# Epistasis and compensatory evolution of antibiotic resistance

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# Epistasis and compensatory evolution of antibiotic resistance

- The role of epistasis between deleterious mutations
- The distribution of compensatory mutations to alleviate the fitness cost of single deleterious mutations

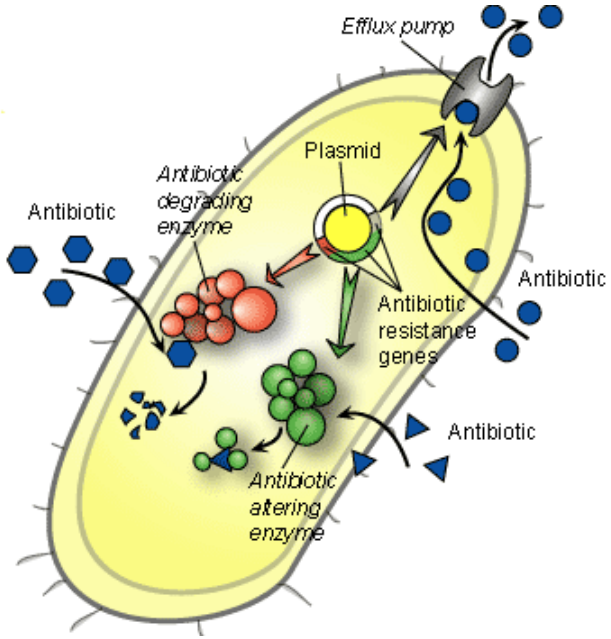
# How do antibiotics inhibit bacterial growth?

Antibiotics inhibit bacterial growth by binding to highly conserved domains of essential proteins to the cell (e.g. ribosome, DNA gyrase, RNA polymerase or cell wall).

**EMERGENCE OF RESISTANCE**

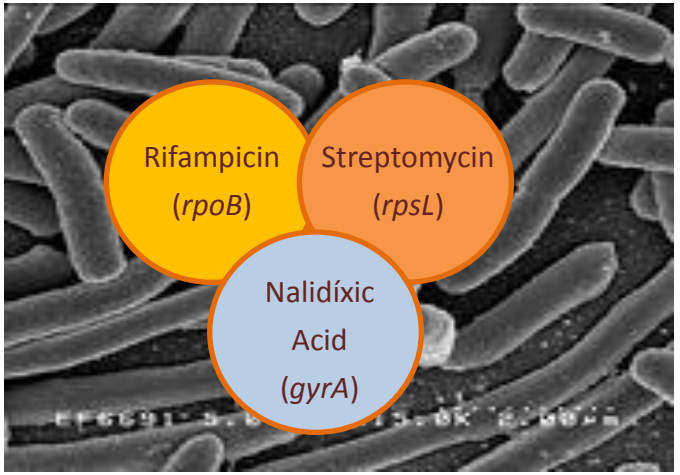
- Target alteration preventing antibiotic binding.
- Enzymatic modification and degradation of antibiotics.
- Reducing antibiotic entry in the cell.

Cost



# What is the cost of a multiple resistance?

Resistance to 2 antibiotics




$$W_{12} = W_1 * W_2$$

EPISTASIS

$$\varepsilon = W_{12} - W_1 * W_2$$

# How can we constrain the evolution of multiple resistance?

Cost of mutation c1


Resistance to antibiotic 1

Cost of mutation c2

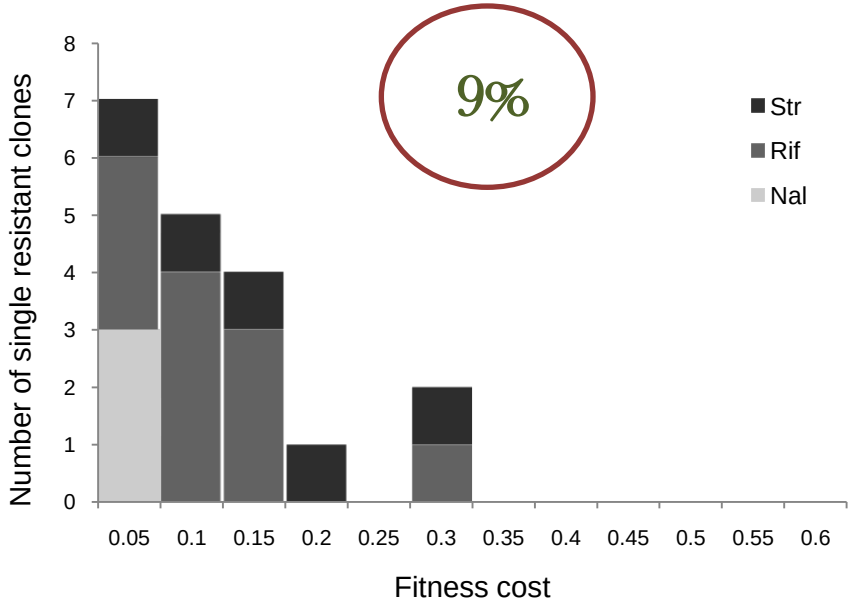

Resistance to antibiotic 2

**Cost of mutation 1 & 2 ?**


Resistance to both antibiotics

| | |
|---|---|
| If $c12 = c1 + c2$ | No epistasis, no interaction $\varepsilon = 0$ |
| If $c12 > c1 + c2$ | Negative epistasis, high cost $\varepsilon < 0$ |
| If $c12 < c1 + c2$ | Positive epistasis, low cost $\varepsilon > 0$ |

