COLLABORATIVE RESEARCH CENTER 1310

Predictability in Evolution

PROBABILISTIC STRUCTURES
IN EVOLUTION

DFG SPP 1590

Evolutionary pathways to antibiotic resistance

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Evolution of antibiotic resistance

- Resistance evolution is a universal response of microbial pathogens to biomedical interventions

- Evolutionary considerations are key for developing strategies that prevent or delay resistance evolution in clinical or environmental settings

- At the same time microbial resistance evolution serves as a model system for addressing broader questions of evolutionary theory

Evolution of antibiotic resistance

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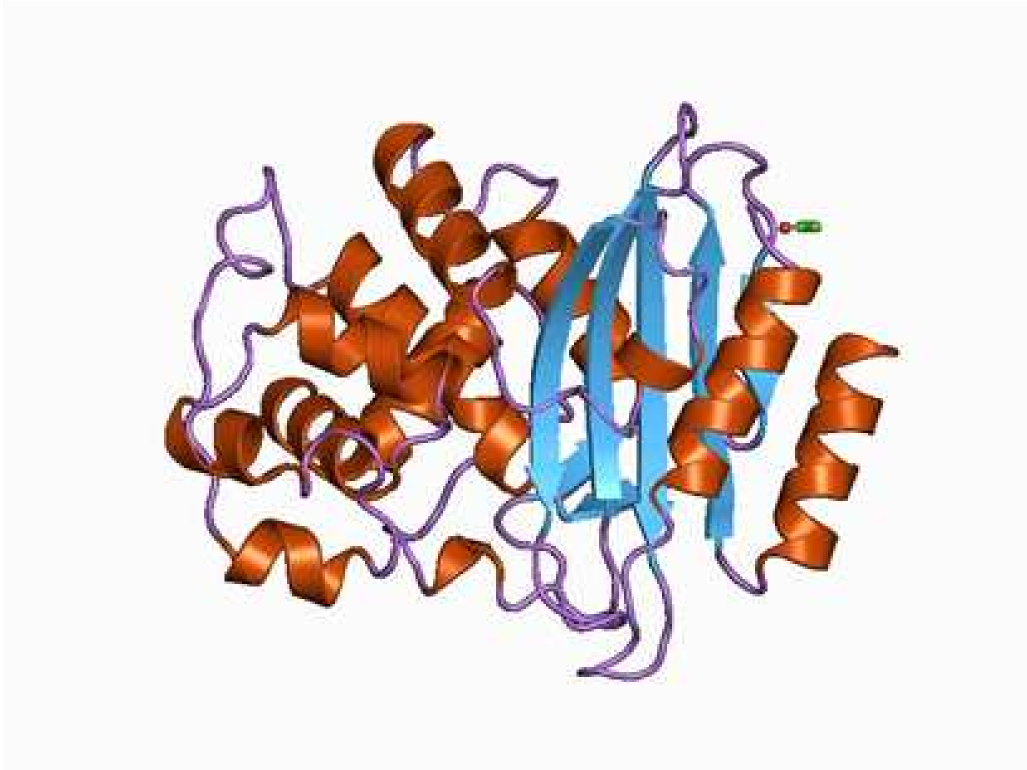
Two case studies

- The resistance landscape of TEM-1 β-lactamase
  joint work with de Visser lab (Wageningen)
- Concentration-dependent evolution of ciprofloxacin resistance
  joint work with Bartek Waclaw and Rosalind Allen (Edinburgh)

Quantifying antibiotic effects

- The effect of a drug is quantified by the dose-reponse curve, the growth rate of a (large) bacterial population as a function of drug concentration
- At the minimal inhibitory concentration (MIC) the growth rate drops to zero
- Resistance mutations increase the MIC
- The combination of multiple mutations along an evolutionary pathway leads to highly resistant strains

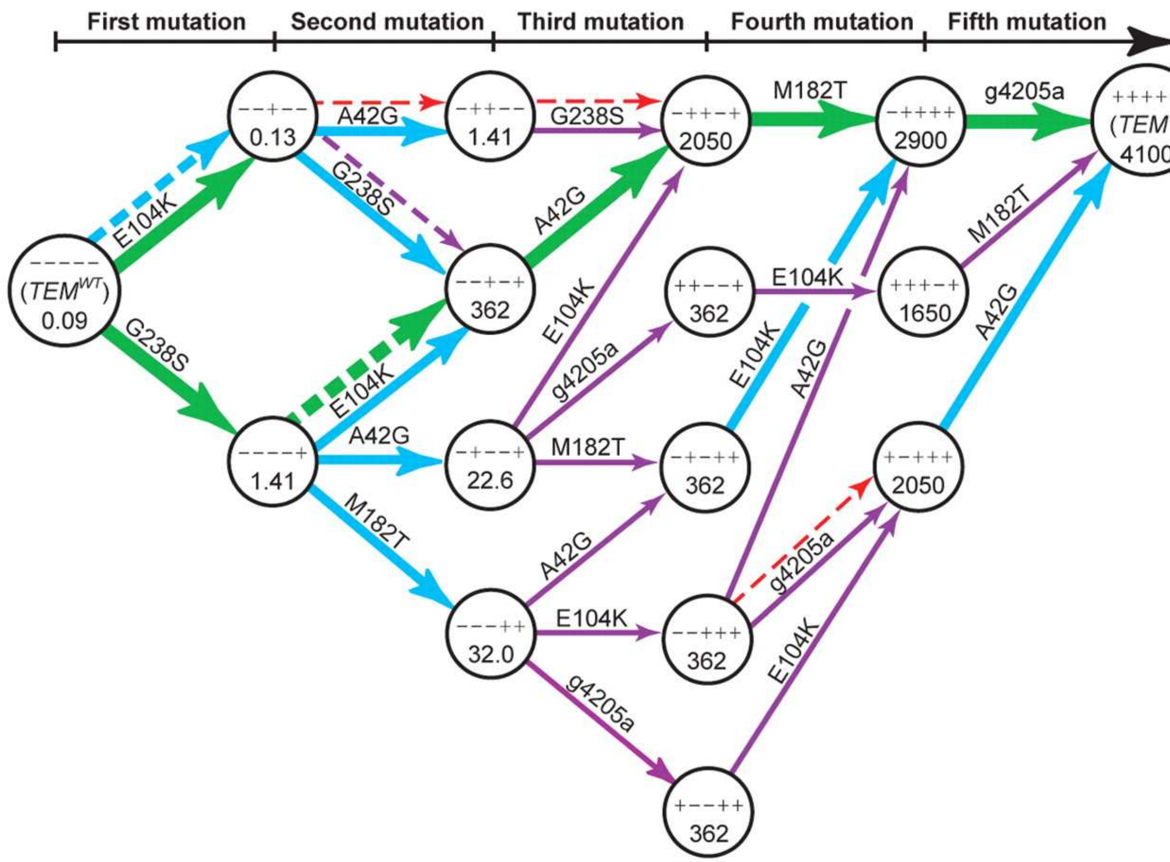
TEM-1 β-lactamase



- β-lactam antibiotics such as penicillin target cell wall synthesis
- TEM-1 β-lactamase confers resistance against ampicillin to *E. coli*
- Experiments study adaptation to novel antibiotic cefotaxime

