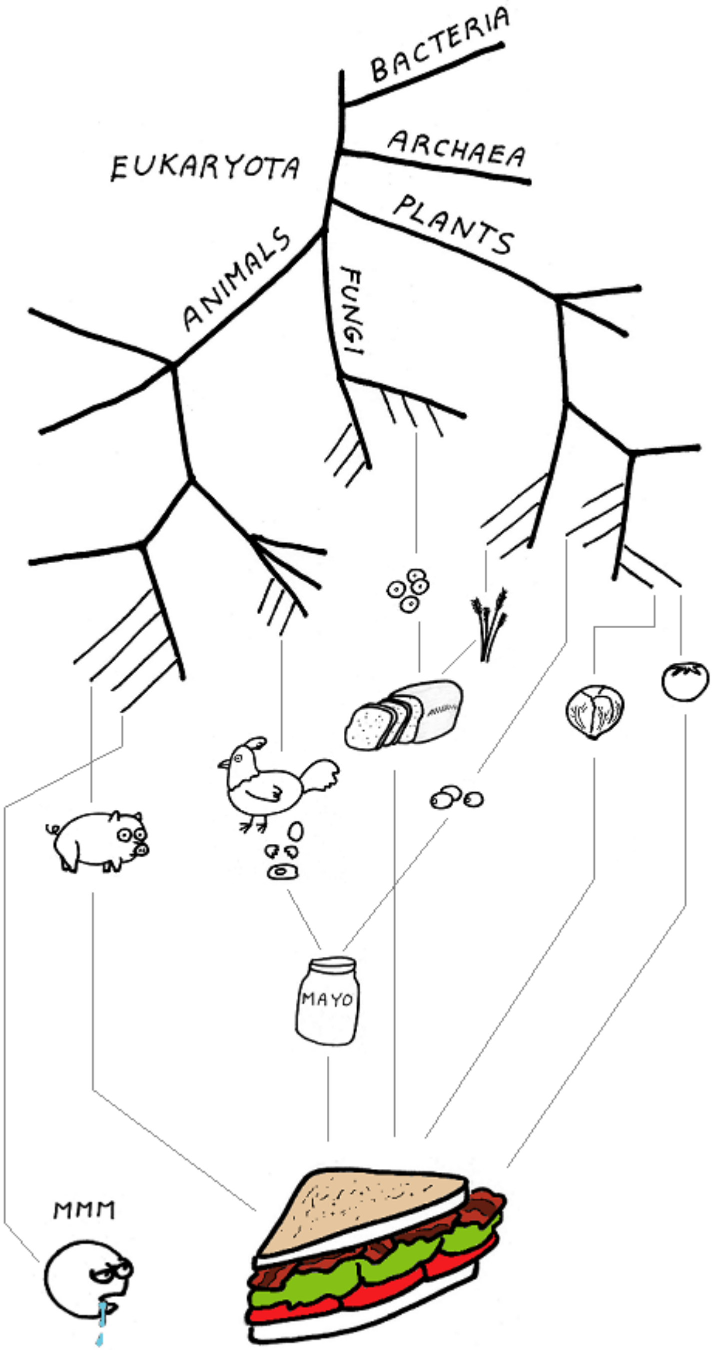
Eco-Evo Lunch

Yoav Ram

Feldman Lab

Stanford University

7 February, 2017



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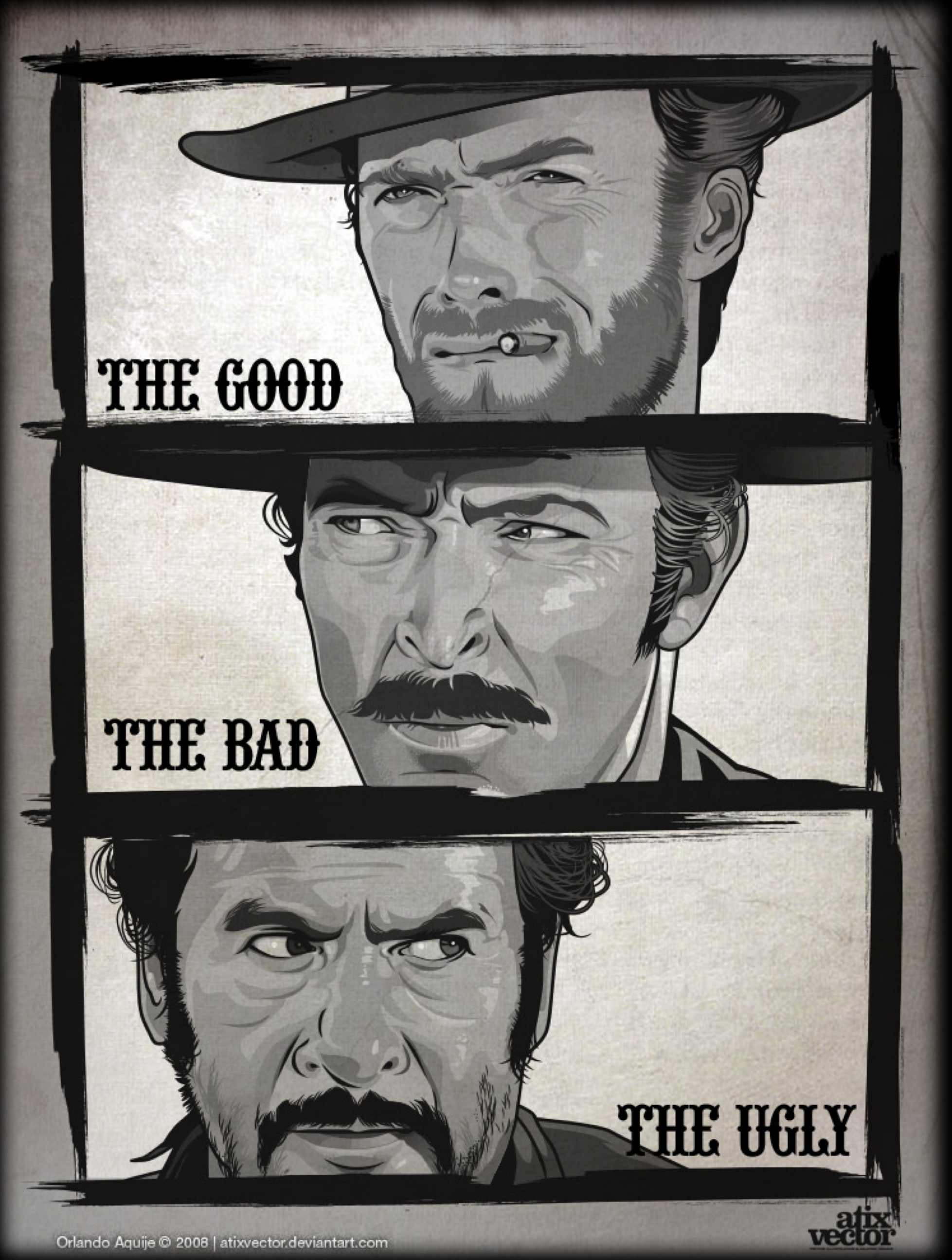
The Evolution of Stress-Induced Mutagenesis



