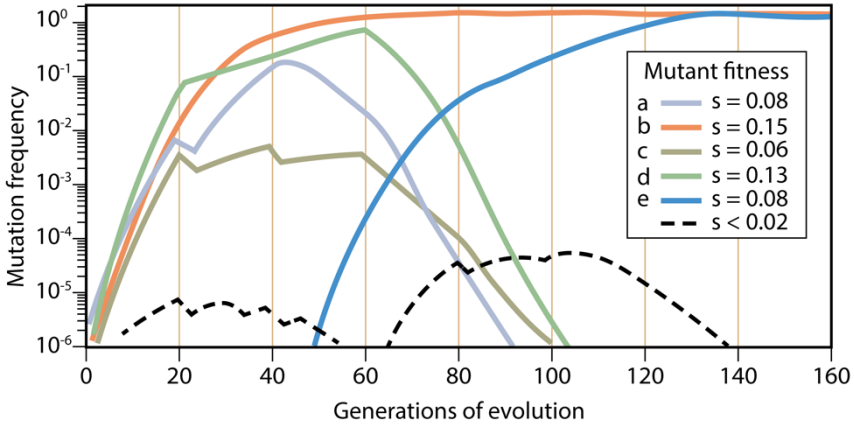
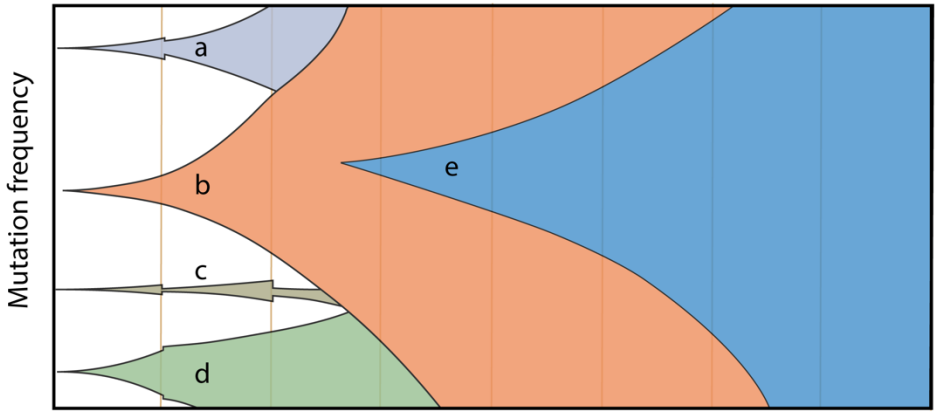
EVOLVINGSTEM

MiGS Microbial Genome Sequencing Center

Experimental evolution, combined with longitudinal population-wide WGS, is:

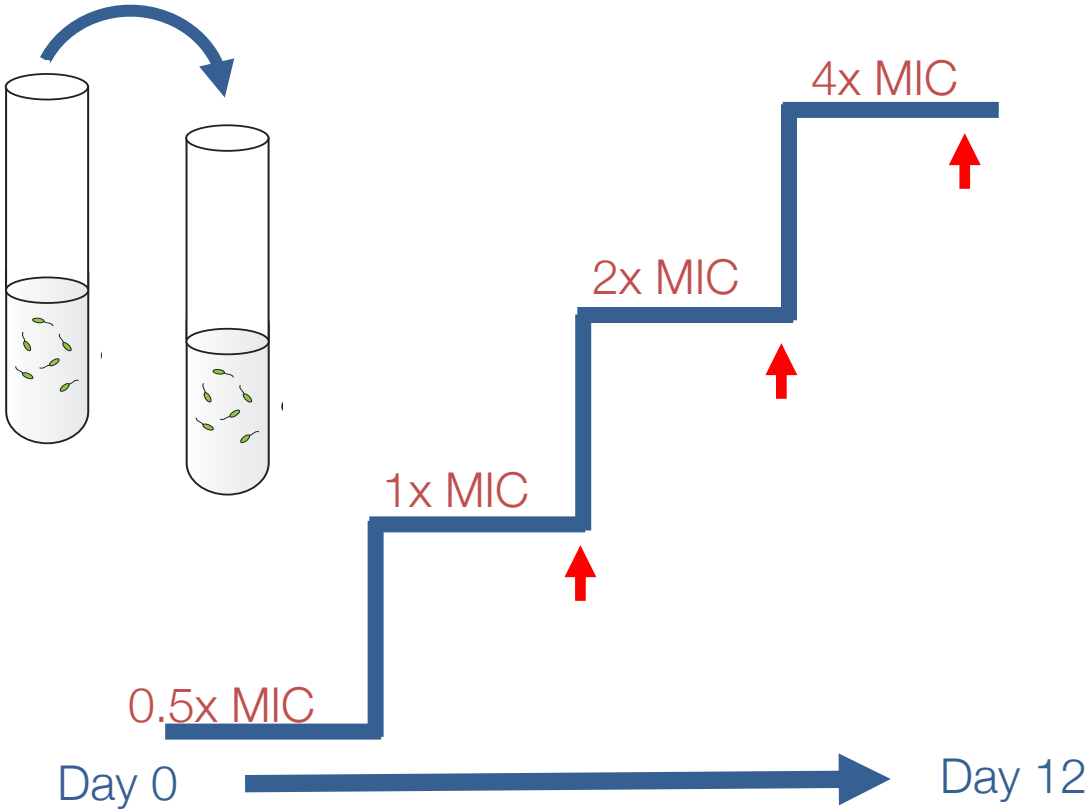
- A forward genetic screen for the best mutations in a given environment
- A method for quantifying relative fitness of competing genotypes