Pathways to TEM-1 resistance

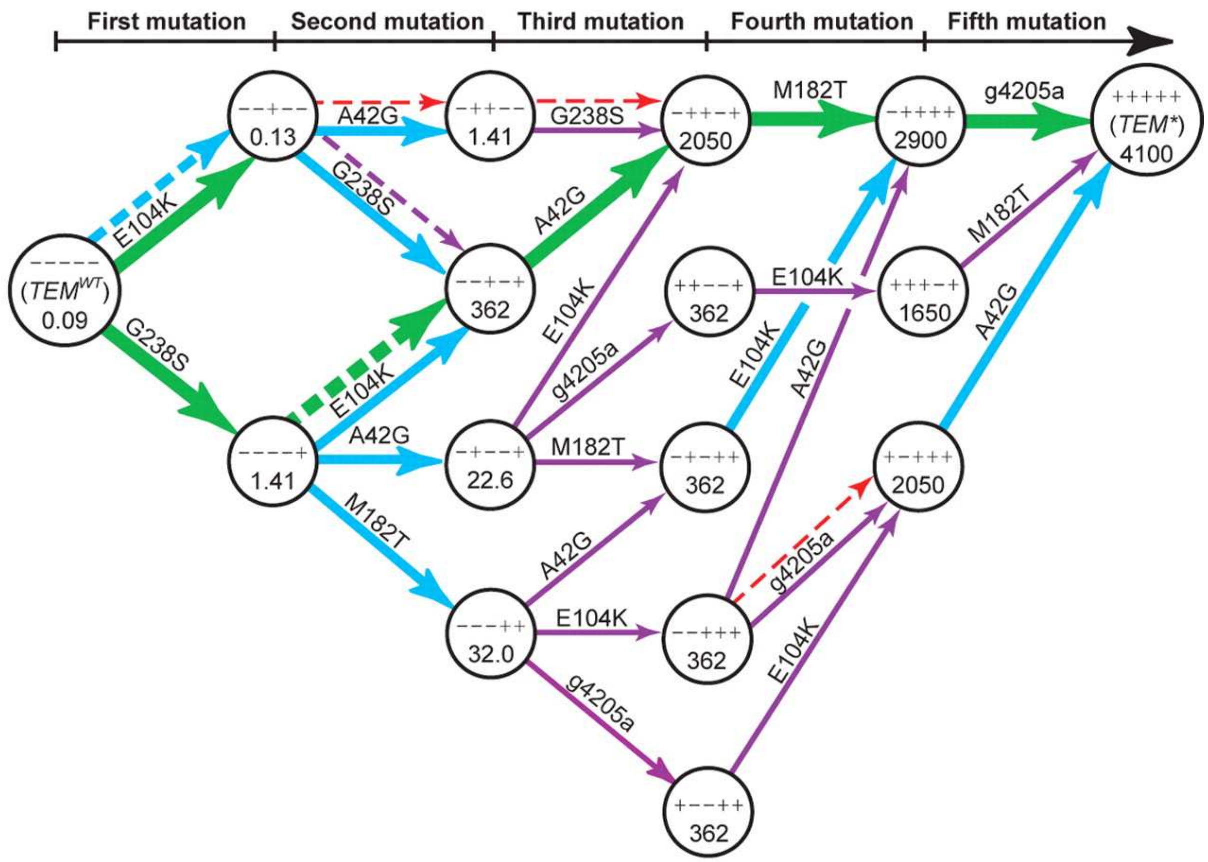
D.M. Weinreich et al., Science **312**, 111 (2006)



- 5 mutations increase the MIC by 4×10^4
- Construct all $2^5 = 32$ combinatorial mutants

Pathways to TEM-1 resistance

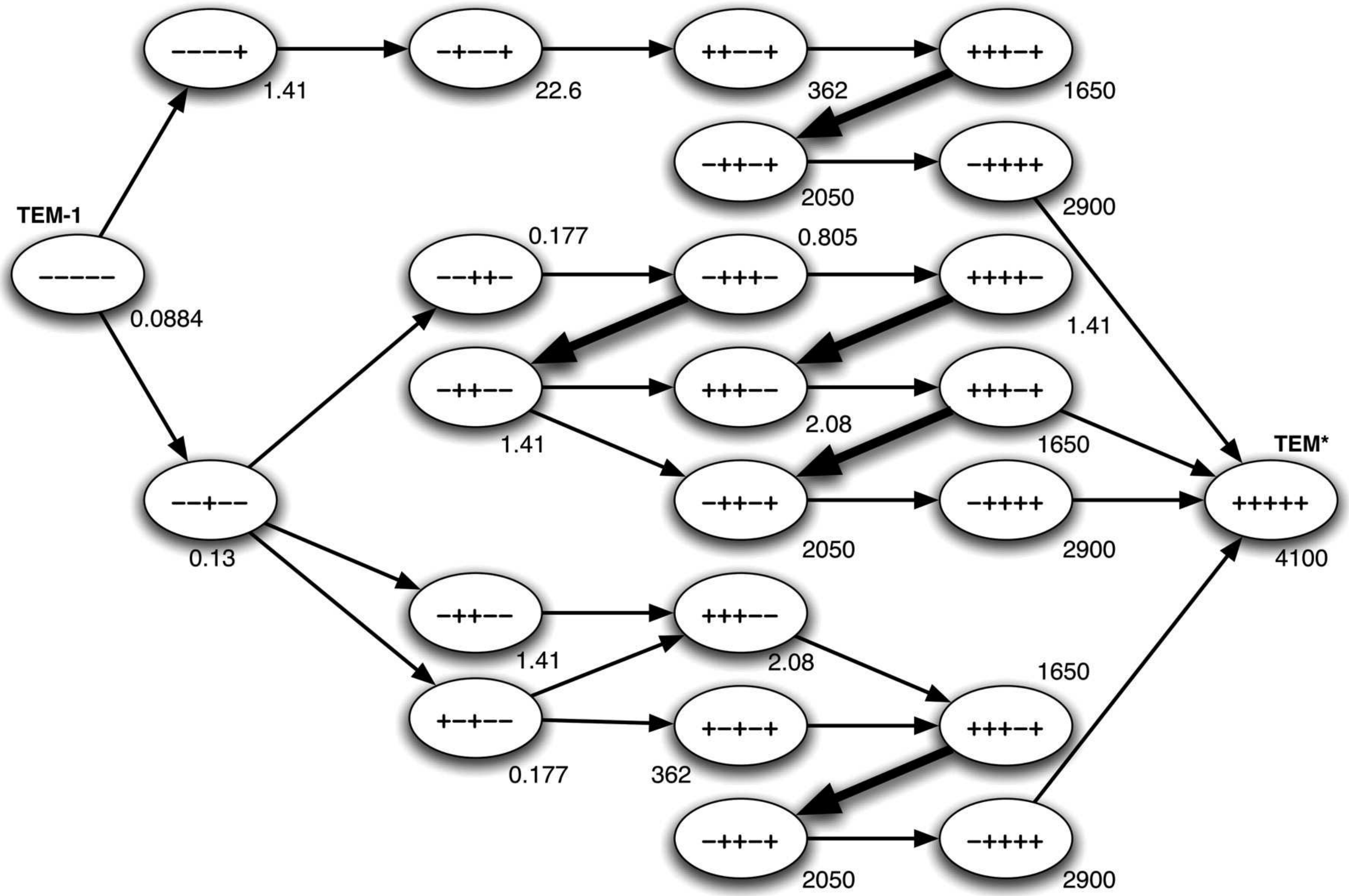
D.M. Weinreich et al., Science **312**, 111 (2006)