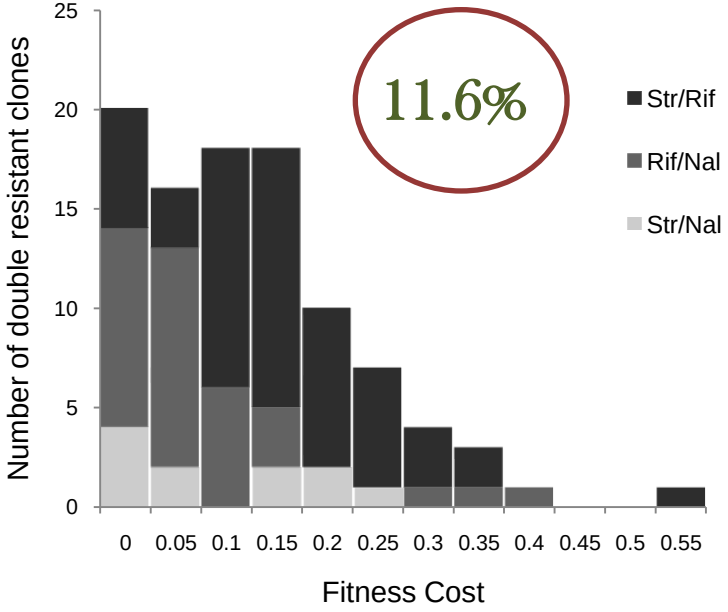
If a pathogenic strain is resistant to antibiotic X, which antibiotic should be administered as a second treatment?