Cooper 2018 https://msphere.asm.org/content/3/3/e00121-18

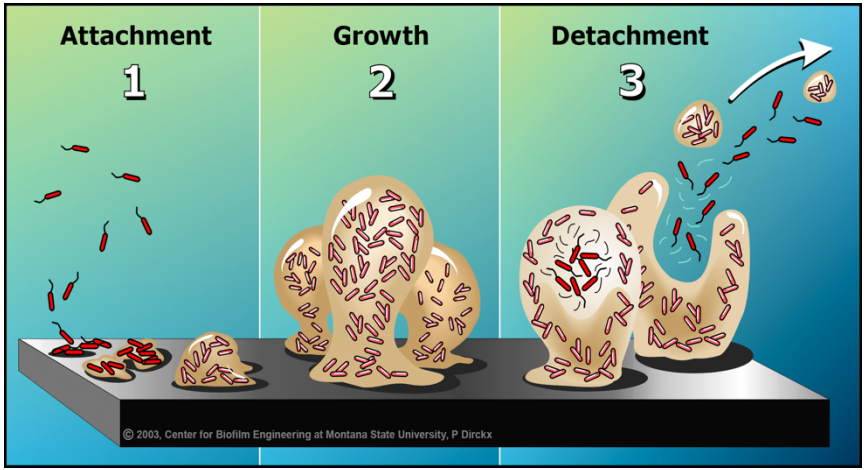
Evolution experiments in antibiotics



MIC =
Minimum
Inhibitory
Concentration

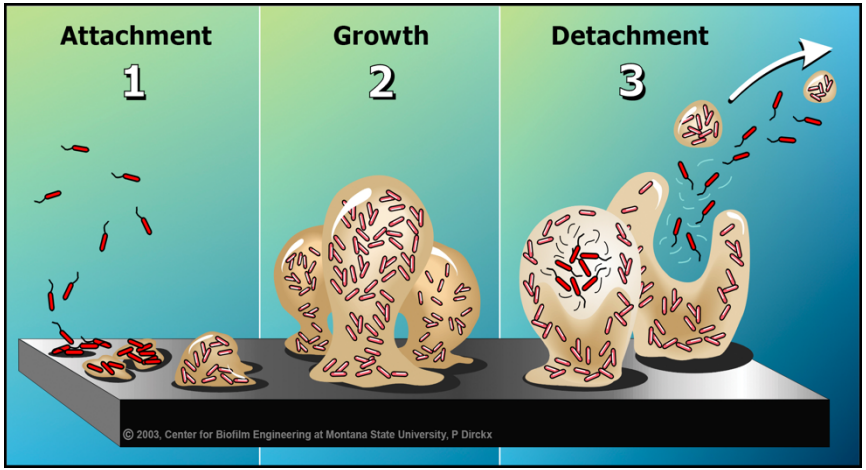
…combined with periodic whole population sequencing

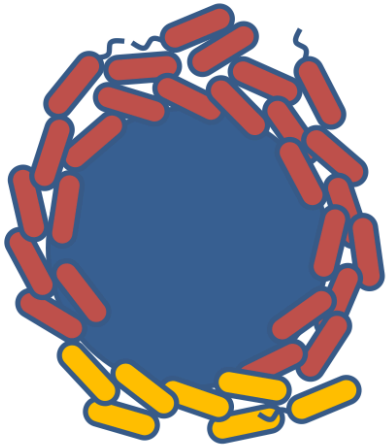
Bead transfer model produces strong biofilm selection