- Only 18 out of $5! = 120$ directed mutational pathways are monotonically increasing in resistance, and only a few of them have appreciable weight

Pathways to TEM-1 resistance

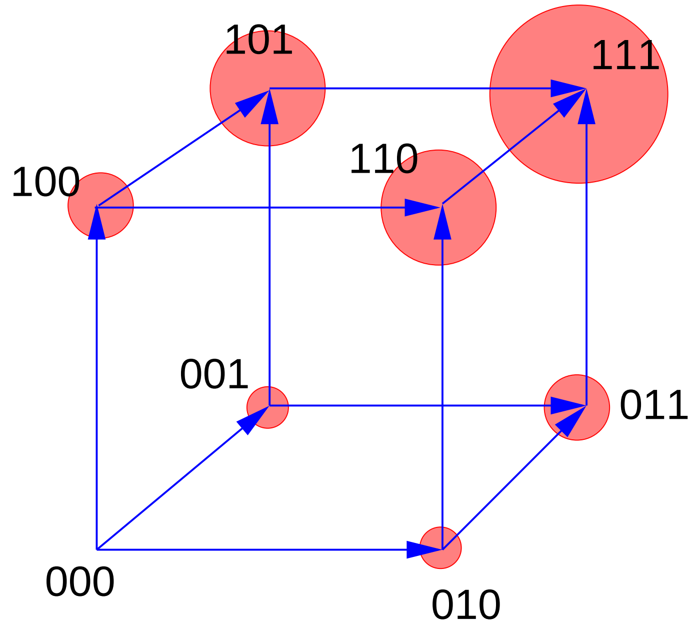
De Pristo et al. 2007



- 27 out of 18651552840 undirected pathways are monotonically increasing

Mathematical framework

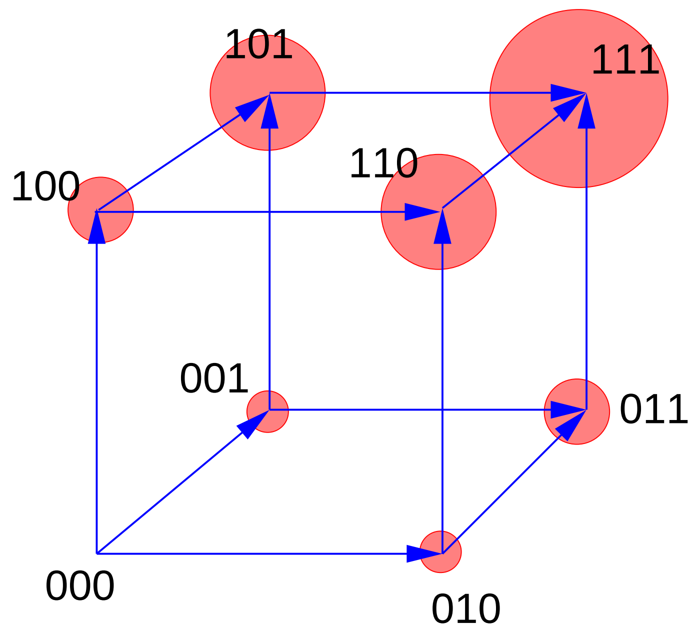
Pathways in fitness landscapes



- Genotypes are encoded by binary sequences $(\sigma_1, \ldots, \sigma_L)$ where $\sigma_i = 1$ ($\sigma_i = 0$) denotes the presence (absence) of a mutation at position i
- A fitness or resistance landscape is a function on the L-dimensional hypercube $\{0,1\}^L$ of genotypes
- The fitness graph is obtained by orienting the links in the direction of increasing fitness

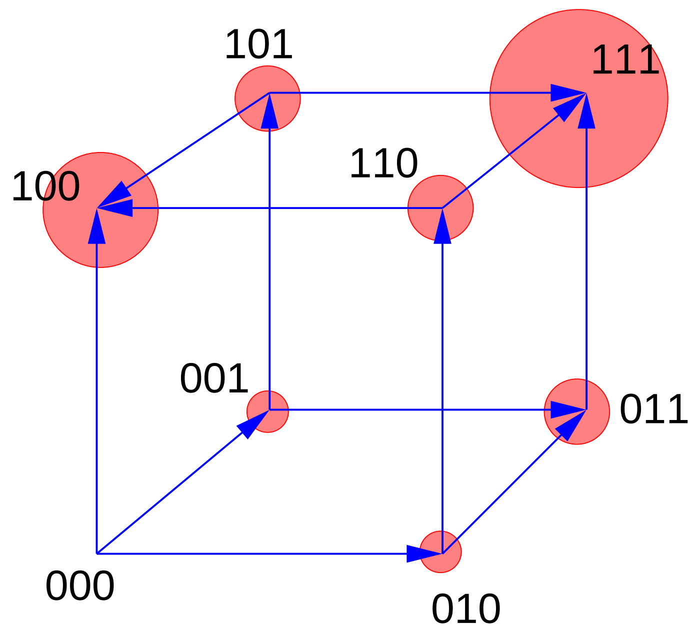
Crona et al. 2013

Pathways in fitness landscapes



- $L = 3$ mutational steps from the wild type 000 to the adapted mutant 111
- Mutations can occur in $3 \times 2 \times 1 = 3! = 6$ different orders corresponding to 6 possible directed pathways
- If all mutations are unconditionally beneficial all pathways are accessible (= increasing in fitness)

Pathways in fitness landscapes



- Sign epistasis occurs if mutations can be beneficial or deleterious depending on the genetic context Weinreich et al. 2005
- This implies that parallel arrows in the fitness graph point in opposite directions
- Sign epistasis reduces the number of direct accessible paths but may increase the number of evolutionary endpoints

Accessibility and predictability

- Pathways are accessible if fitness/resistance increases monotonically

- Existence of a small but nonzero fraction of accessible pathways implies high (retrospective) predictability

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Questions for theory

- How does accessibility depend on the structure of the fitness landscape and on the boundary conditions of the paths?
- How typical is it that a small but nonzero fraction of pathways are accessible?

Accessibility percolation

JK, arXiv:1903.11913

- Null model: assign fitness at random to genotypes — Kauffman & Levin 1987

- Probability of existence of accessible paths generically displays a sharp percolation transition from 0 to 1 at a critical value β^* of the fitness quantile $\beta \in [0,1]$ between initial and final genotype

- For directed paths on the hypercube $\beta^* = 1 - \frac{\ln L}{L} \to 1$ for $L \to \infty$
  Hegarty & Martinsson 2014

- Mutational reversions increase accessibility such that $\beta^* < 1$ for $L \to \infty$
  Berestycki et al. 2017

- For sequences with a alleles per site — B. Schmiegelt, JK, 2019

$$\beta^* \approx \frac{\ln a}{a} + \frac{1 + \ln a}{a^2} \to 0 \quad \text{for} \quad a \gg 1$$