**SINGLE MUTANTS - 19**

9% x 2 = 18%

11.6% < 18%




# EPISTASIS

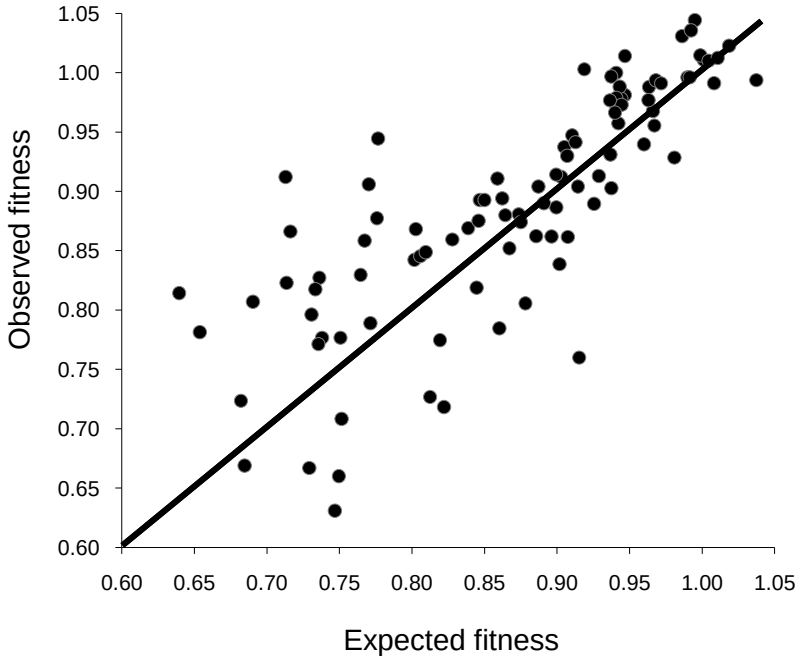
**DOUBLE MUTANTS - 103**

Trindade *et al* (2009)
*PLoS Genetics*

$$\varepsilon = W_{R1R2} - W_{R1} * W_{R2}$$

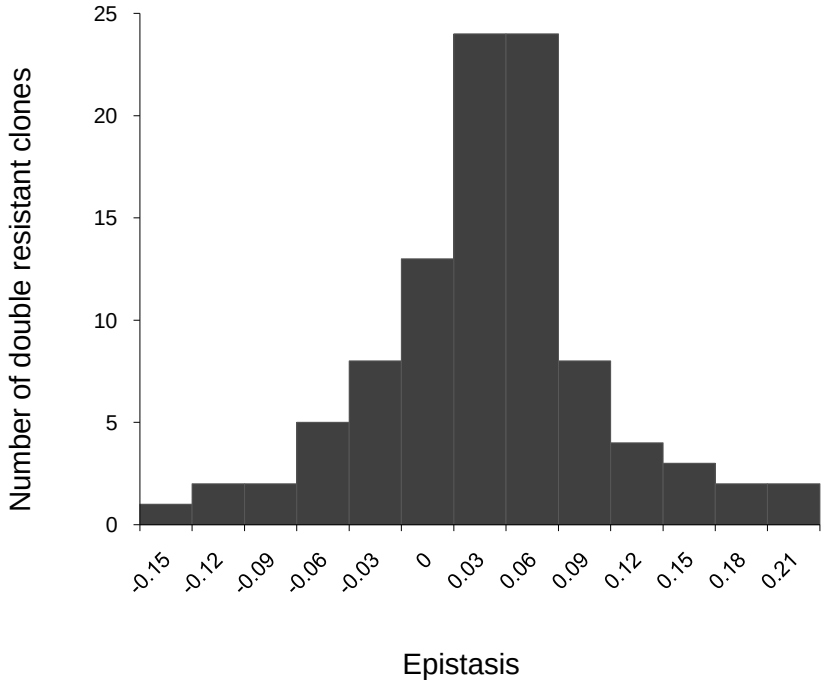
$\varepsilon = 0$

$$W_{R1R2} = W_{R1} * W_{R2}$$




**Median = 0.025 < 0.09**

Bootstrap 95% CI [0.016; 0.032]