Biofilm

Bead transfer model produces strong biofilm selection





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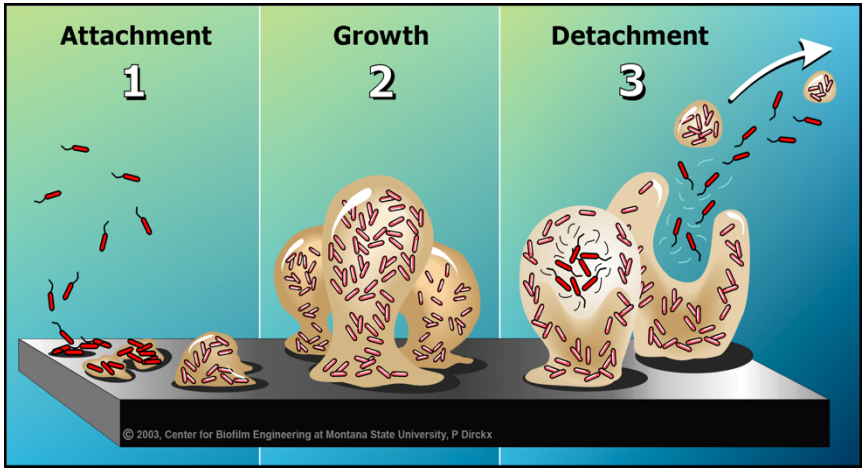

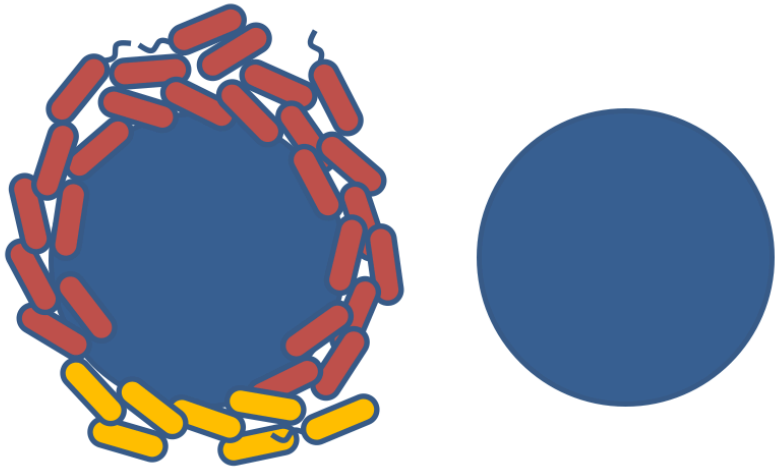

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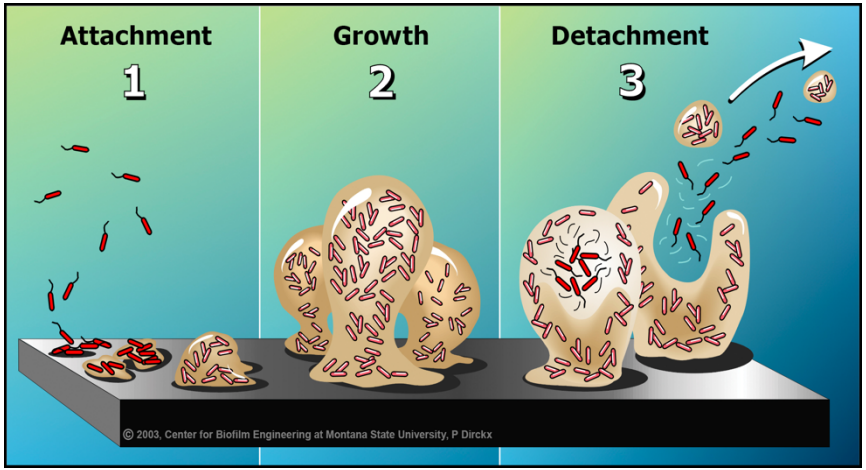
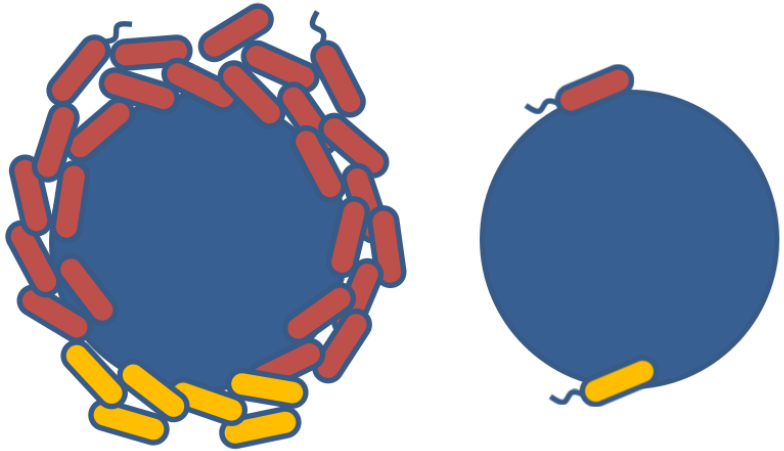
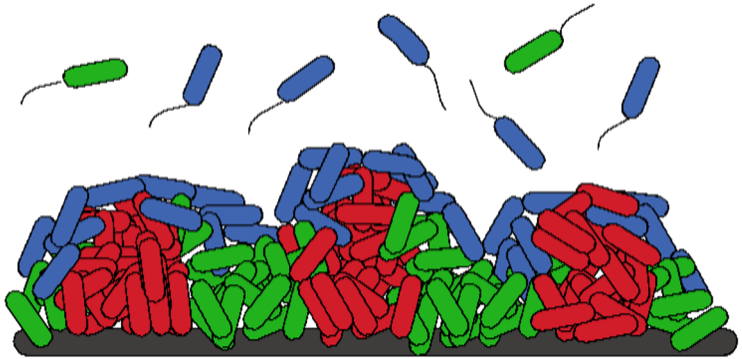