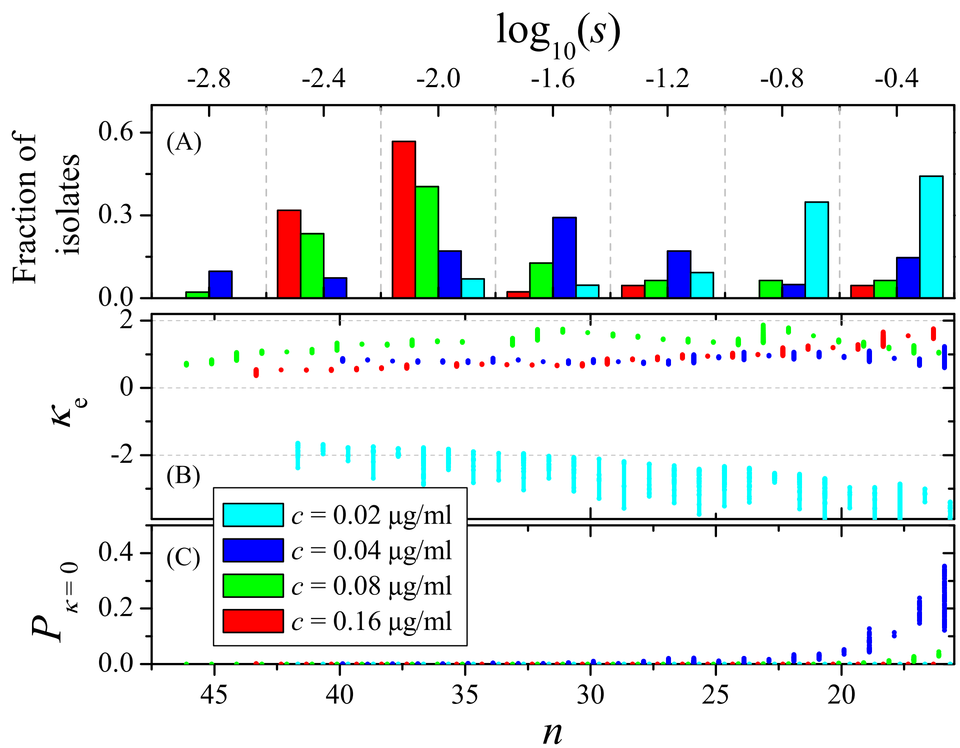
- Near $\beta = \beta^*$ the number of accessible paths is small and hence predictability is high

Exploring the TEM-1 resistance landscape

A panel of resistance mutations

M.F. Schenk et al., PLoS Genet. 2012

- At least 48 out of 2583 point mutations increase resistance against cefotaxime



- Analysis using extreme value theory yields power law distribution of mutational effects with an exponent ~ 1

Construction of combinatorial resistance landscapes

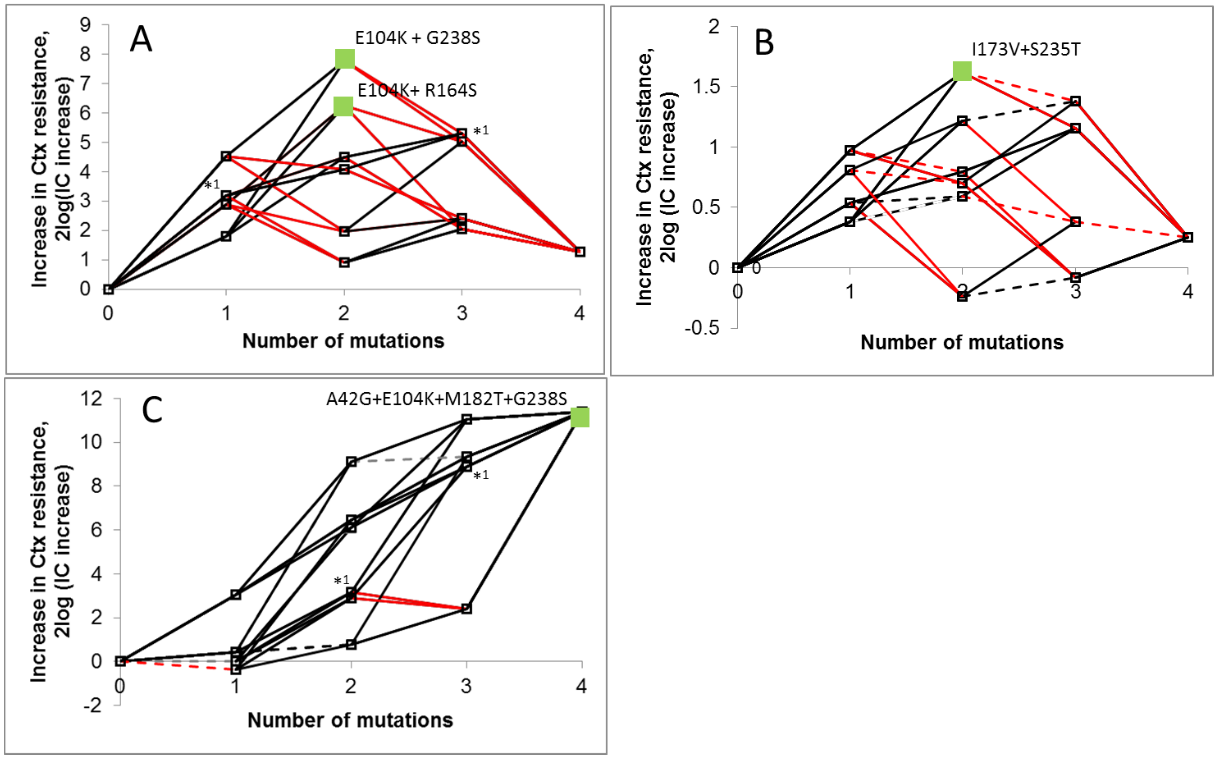
- Constructing all possible $2^{48} \approx 2.8 \times 10^{14}$ combinatorial mutants is obviously unfeasible

- The choice of a subset of mutations is expected to bias the structure of the fitness landscape: de Visser & Krug, Nat. Rev. Genet. 2014
  - singly beneficial vs. singly deleterious mutations
  - mutations chosen for individual or collective effects
  - mutations occurring along an adaptive trajectory

- Here we consider 4-dimensional landscapes constructed from three subsets of individually beneficial mutations: Schenk et al. 2013
  - non-synonymous mutations of strong effect
  - non-synonymous mutations of weak/typical effect
  - synonymous mutations of strong effect

Mutations chosen for individual vs. collective effect

M.F. Schenk et al., Mol. Biol. Evol. (2013)