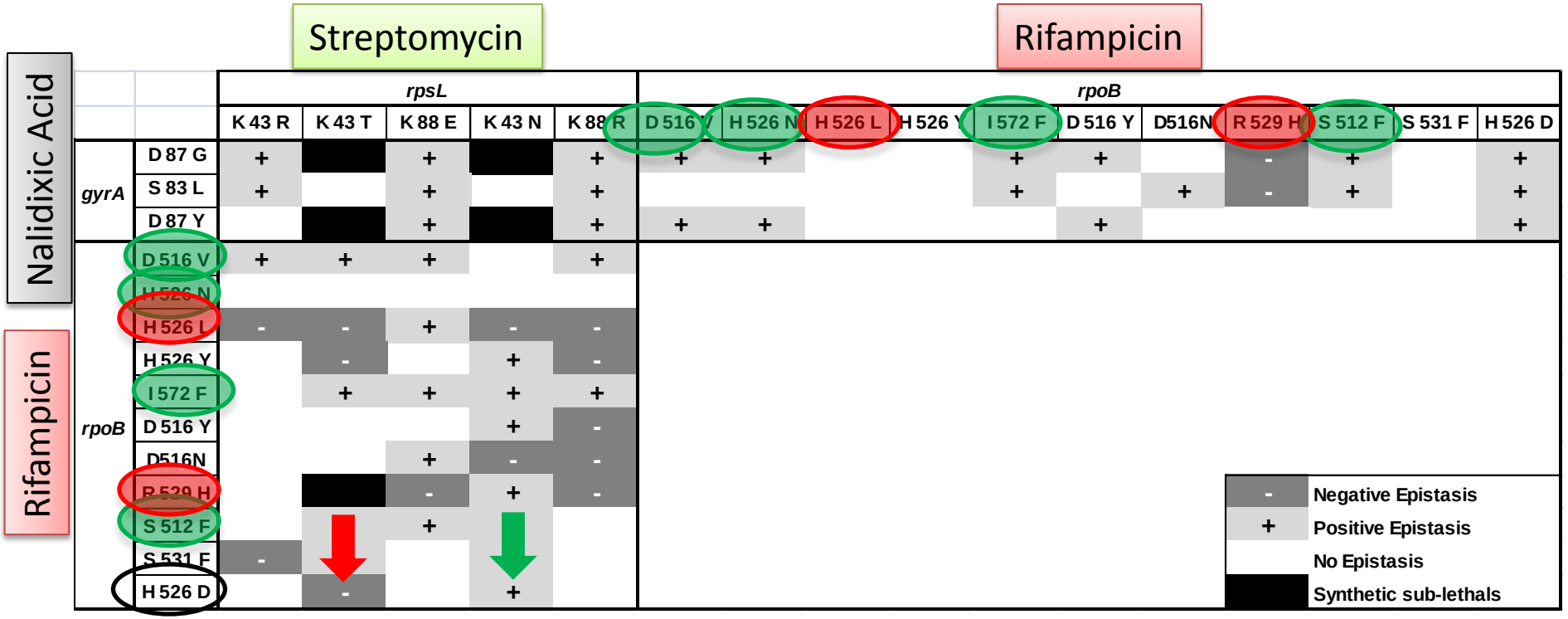



53% → **Epistasis**

- 73% Positive Epistasis
- 27% Negative Epistasis

Trindade *et al.*, 2009

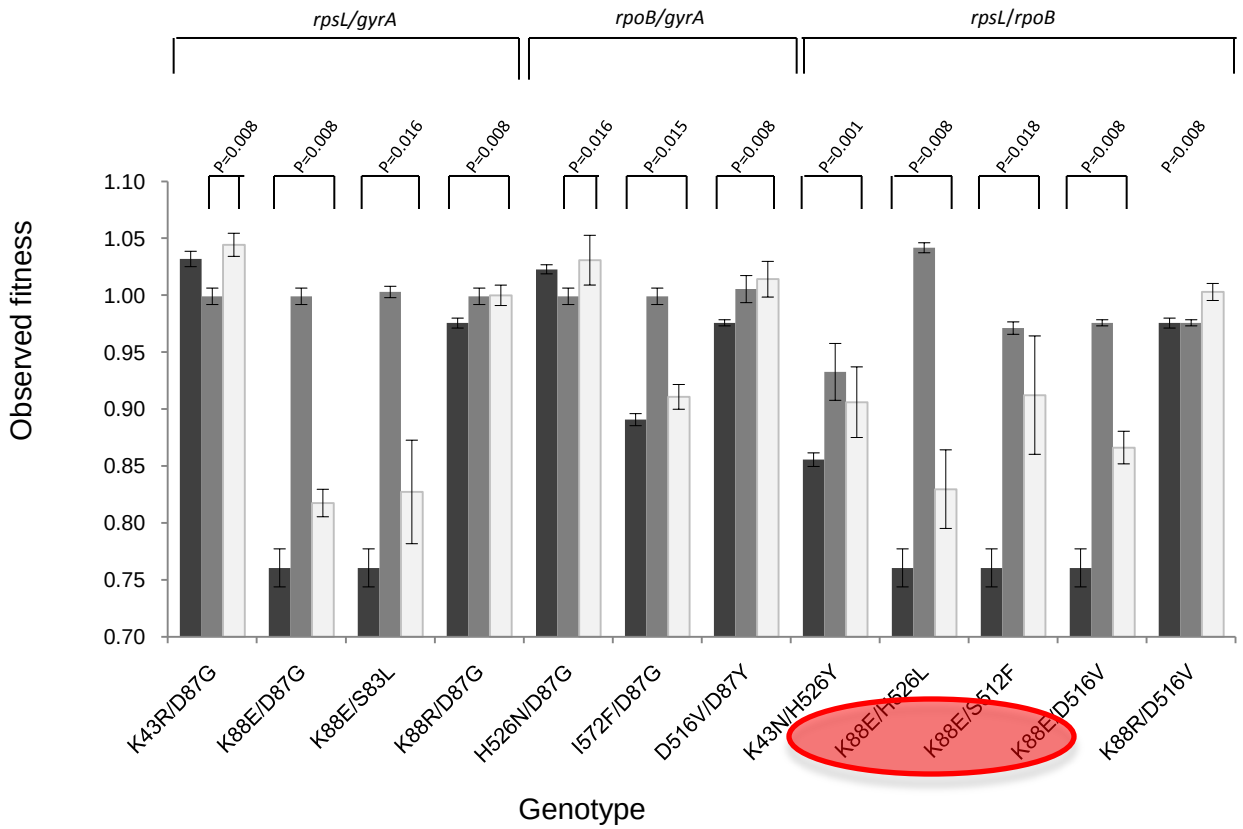
# Interactions between resistances are allele specific




H526D has been found in multi-resistant strains of *Mycobacterium tuberculosis*
(also dependent on the genetic background)

# Sign Epistasis

## 12% mutants



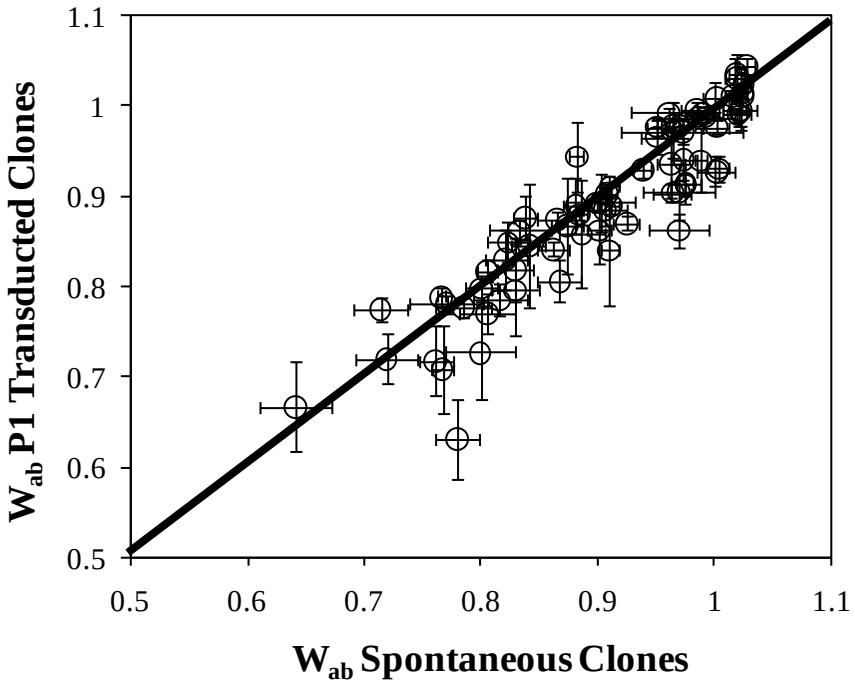

Many clones of *Mycobacterium tuberculosis* segregating in nature are resistant to Streptomycin (*rpsL*), Levofloxacin (*gyrA*) and Rifampicin (*rpoB*)

## Conclusions

- **Positive epistasis is pervasive** among antibiotic resistance mutations.

- The type of interactions is not gene but **allele specific**.

- Presence of **sign epistasis** in the cost of multi-drug resistance involving all the antibiotics studied. This means that for a small fraction of resistants having two resistances is less costly than at least one of the resistances.