See protocol description and videos at http://evolvingstem.org

Biofilms can produce intrinsic tolerance to antibiotics



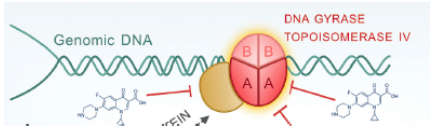
Matrix of exopolysaccharides, eDNA, and protein enabling cells to attach to surfaces

Hypothesis: lifestyle influences the dynamics and genetic routes to antimicrobial resistance

Experimental evolution of antibiotic resistance: Design

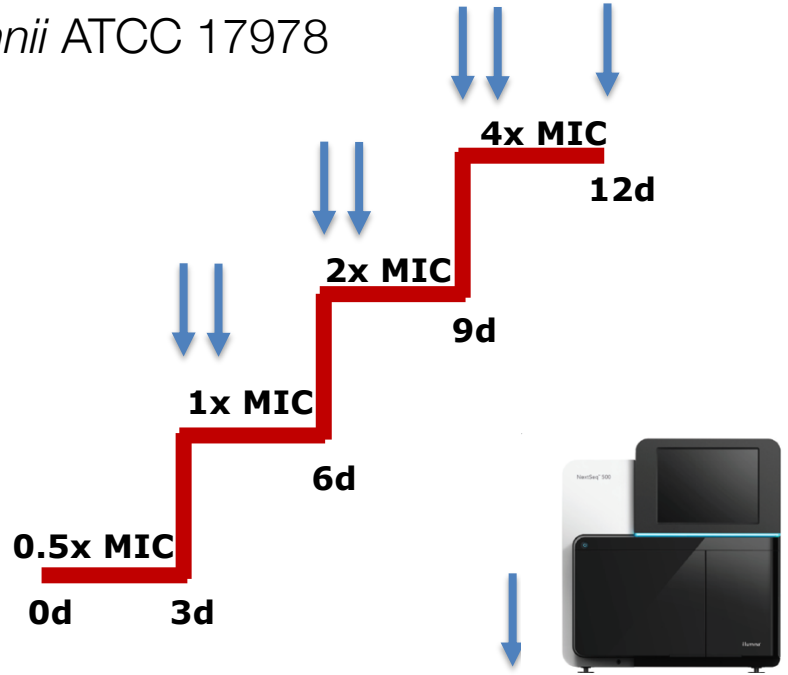
Acinetobacter baumannii ATCC 17978

CIP: Fluoroquinolone, topoisomerase inhibitor