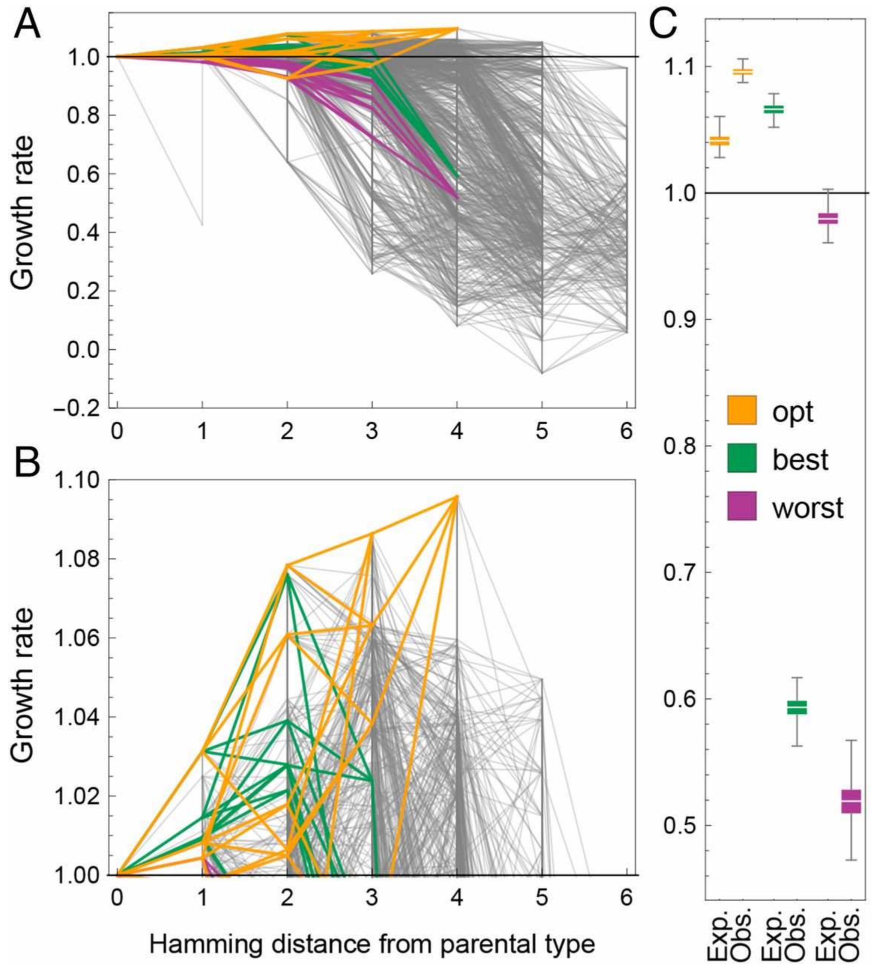


A: Large effect B: Small effect C: Weinreich 2006

- Mutations chosen for individual effect interact more strongly and negatively than mutations chosen "with hindsight" because of their collective effect

Similar patterns observed in yeast

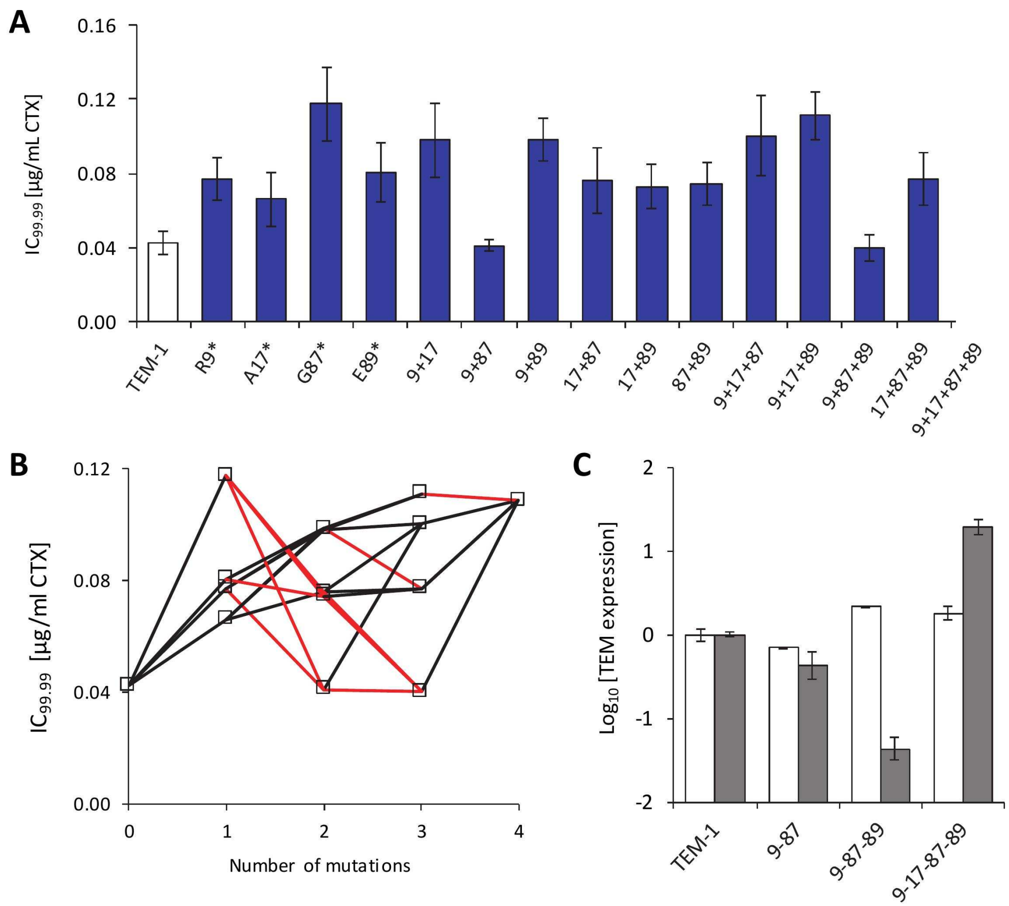
Bank et al., PNAS 2016



- Combinatorial study of 13 mutations in the Hsp90 heat shock protein

Synonymous resistance landscape

M.P. Zwart et al., Heredity (2018)



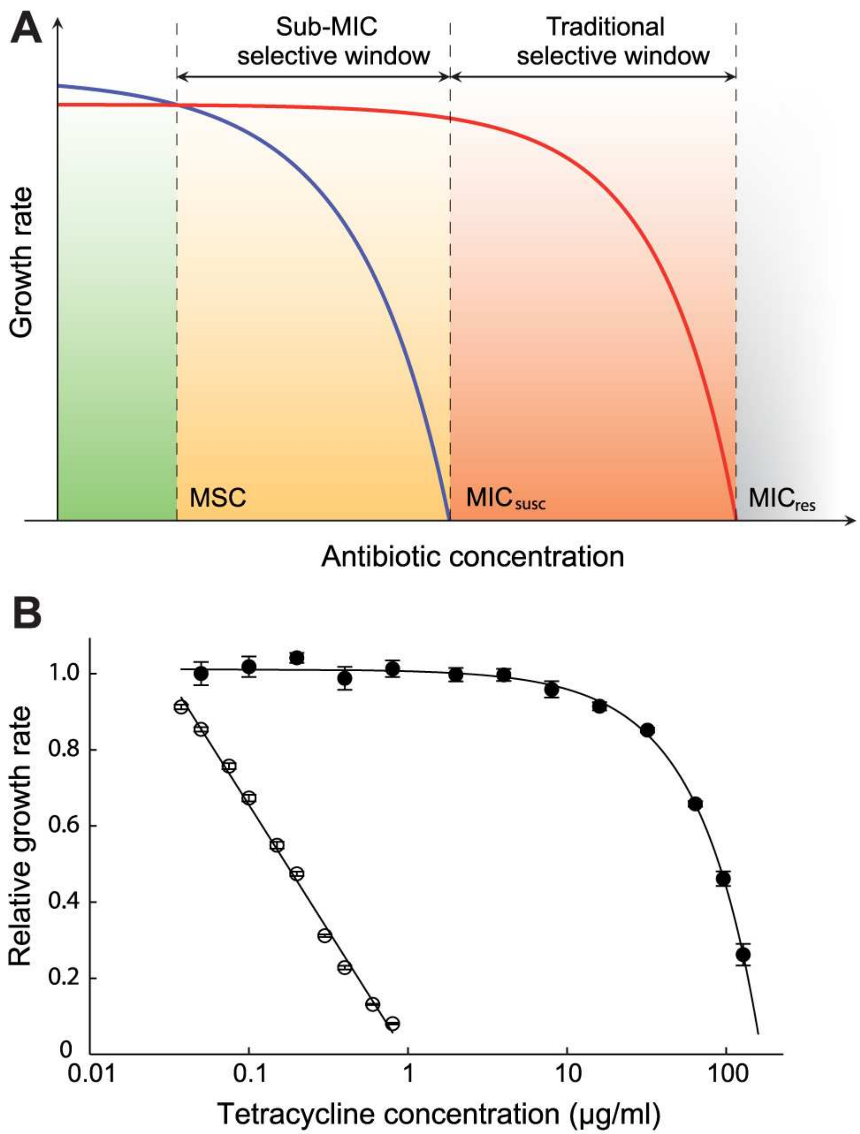
- Landscape displays a “layered” structure that may be related to translational bottlenecks