Mutation rate evolution 101

Constant environment

- High mutation rates reduce *adaptedness* of populations
- The **reduction principle** - selection will reduce the mutation rate Liberman & Feldman 1986

Changing environment

- Natural selection favors *adaptability* – ability to adapt

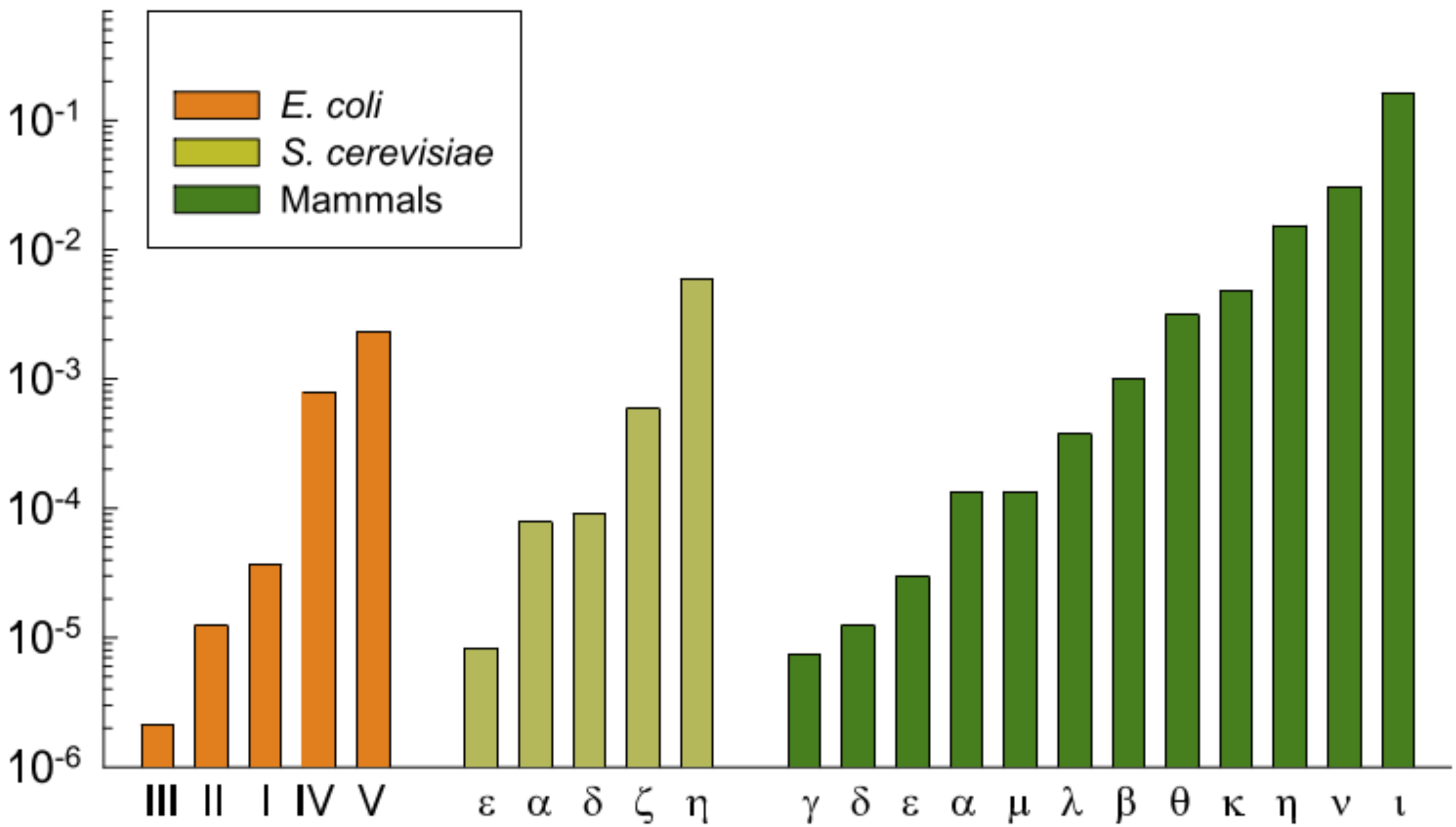
Trade-off

- The mutation rate must balance between *adaptability* and *adaptedness* Leigh 1973