Predictable target: *gyrA* S81/83L

Also: efflux-mediated

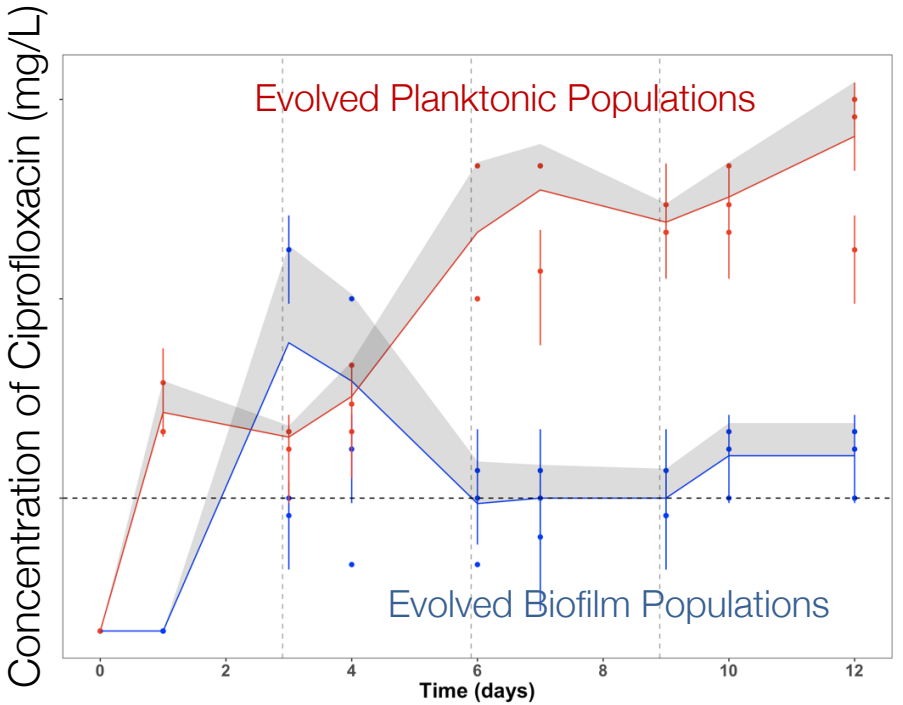


Experimental evolution of resistance: MICs

Planktonic = high resistance 2+ jumps

Biofilm = one low resistance jump

MIC increases in biofilm:
P: >1024x
B: 256x

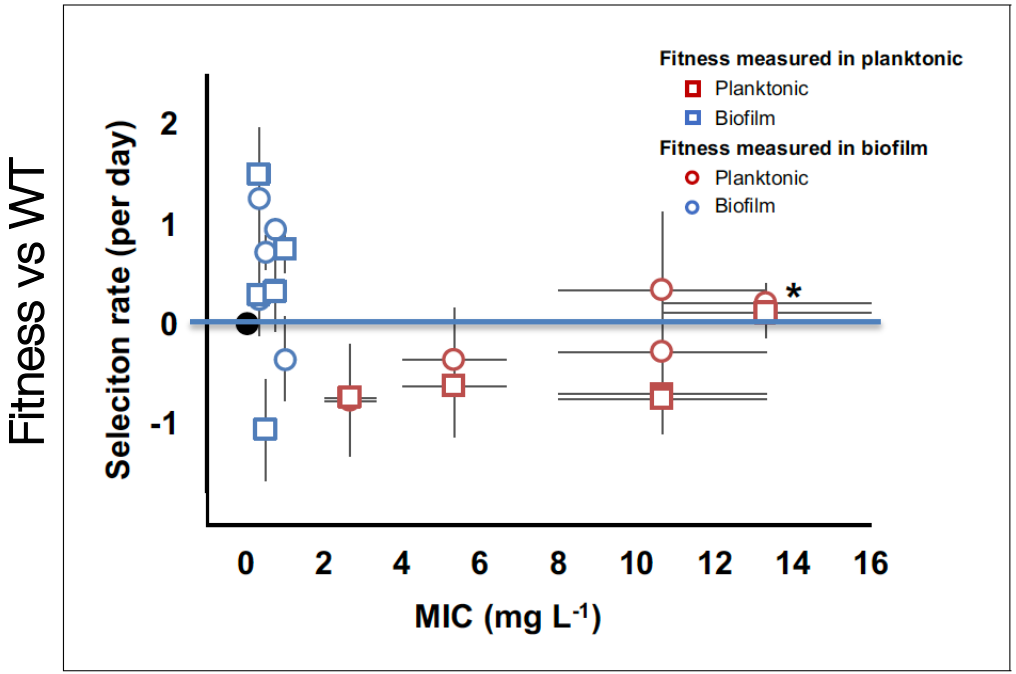


150x MIC

4x MIC

Santos-Lopez, Marshall et al. eLife 2019

Evolved tradeoff between resistance and fitness



Santos-Lopez et al, Marshall, et al, eLife 2019 https://elifesciences.org/articles/47612