M. Josupeit, JK, arXiv:2009.10621

Concentration-dependent fitness landscapes

S. Das, S. Direito, B. Waclaw, R. Allen, JK, eLife 9:e55155 (2020)

Dose-response curves



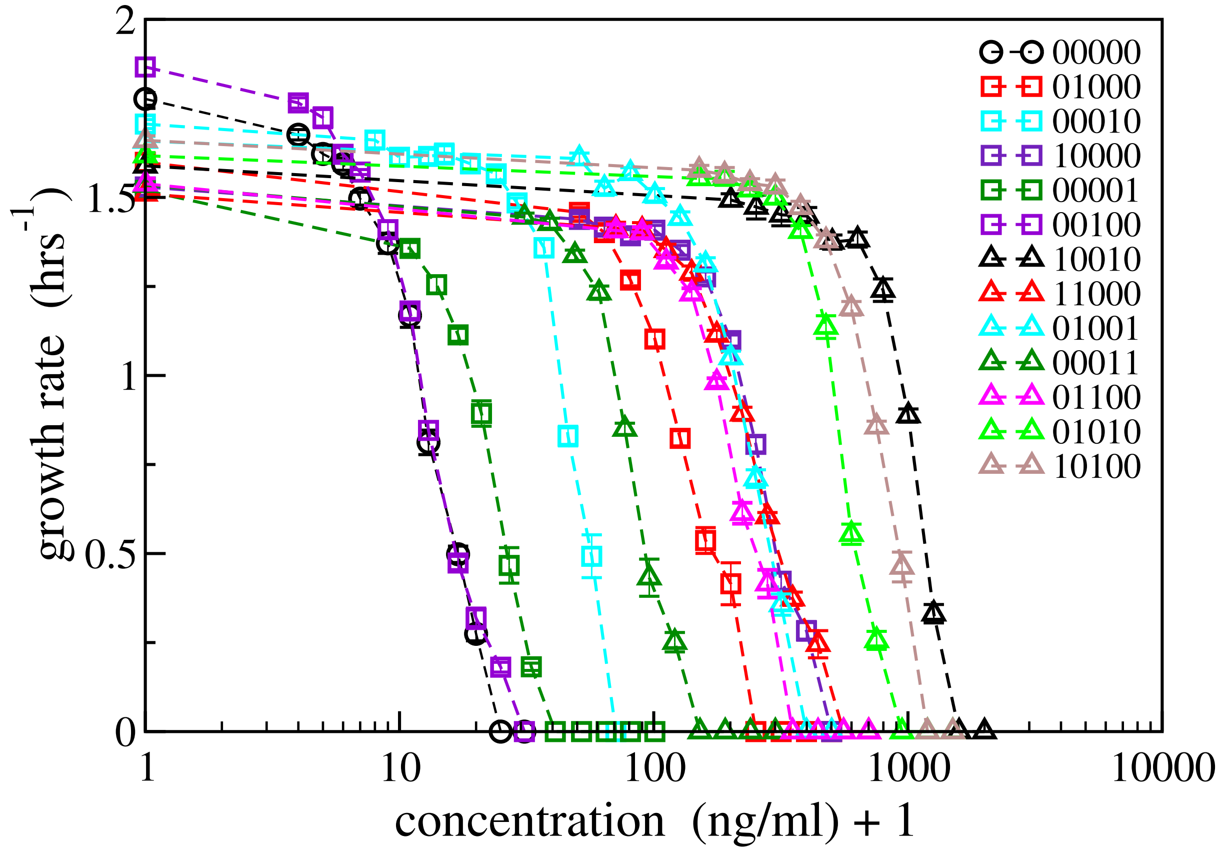
Gullberg et al., PLoS Pathogens 2011

- Mutations that increase resistance often decrease growth rate in the absence of antibiotic (null fitness)
- As a consequence the dose-response curves of susceptible and mutant strains cross at the minimal selective concentration (MSC)
- The mutant selection window is the concentration range

$$\text{MSC} < c < \text{MIC}_{\text{res}}$$

Observation 1: Scaling of dose-response curves

- Single and double mutations in *Escherichia coli* conferring resistance against ciprofloxacin S. Direito, B. Waclaw, R. Allen



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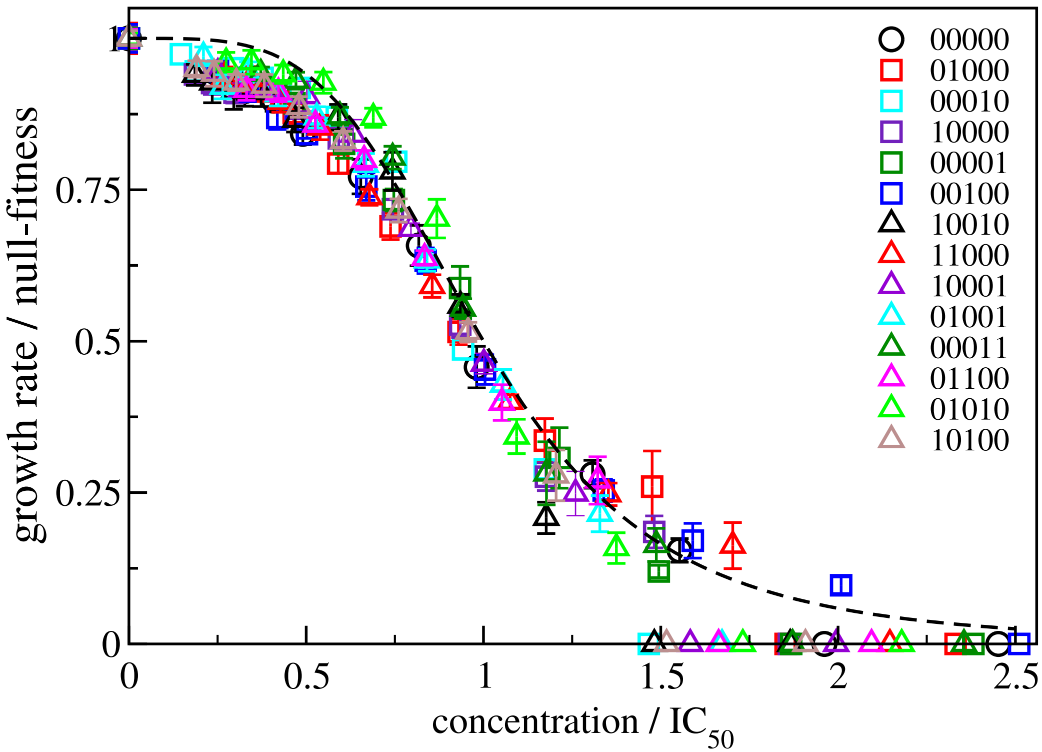