Variability in mutation rates

Within individuals

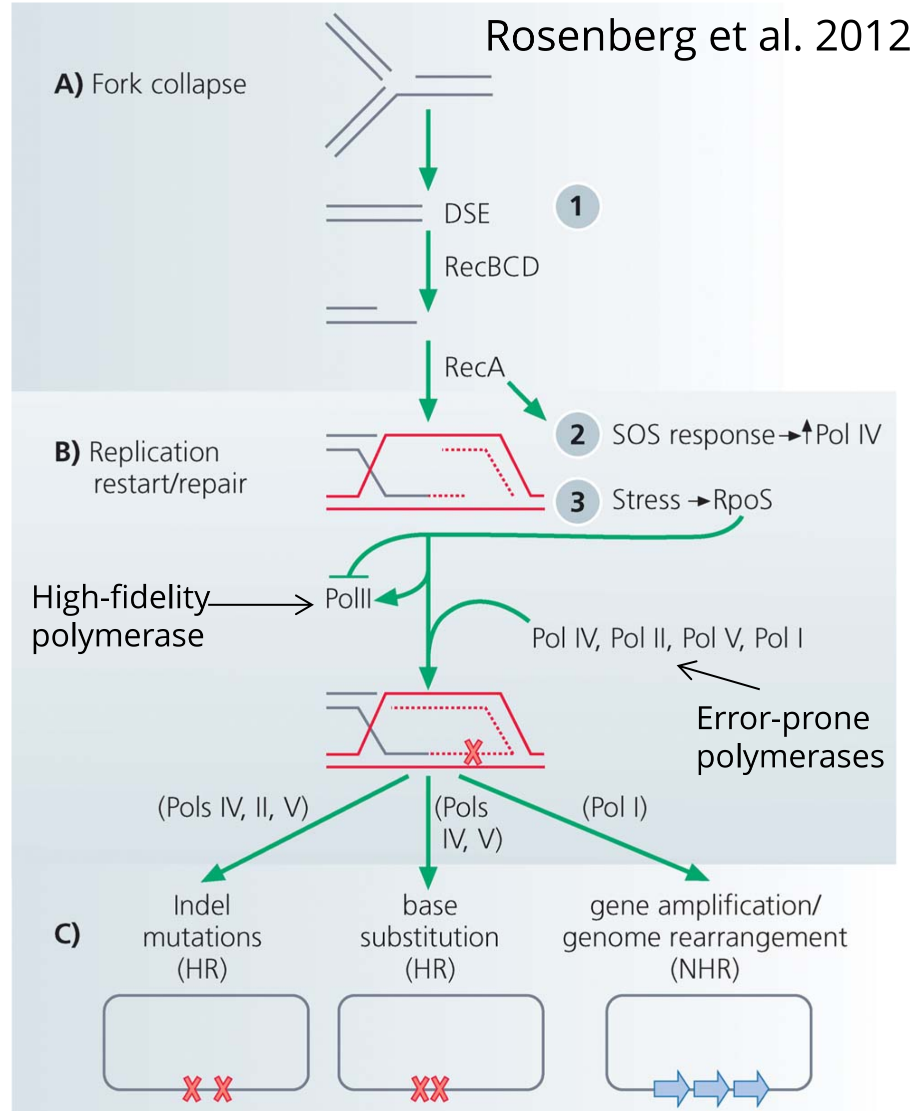
DNA polymerase error rate   Lynch 2011

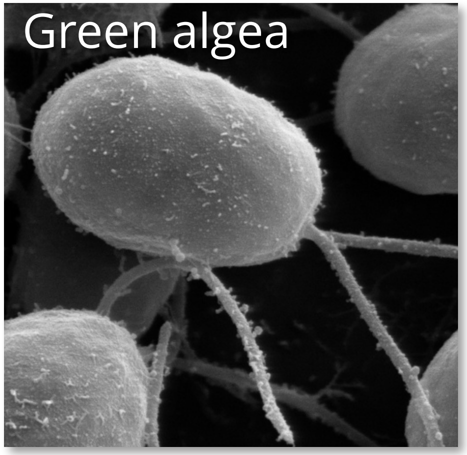


Stress-induced mutagenesis

In *E. coli:*

- Error prone polymerase induced by stress responses:
  - SOS response
  - DNA damage
  - Starvation
- Mismatch repair system
- Other mechanisms:
  - Galhardo et al. 2007
  - Al Mamun, Science 2012