Evolution of Antibiotic Resistance: Dynamics

Planktonic

Blues: *adeN*

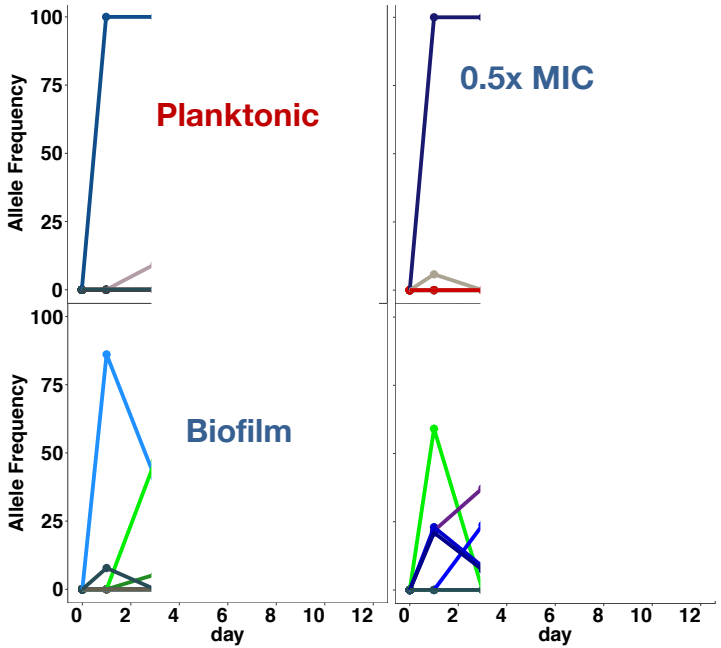
Biofilm

Greens: *adeL*

Blues: *adeN*

Purple: *adeS*

Ade = Acinetobacter Drug Efflux



Santos-Lopez, Marshall et al. eLife 2019

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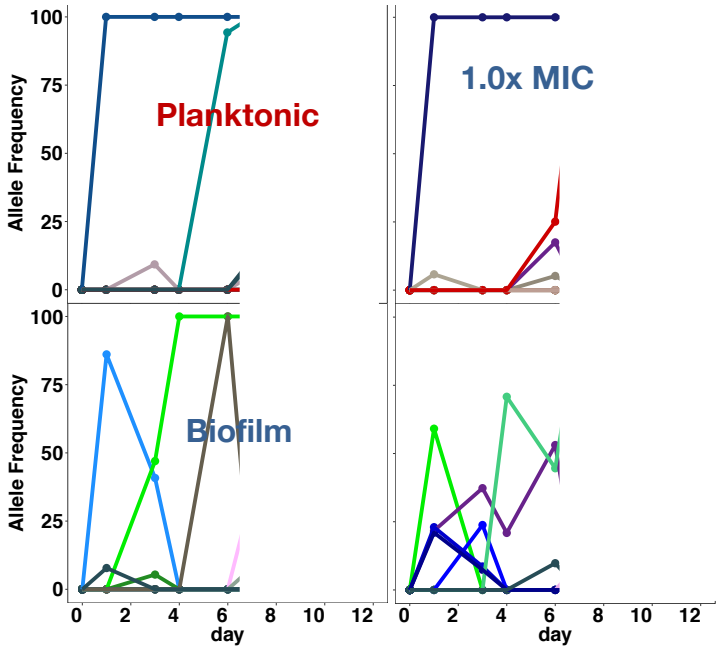

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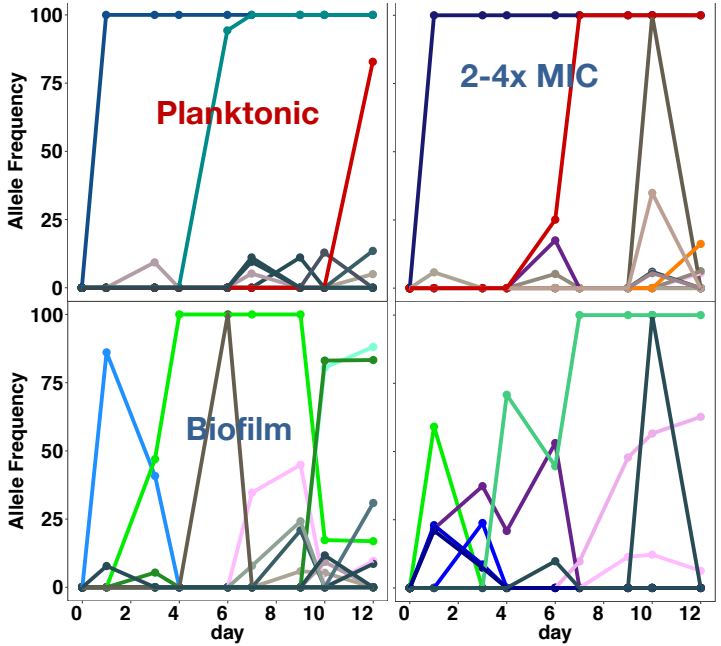

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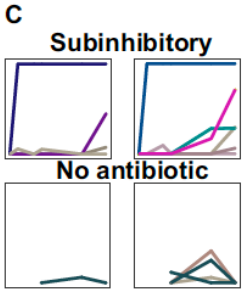
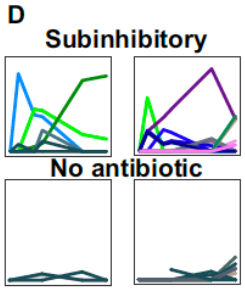