- Shape of dose-response curve is a Hill function $f(x) = (1 + x^4)^{-1}$

Observation 2: Independent marginal phenotypes

- Null-fitness and MIC of multiple mutants combine multiplicatively or display negative interactions data from Marcusson et al., PLoS Pathogens 2009

Strain	String	log(null-fitness)	Non-epistatic	log(MIC)	Non-epistatic
MG1655	00000	0.00 (± .004)		0.00 (± .35)	
LM378	10000	0.01 (± .016)		3.17 (± .70)	
LM534	01000	-0.01 (± .018)		2.75 (± .70)	
LM202	00010	-0.19 (± .020)		0.69 (± .70)	
LM351	00001	-0.094 (± .014)		1.08 (± .70)	
LM625	11000	-0.030 (± .011)	0.0 (± .038)	3.17 (± .70)	5.92 (± 1.1)
LM421	10010	-0.15 (± .019)	-0.18 (±.040)	4.13 (± .70)	3.56 (± 1.1)
LM647	10001	-0.051 (± .013)	-0.084 (± .034)	3.44 (± .70)	4.65 (± 1.1)
LM538	01010	-0.19 (± .020)	-0.20 (± .042)	4.13 (± .70)	3.46 (± 1.1)
LM592	01001	-0.083 (± .015)	-0.10 (± .036)	3.16 (± .70)	3.83 (± 1.1)
LM367	00011	-0.20 (± .026)	-0.28 (± .038)	2.06 (± .70)	1.77 (± 1.1)
LM695	11010	-0.24 (± .017)	-0.19 (± .058)	3.85 (±. 70)	6.61 (± 1.1)
LM691	11001	-0.073 (± .013)	-0.094 (± .052)	3.85 (±. 70)	7.00 (± 1.4)
LM709	10011	-0.24 (± .027)	-0.274 (± .054)	4.54 (±. 70)	4.94 (± 1.4)
LM595	01011	-0.51 (± .051)	-0.294 (± .056)	4.54 (±. 70)	4.52 (± 1.4)
LM701	11011	-0.42 (± .037)	-0.284 (±.072)	4.83 (±. 70)	7.69 (± 1.8)

- This pattern was first observed for resistance mutations in *Salmonella enterica* and *E. coli* Knopp & Andersson, mBio 2018

Model with predictable concentration dependence

- L resistance mutations $i = 1, \ldots, L$ characterized by null-fitness $r_i < 1$ and resistance $m_i > 1$ relative to the wild type

- Dose-response curve of a mutant $\sigma = (\sigma_1, \ldots, \sigma_L)$ is given by

$$w_\sigma(x) = r_\sigma f(x/m_\sigma)$$

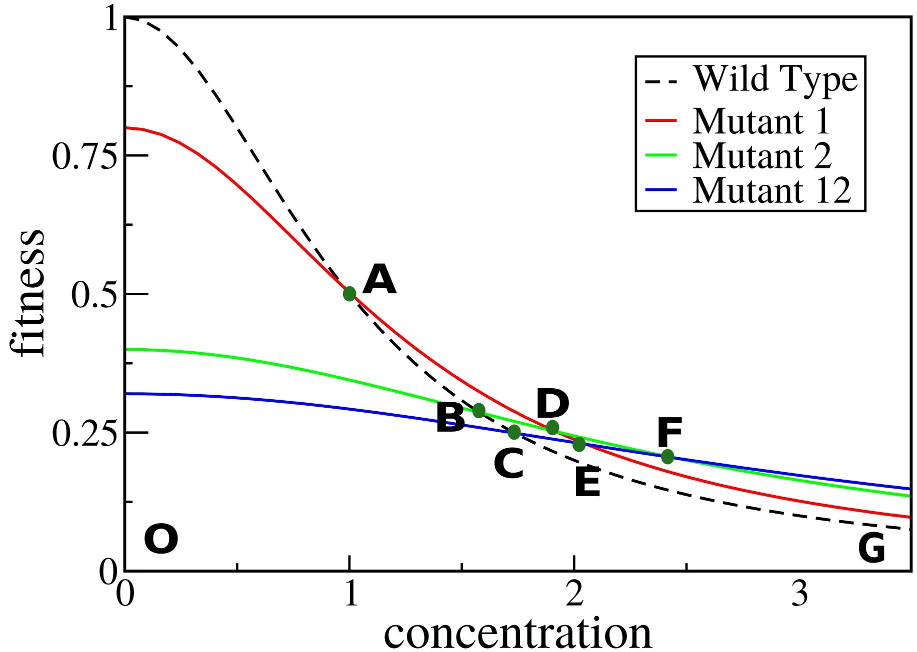
  where the function $f(x)$ is independent of σ and the marginal phenotypes combine multiplicatively as

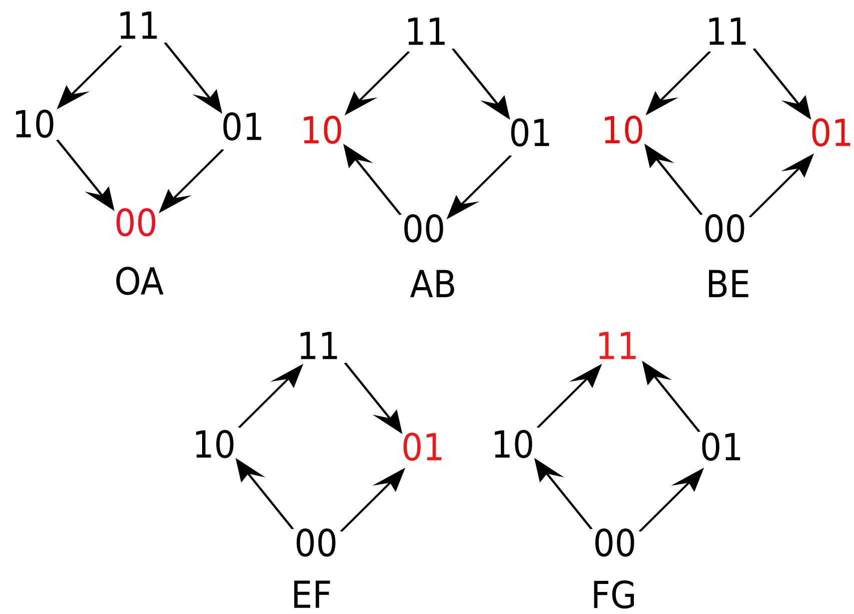
$$r_\sigma = \prod_{j=1}^{L} (r_j)^{\sigma_j} \quad \text{and} \quad m_\sigma = \prod_{j=1}^{L} (m_j)^{\sigma_j}$$

- Resistance is quantified by the concentration at which growth drops by 50% (IC_{50}), which implies that $f(1) = \frac{1}{2}$

- In this way 2^L concentration-dependent fitness values can be predicted from $2L$ single mutant phenotypes and one shape function