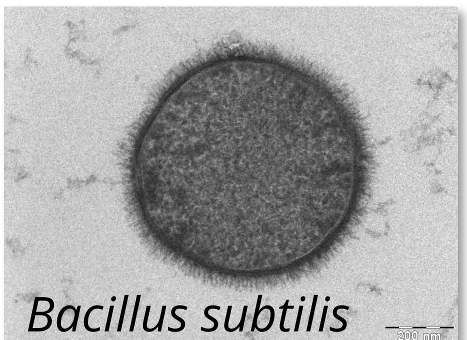




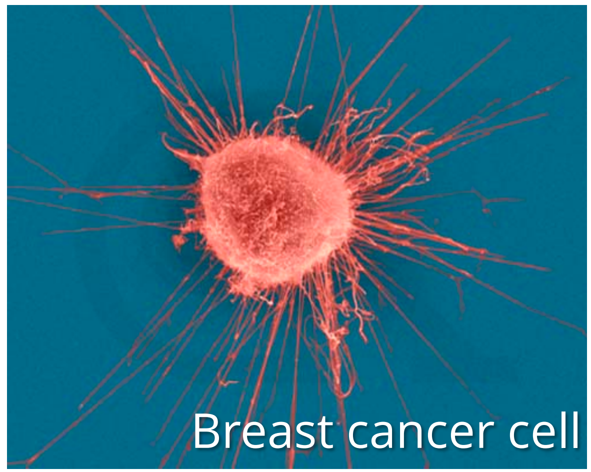
Green algea

Evidence

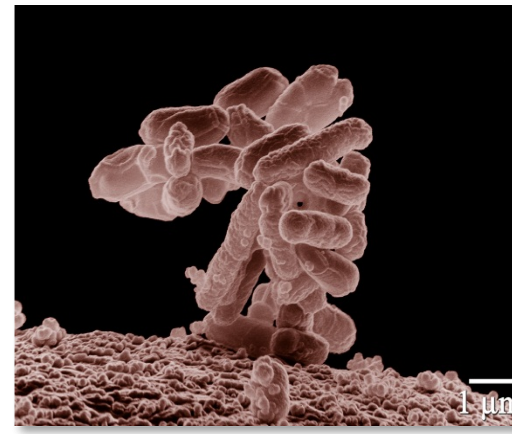
Nematodes



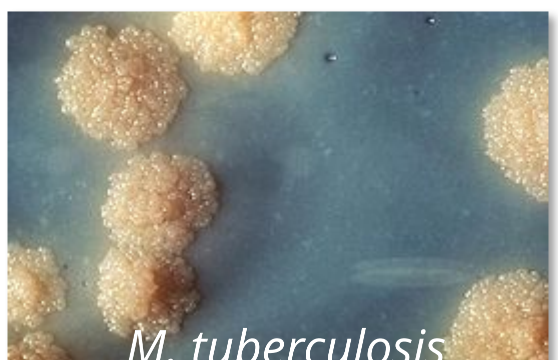
Bacillus subtilis



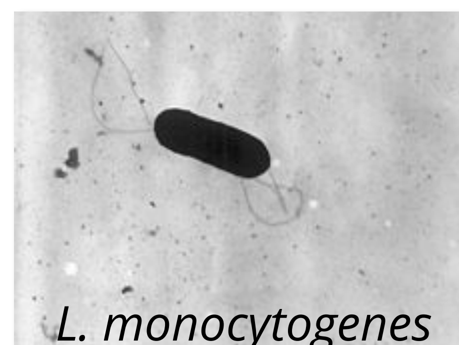
Breast cancer cell



D. Melanogaster



M. tuberculosis



L. monocytogenes

Evolution of stress-induced mutagenesis

Null hypothesis

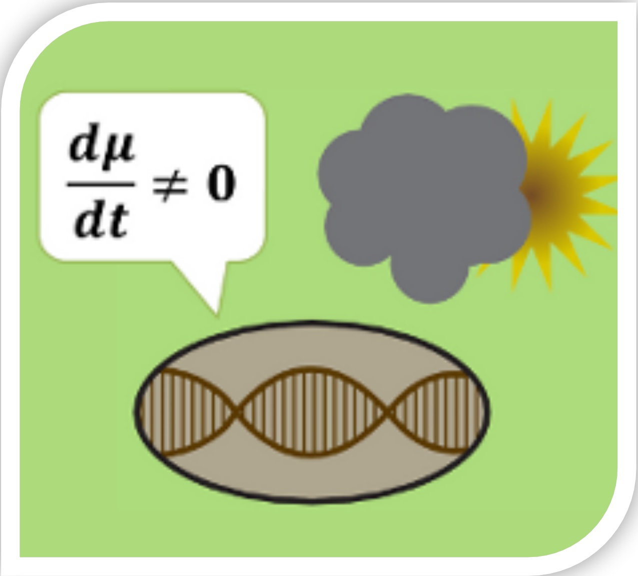
- Mutagenesis is the by-product of stress

Alternative non-adaptive hypotheses

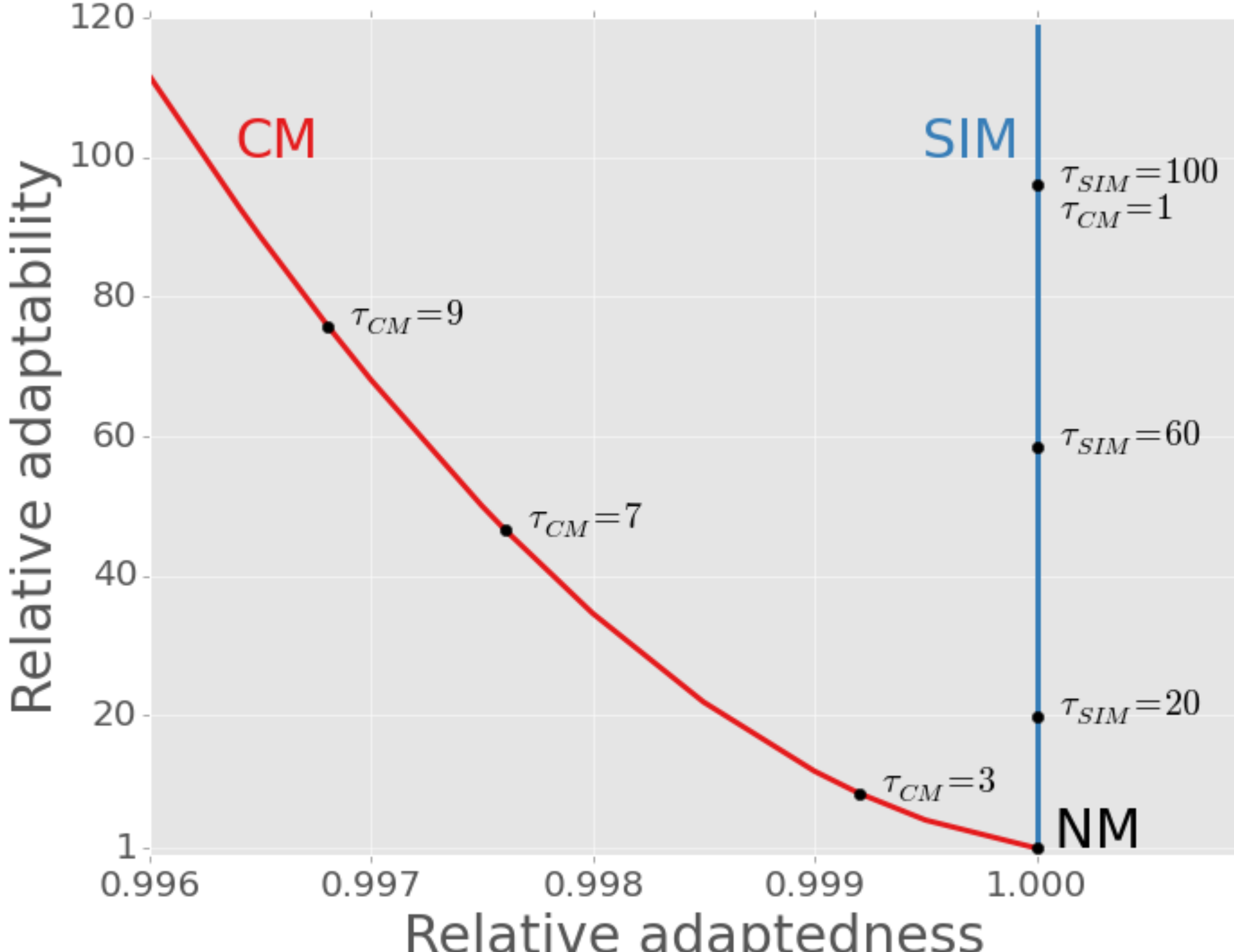
- Cost of replication fidelity
- Drift barrier hypothesis
- Pleiotropy