More diversity

Planktonic

Blues: *adeN*

Red: *gyrA*

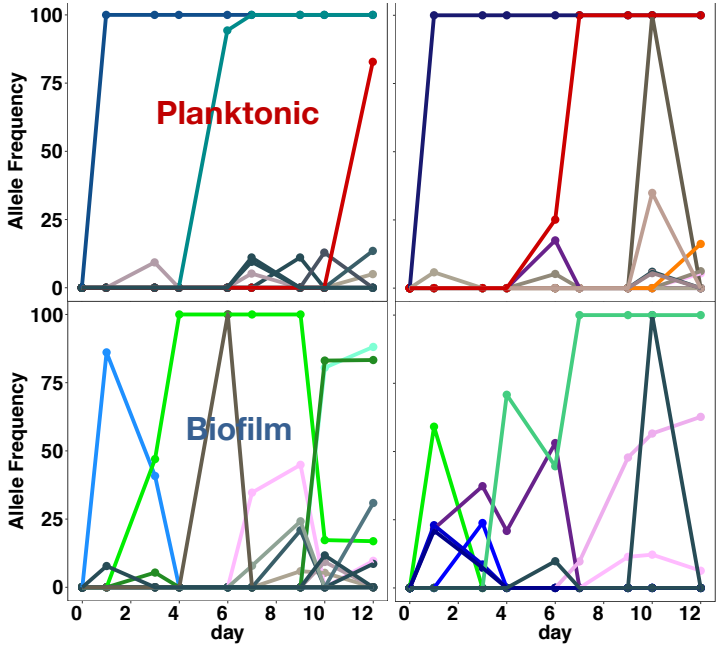
Biofilm

Greens: *adeL*

Blues: *adeN*

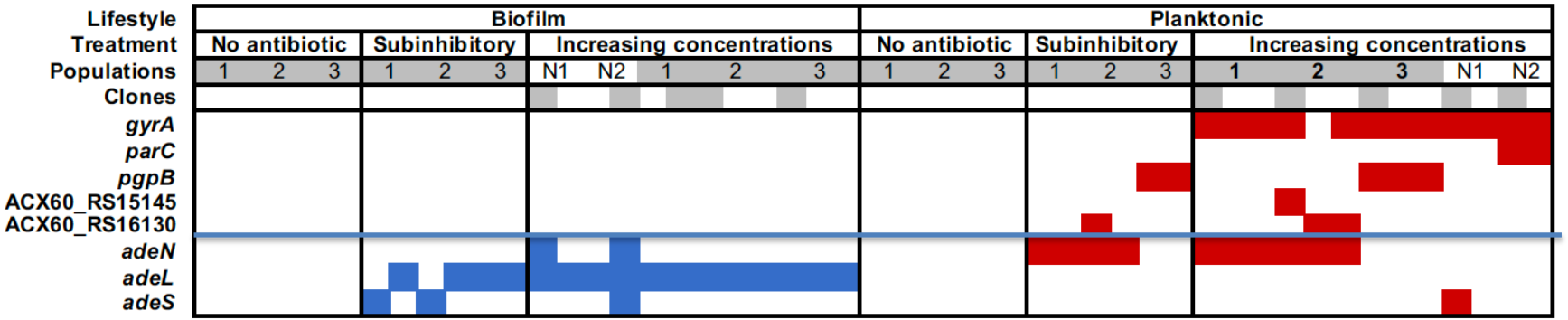
Purple: *adeS*

Ade = Acinetobacter Drug Efflux



More diversity

Evolution of Antibiotic Resistance: Genotypes differ w/ Lifestyle

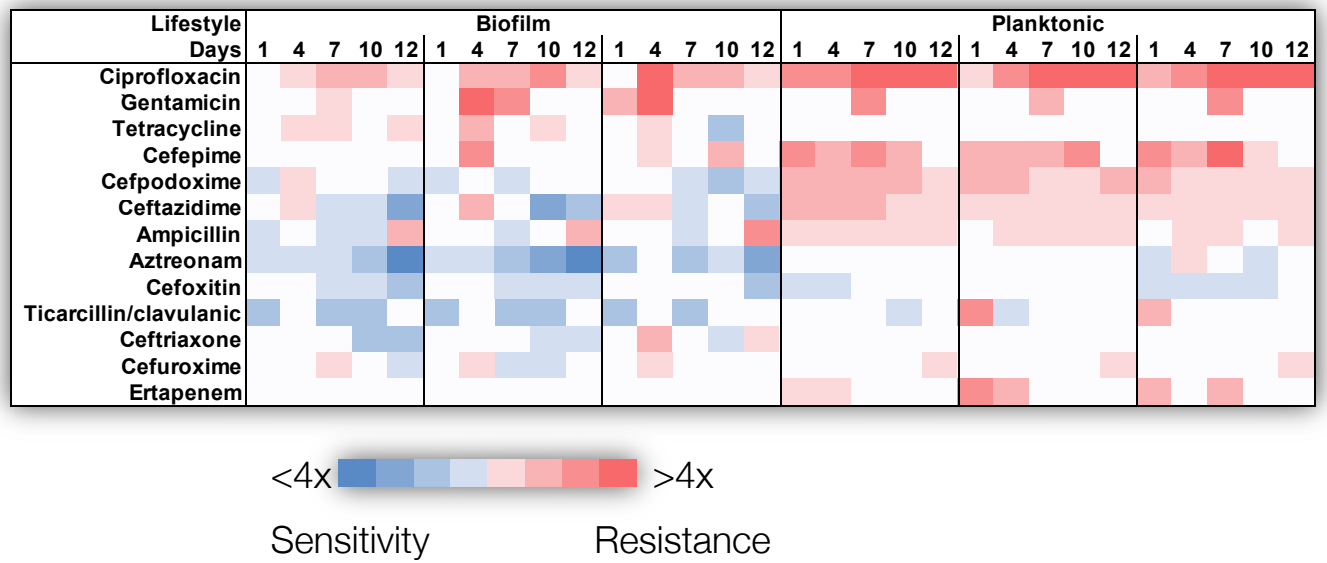


adeL and adeS dominate

gyrA and adeN dominate

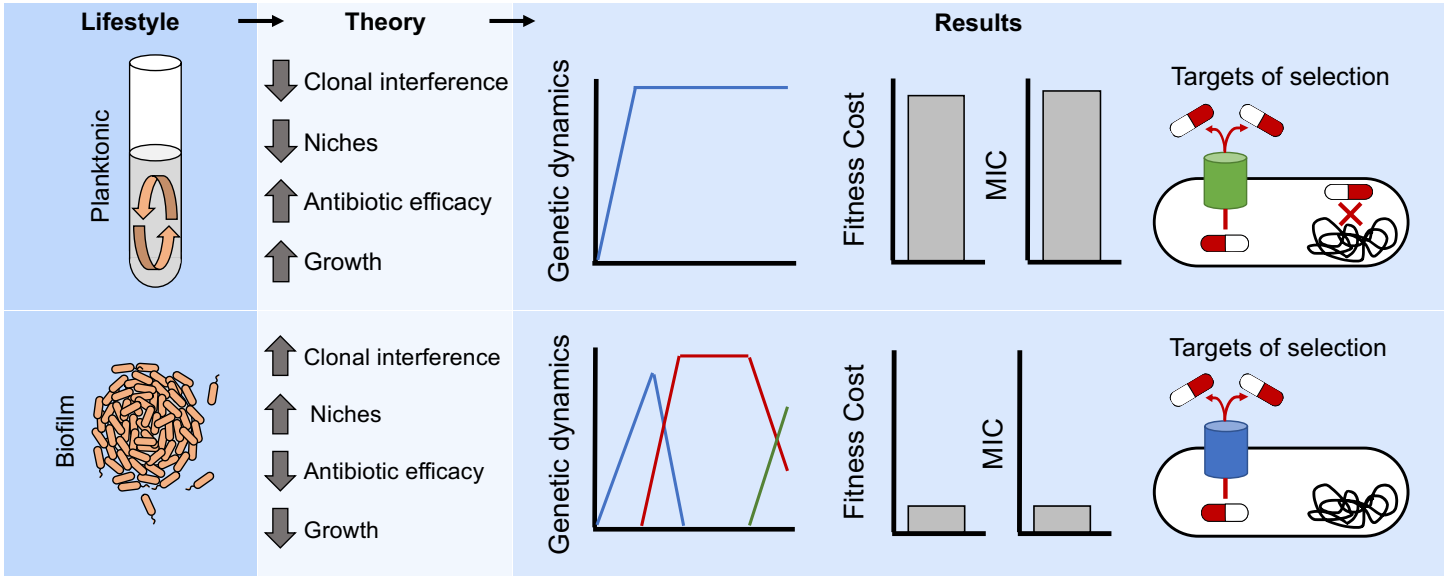
Evolution of Antibiotic Resistance: Interactions

Biofilm-evolved populations evolved collateral sensitivity to β-lactams



Lifestyle affects collateral effects: *adeN/gyrA* = resistance, *adeL* = sensitivity

Selection (predictably) acts on different mutations with different effects in different environments



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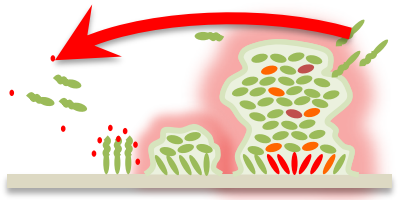

Why resistance evolution in biofilms is different

1. Environmental structure increases genetic diversity
2. Greater ecological heterogeneity = multiple niches
3. Biofilms provide intrinsic protection to external stresses like antibiotics

Related project about aminoglycoside resistance evolution in *A. baumannii* and *P. aeruginosa*, see:

Parallel evolution of tobramycin resistance across species and environments

Michelle R. Scribner, Alfonso Santos-Lopez, Christopher W. Marshall, Christopher Deitrick, Vaughn S. Cooper

doi: https://doi.org/10.1101/758979

Now accepted for publication in *mBio*