5 spontaneous resistance clones have higher fitness than the corresponding P1 transducted clones.

⇩

Candidates to carry **compensatory mutations**.

A deleterious mutation has several different possible fates:

1. It may go extinct.
2. Revert back to its ancestral state.
3. Be compensated by additional mutations.

**Compensation** is of special interest with regard to the potential reversibility of antibiotic resistance, as antibiotic-resistant bacteria may adapt genetically to the costs by acquiring mutations that restore fitness. A possible, and medically unwanted, consequence of compensation is that the resistant bacteria are stabilized in the population and resistance becomes less reversible, or even irreversible, at the population level.

It has been estimated that for every reversion there are aproximatelly **11** possible **compensatory mutations** .

Poon *et al*. (2005)

# How can we predict the rate of adaptation?

Compensatory adaptation is a very important phenomenon when considering antibiotic resistance evolution and it explains why resistance alleles persist in bacterial populations long after its clinical use has been withdrawn.

The rate of adaptation to the cost of antibiotic resistance might be inferred by the **distribution of the effects of compensatory mutations.**

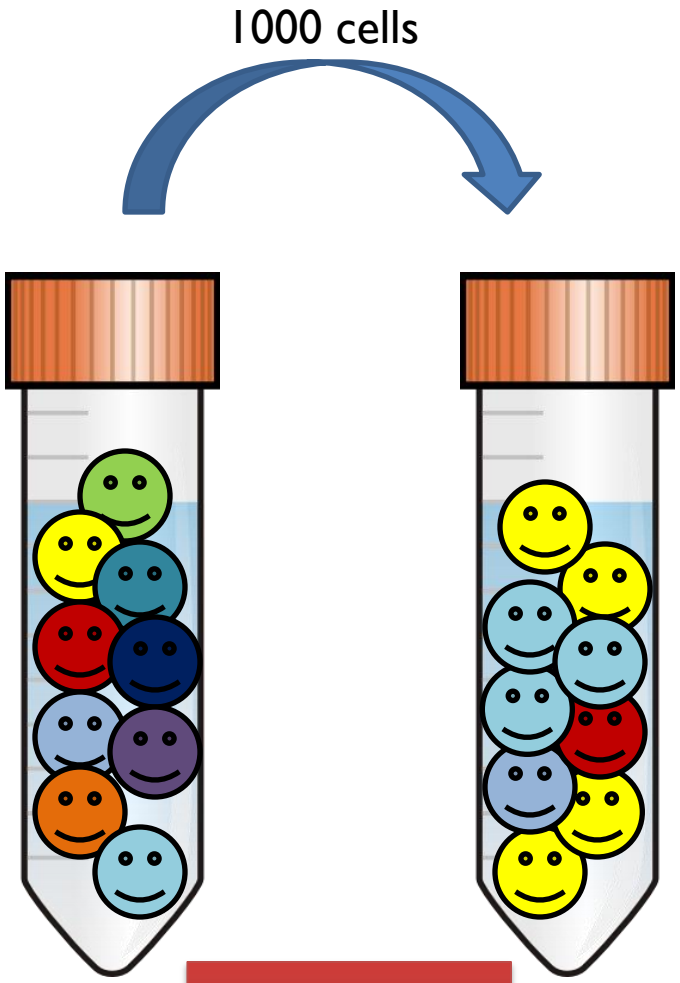
**FAST**

When the distribution contains many mutations or when it is skewed to the right implies that mutations of large effect are relatively common.

**SLOW**

When the distribution has few mutations or when it is skewed to the left, presents an excess of small effect mutations.

# EXPERIMENTAL SETUP

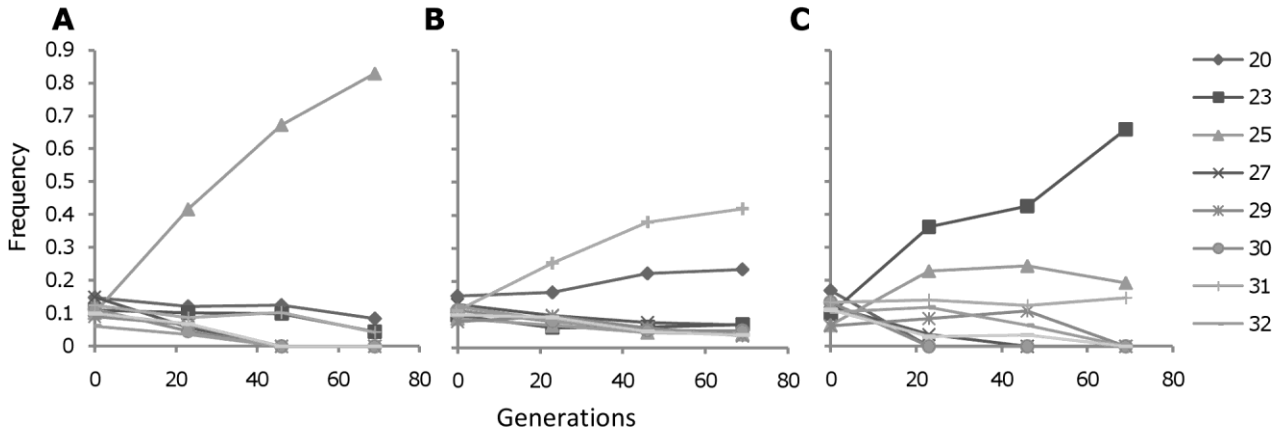



- Model organism: *Escherichia coli* MG1655
- 30 independent populations for each mutation (*rpsL* , $Str^r$ )
- K43N ~18%; K88E ~27%
- 9 Neutral markers – microsatellite sequence inserted in plasmid pBR322, stable over the time scale of the experiment