Adaptive hypothesis

- 2^{nd} order selection

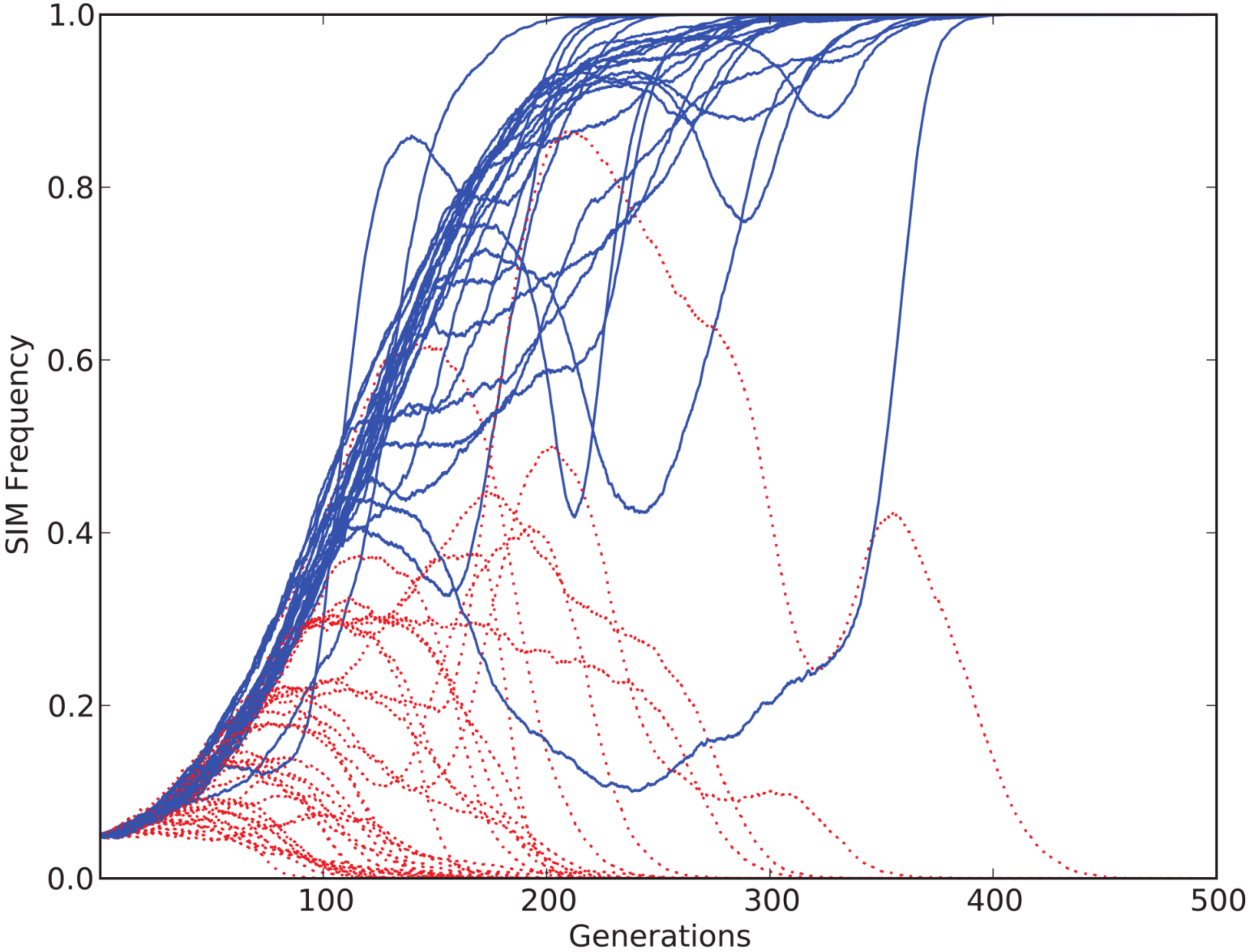


SIM Breaks the *adaptability-adaptedness* trade-off



Ram & Hadany, PRSB 2014

SIM wins competitions



Ram & Hadany, Evolution 2012

Conclusions

- **Stress-induced mutagenesis evolves:**
  - In constant & changing environments
- **2nd order selection** can lead to the evolution of stress-induced mutagenesis in asexual populations   Ram & Hadany, Evolution 2012

- **More:**
- SIM & adaptive peak shifts Ram & Hadany, PRSB 2014
- SIM in presence of recombination in preparation
- SIM with of regulation errors in preparation

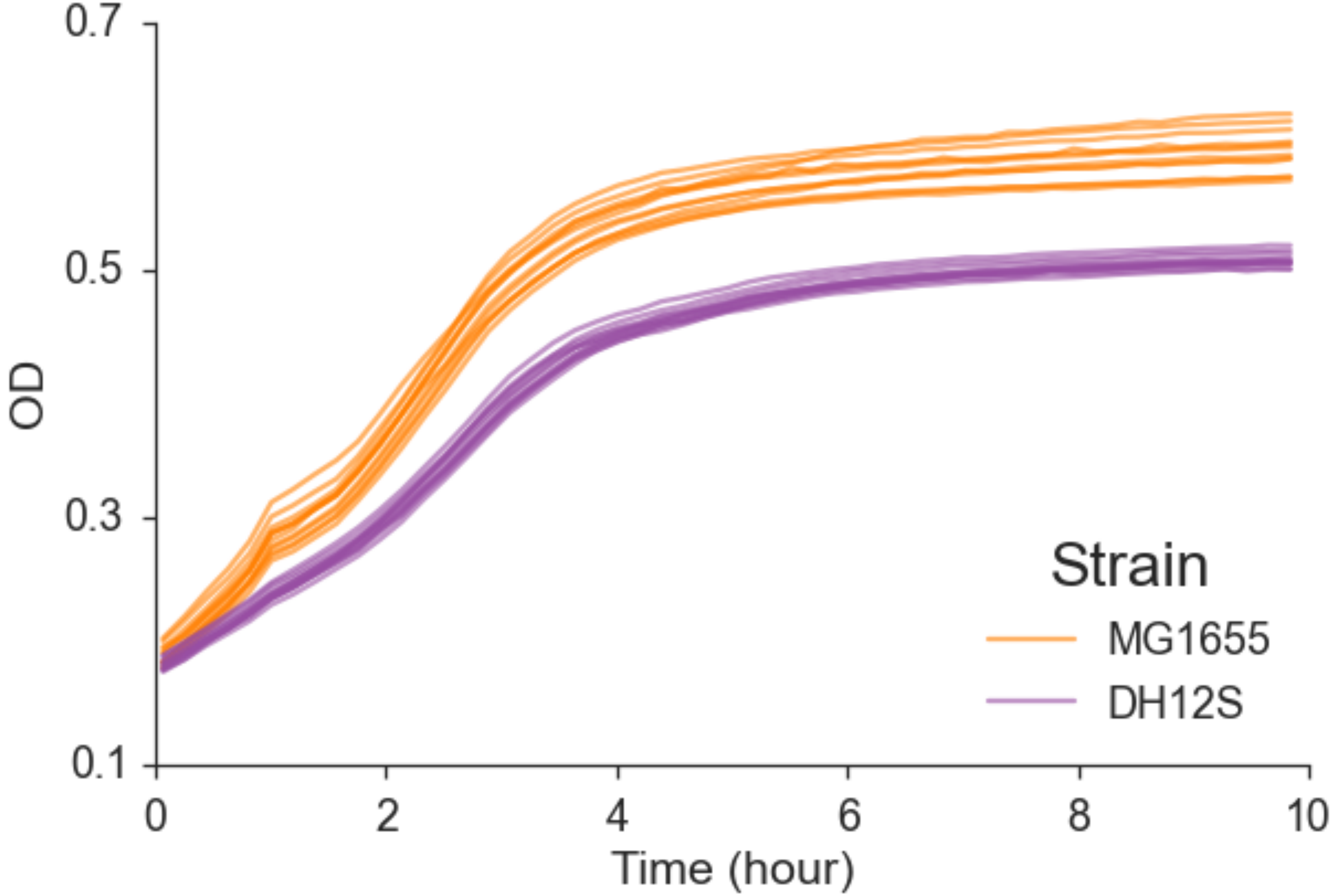
Predicting microbial growth in a mixed culture