Two resistance mutations



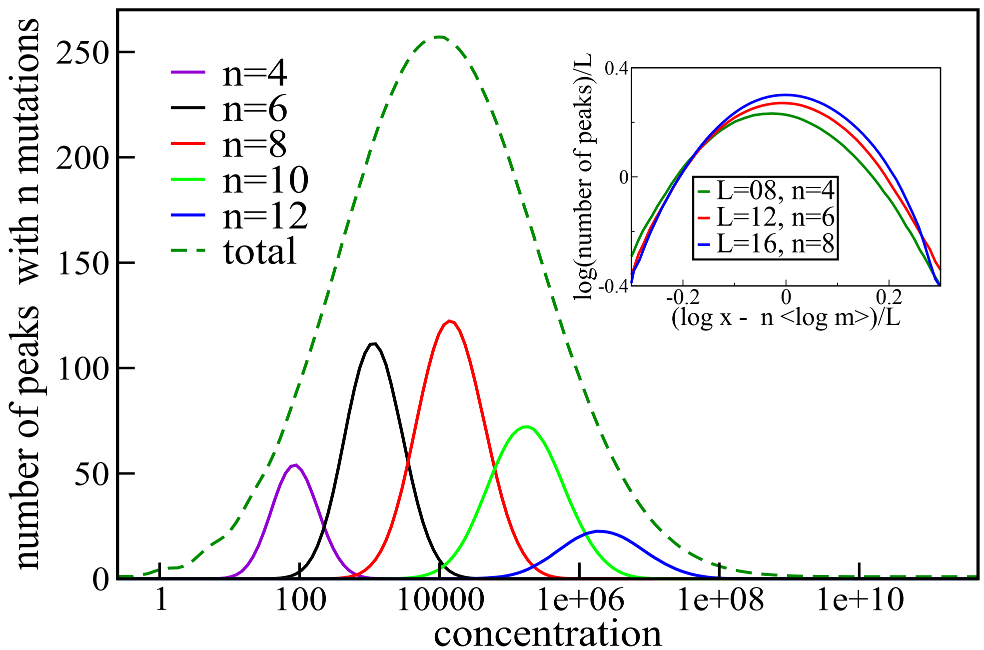


- Fitness landscape evolves from single-peaked to two-peaked and back
- Not all rank orders can appear in this process

Maximal ruggedness at intermediate concentrations

- $L = 16$ mutations with randomly distributed r_i, m_i

- Quantify ruggedness by the number of local fitness peaks



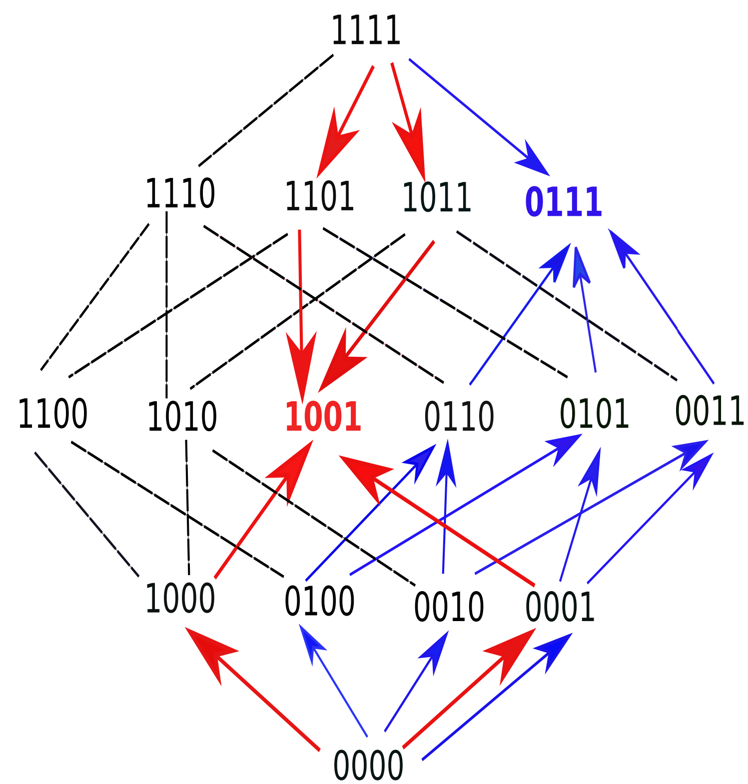
- Typical fitness peaks carry n mutations at $\ln x \sim n\langle \ln m_i \rangle$, and the maximal number of peaks grows exponentially with L

Landscapes are nevertheless highly accessible

- Scaling and absence of positive marginal epistasis imply that certain rank orders are forbidden at any concentration

- As a consequence, any peak genotype is accessible from all its sub- and supersets

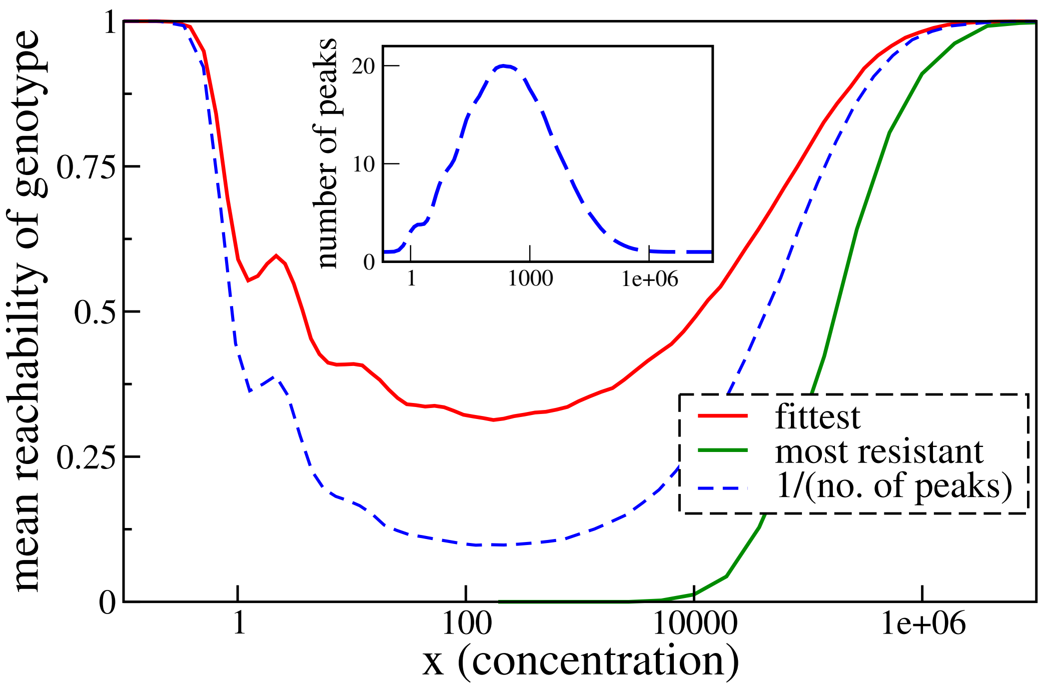
- In particular, the fittest type is always accessible from the wild type



Two-peaked landscape at an intermediate concentration

Reachability of the fittest and most resistant mutant

- $L = 10$ mutations with randomly distributed r_i, m_i



- Probability of reaching the fittest/most resistant mutant from the wild type using strong selection/weak mutation dynamics

Summary

- Evolution of antimicrobial resistance through multiple mutational steps is a model for evolutionary predictability

- Combinatorial construction of resistance landscapes reveals a systematic dependence on the choice of the combined mutations

- Tradeoff between resistance and growth rate induces rugged fitness landscapes at intermediate antibiotic concentration

- Despite their ruggedness these landscapes are remarkably accessible and the evolution of high levels of resistance remains facile

- Outlook: Consider time-dependent antibiotic concentrations as a model for evolution in changing enviroments

Thanks to

- Cologne:
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