$(GA)_{20}$ $(GA)_{23}$ $(GA)_{25}$
$(GA)_{27}$ $(GA)_{29}$ $(GA)_{30}$
$(GA)_{31}$ $(GA)_{32}$ $(GA)_{34}$

~ 69 generations

# Dynamics of adaptation




**Single strong mutation**

⇩

***Periodic selection regime***

**Two beneficial mutations competing**

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***Classical clonal interference regime***

**Two beneficial mutations occurring sequentially in the same clone**

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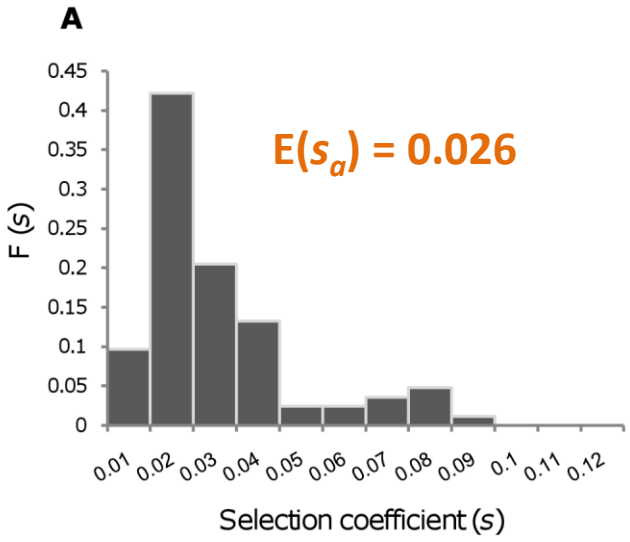
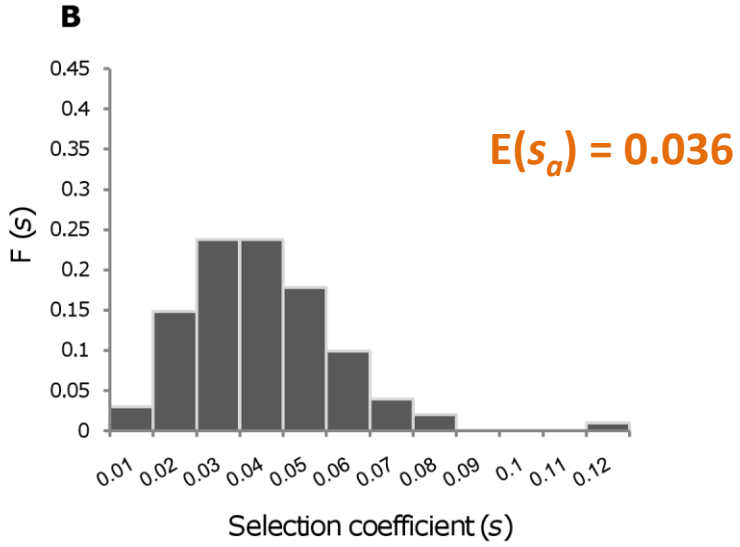
***Clonal interference in the multiple mutations regime***