Yoav Ram

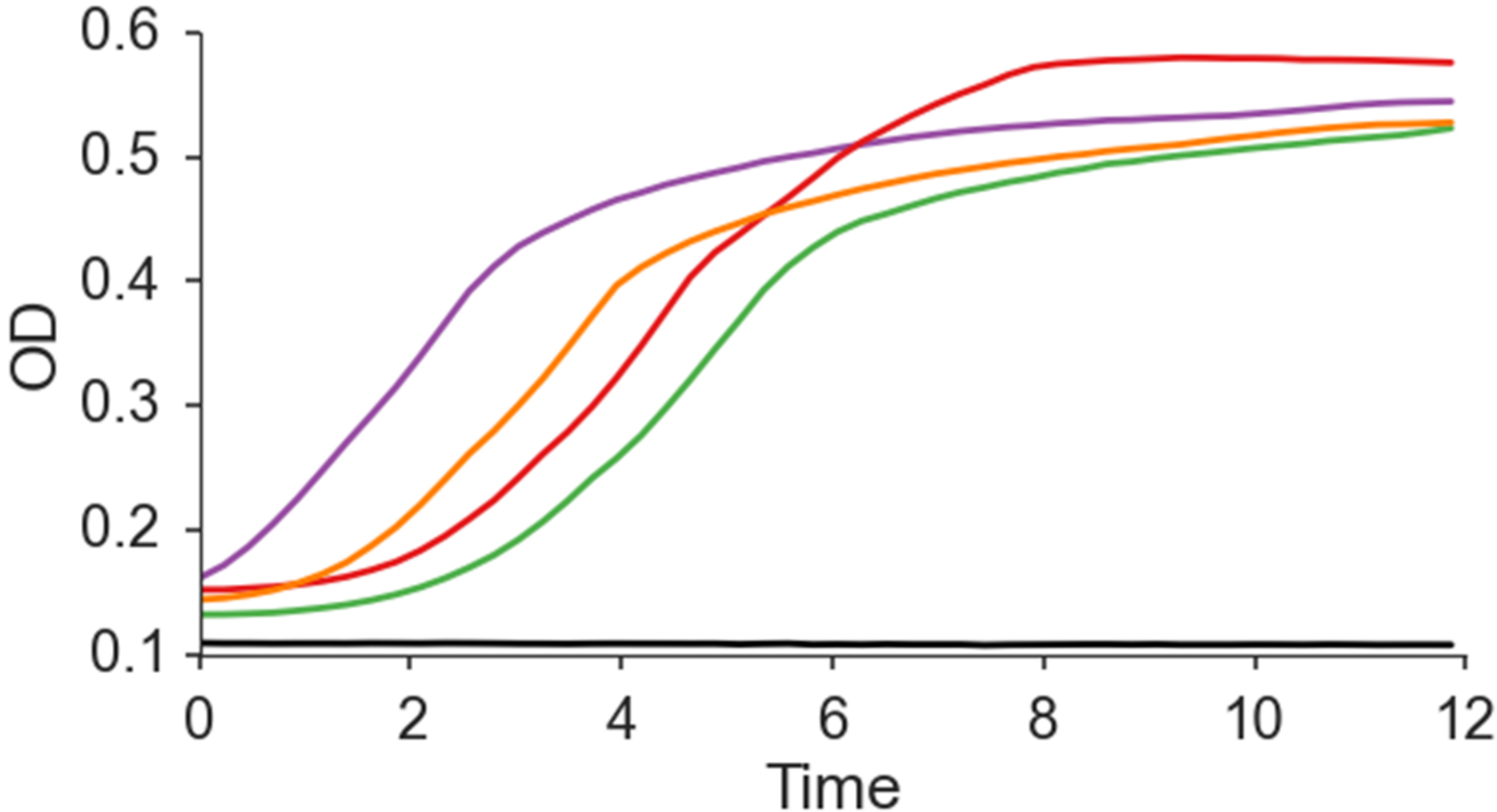
Feldman Lab @ Stanford

Eco-Evo Lunch, 7 February, 2017

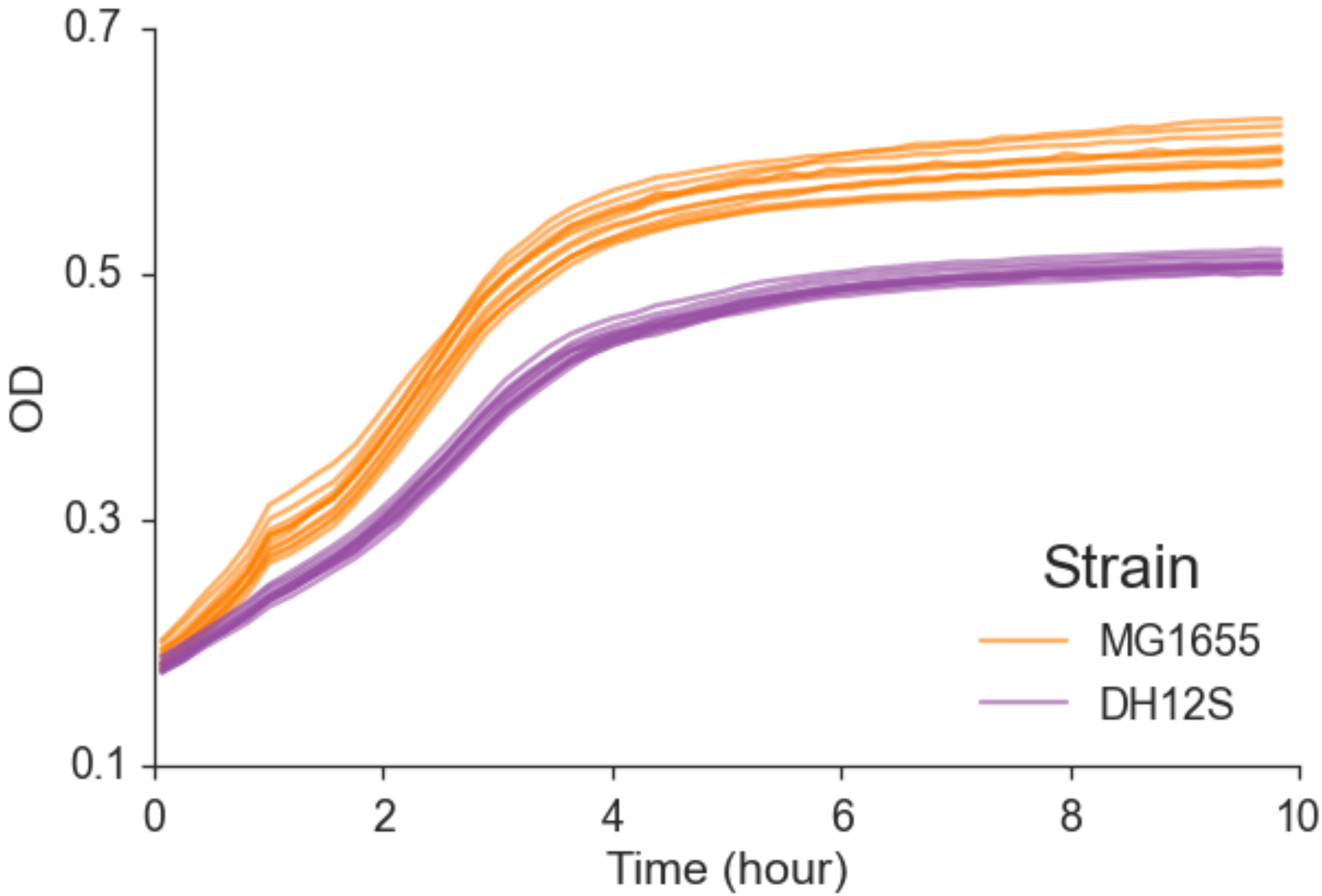
Which is fitter?



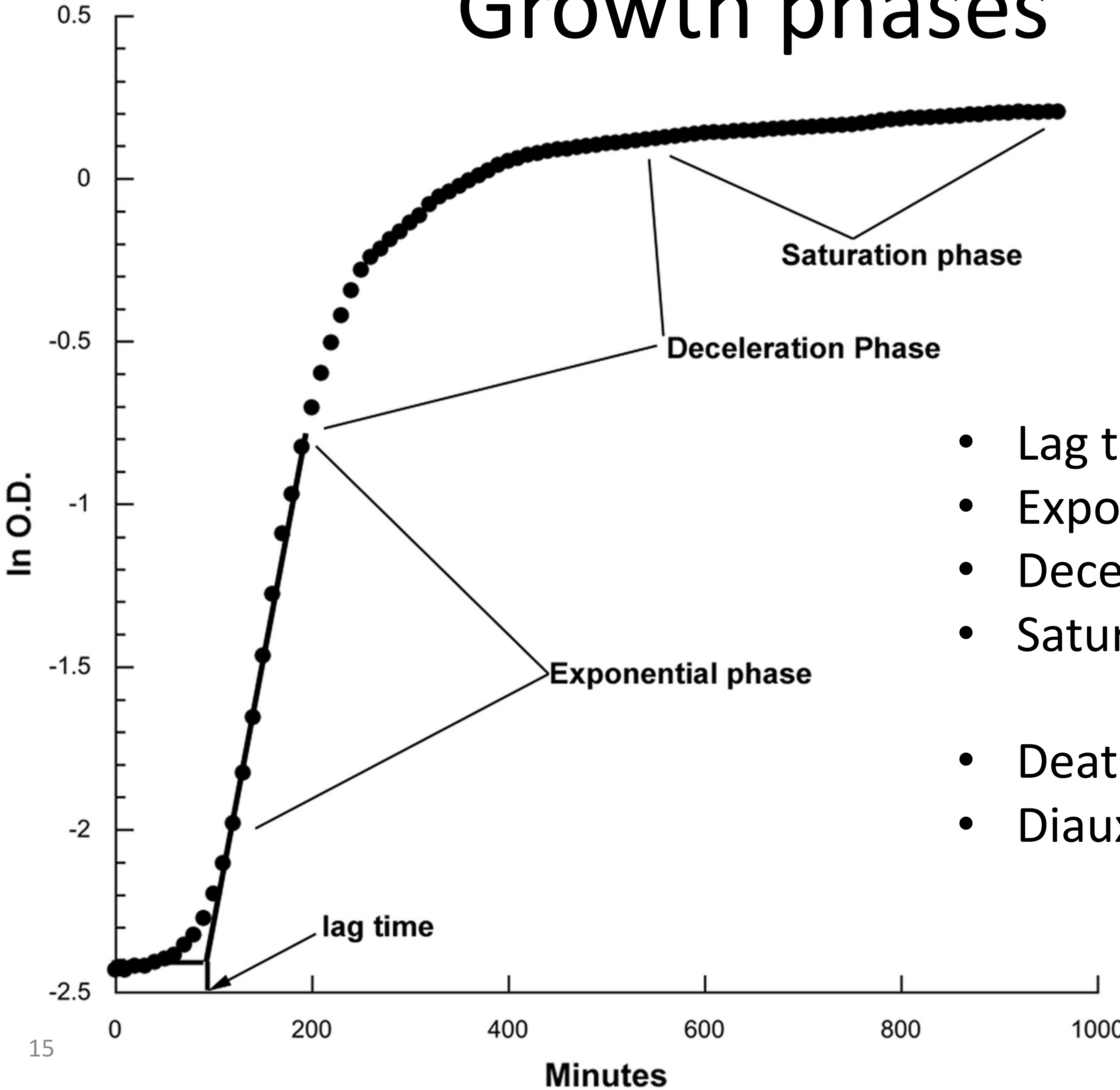
Which is fitter?