K43N – low cost mutation




K88E – high cost mutation



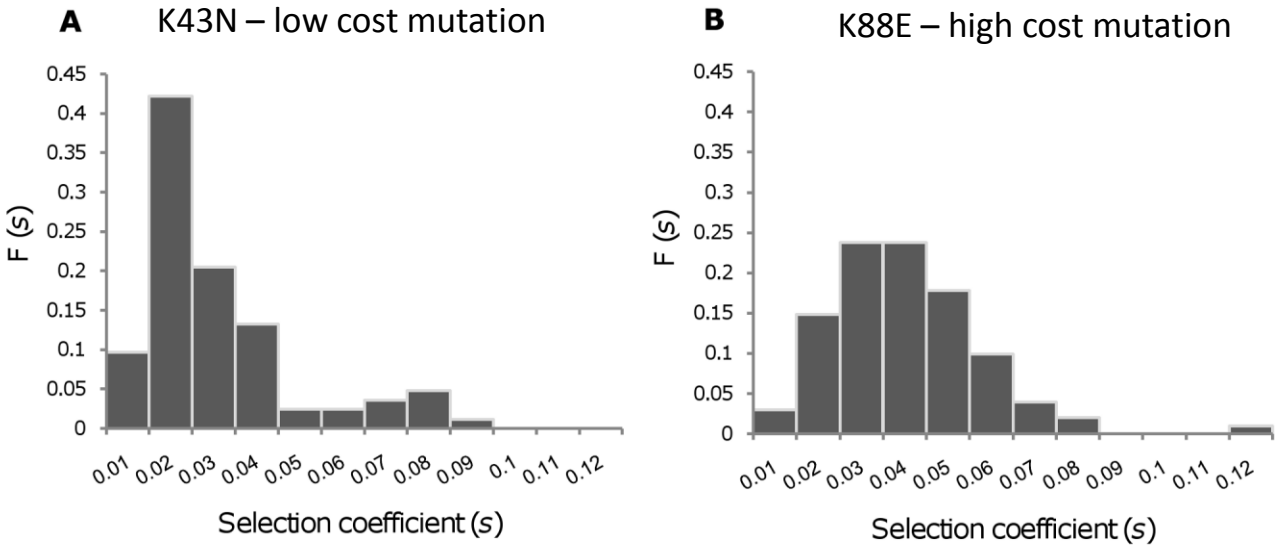

- The distributions are significantly different (Kolmogorov-Smirnov, $P=1.8 \times 10^{-6}$)

# Fisher's model predictions

| | E(sd) = 0.0125* | | | E(sd) = 0.03** | |
|---|---|---|---|---|---|
| | Cost of resistance | | | Cost of resistance | |
| | 0.18 | 0.27 | | 0.18 | 0.27 |
| n | | | n | | |
| 4 | 0.053 | 0.069 | 4 | 0.074 | 0.100 |
| 5 | 0.047 | 0.062 | 6 | 0.060 | 0.081 |
| 6 | 0.043 | 0.056 | 14 | 0.038 | 0.051 |
| 7 | 0.039 | 0.053 | 15 | 0.037 | 0.049 |
| 10 | 0.032 | 0.043 | 20 | 0.030 | 0.041 |
| 13 | 0.029 | 0.037 | 22 | 0.027 | 0.038 |
| 14 | **0.027** | **0.036** | 25 | **0.026** | **0.036** |
| 15 | 0.025 | 0.034 | 26 | 0.026 | 0.036 |

*Kibota and Lynch (1996)
*Nature*

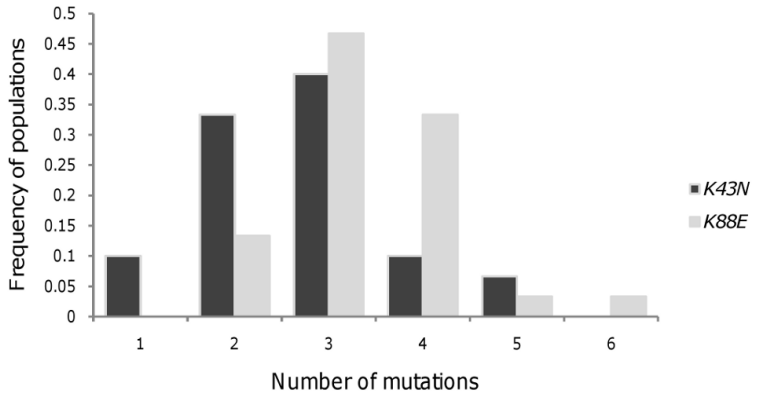
**Trindade *et al* (2010)
*Phil Trans Roy Soc Biol Sci*




| | K43N | | | | K88E | | | |
|---|---|---|---|---|---|---|---|---|
| **Distribution** | **p1** | **p2** | **Log Lik** | **AIC** | **p1** | **p2** | **Log Lik** | **AIC** |
| Beta, p1=2 | 2 | 75±6 | 235.7 | -470 | 2 | 54±4 | 263.7 | -523 |
| Beta | 2.6 ± 0.4 | 98.22±15.8 | 237.4 | -471 | 4.5±0.6 | 120±17 | 277.6 | -551 |
| Beta trunc | 1.7 ± 0.5 | 71±17 | 243.0 | -482 | 4.3±0.6 | 117±18 | 277.9 | -552 |
| Lognorm | -3.84±0.07 | 0.61±0.05 | 242.9 | -482 | -3.44±0.05 | 0.49±0.03 | 275.4 | -547 |
| Gamma | 2.7±0.4 | 104.47±16.83 | 237.7 | -471 | 4.6±0.6 | 129±18 | 277.6 | -551 |
| Weibull | 0.03±0.002 | 1.59±0.13 | 233.2 | -462 | 0.04±0.002 | 2.2±0.2 | 274.6 | -545 |
| 2sHalfnorm | 56± 2 | | 228.5 | -455 | 45± 2 | | 273.5 | -545 |
| Exponential | | 38±4 | 219.9 | -438 | | 28±3 | 234.9 | -468 |
| Normal | 0.03±0.002 | 0.02±0.001 | 215.3 | -427 | 0.036±0.002 | 0.017±0.001 | 268.9 | -534 |

The beta distribution describes reasonable well the data for both mutations:
Kolmogorov-Smirnov test P=0.2 for K43N, P=0.7 for K88E

K43N – 2.7
K88E – 3.4




$$Nmut = T_{obs} NU \int_0^1 Beta(\alpha, \beta, s)\pi(s)e^{-I(U,N,s)}$$

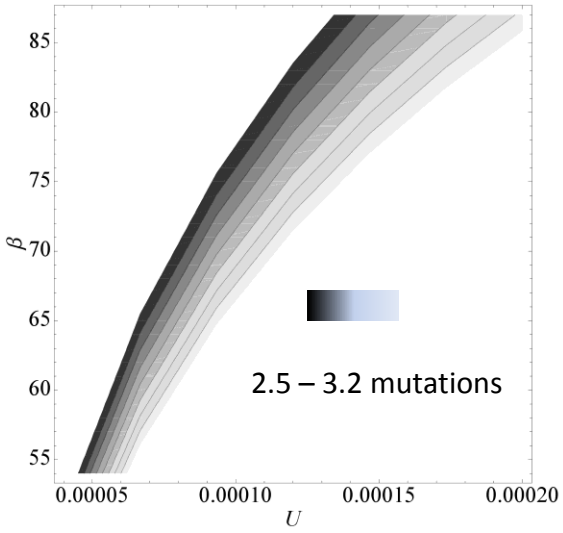
where $I(U,N,s) = e^{\gamma} NU\left(\frac{1-e^{sT_{obs}}}{s}\right)\int_s^1 Beta(\alpha,\beta,s)\pi(s)$

and γ is the Euler constant

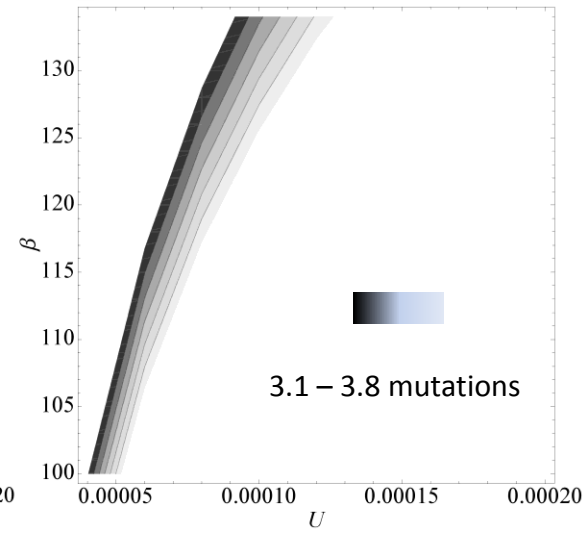
Gerrish and Lenski, 1998
*Genetica*

Range of values of β for the "Beta truncated" distribution

**A** K43N – low cost mutation




**B** K88E – high cost mutation



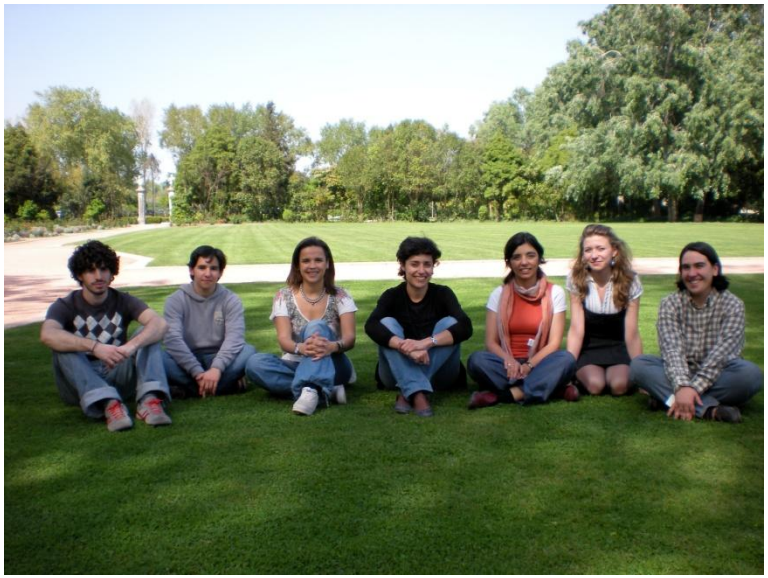

$U_a$ = [5x10$^{-5}$, 2x10$^{-4}$]

$U_a$ = [4x10$^{-5}$, 1x10$^{-4}$]

# Conclusions

- Mutation rate is not distinguishable between the two mutations and is of the order of $10^{-5}$ but the mean effect of mutations is larger for the higher cost mutation.
- The maximal value of the mutations for the compensation of *K43N* resistance detected was 0.08 and for the *K88E* resistance 0.12. Given the fitness costs of each resistance the maximum expected values, corresponding to a reversion *N43K* and *E88K*, would be 0.18 and 0.27, respectively. Adaptive mutations compensated 13 to 14% of the fitness cost of the resistant mutation on average, and at most 44% of the cost.
- Rate of compensation per deleterious mutations ~ rate of production of beneficial alleles when adapting to new environment.
- Given total rate of mutation for *E. coli,* 1% of new mutations is either beneficial or compensatory.
- Remarkably similar to yeast estimates (Shaw et al, Desai Fisher and Murray).

## Evolutionary Biology Group



http://eao.igc.gulbenkian.pt/EB/index.html

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