How much fitter???



Growth phases



- Lag time
- Exponential phase
- Deceleration phase
- Saturation phase

- Death phase?
- Diauxic shift?

Hall et al., MBE, 2014

Competition experiments

- Growth in a mixed culture
- Capture all relevant growth phases by direct competition
- Also captures direct interactions

CBS Boston

Direct interactions

Cooperation (++)
bread & spread

Interference (--)
The opposite game

Spite (0-)
Quiet please

Parasitism (-+)
Milk & Cookies

Charity (0+)
If I knew you were coming

Competition experiments

Strains must have a genotypic or phenotypic marker

- Fluorescence and flow cytomery

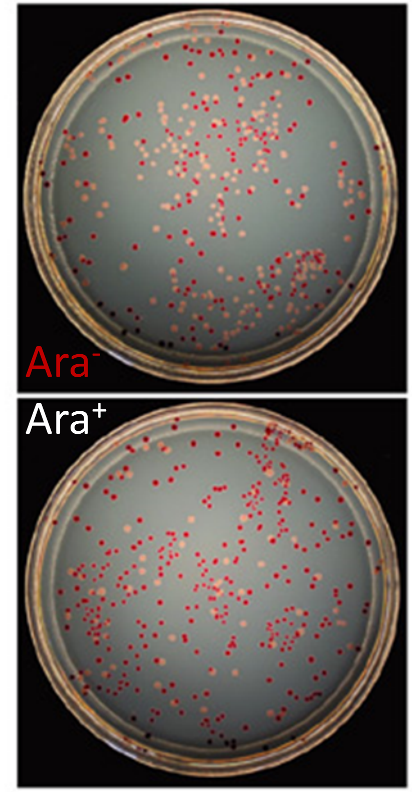
  Gallet et al., Genetics 2012

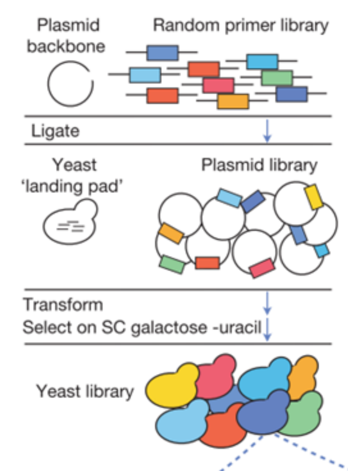
- LTEE: arabinose utilization phenotype

  Lenski et al., Am Nat 1991

- Lineage tracking with deep sequencing

  Bank et al., Evolution 2013; Levy et al., Nature 2015



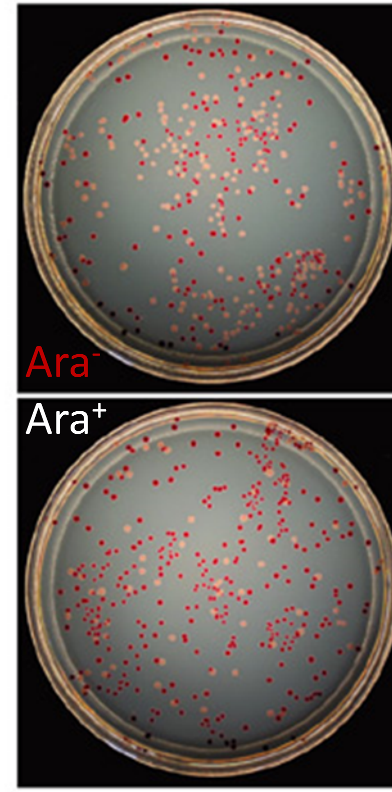


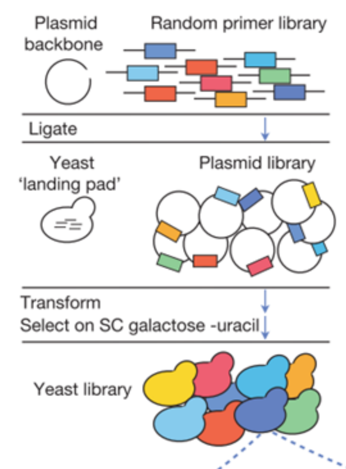
Competition experiments

Strains must have a genotypic or phenotypic marker

Laborious and Costly

More so for <u>non-model</u> organisms



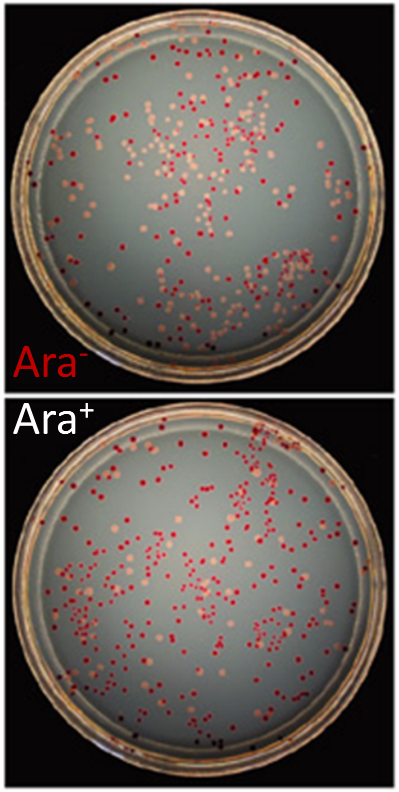


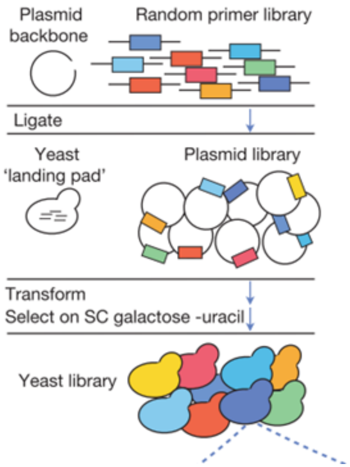
Competition experiments

Problem: Laborious and Costly

Our Solution: Computational framework that predicts competition results

1. Fit growth models to growth curves
2. Predict competition results
3. Infer fitness

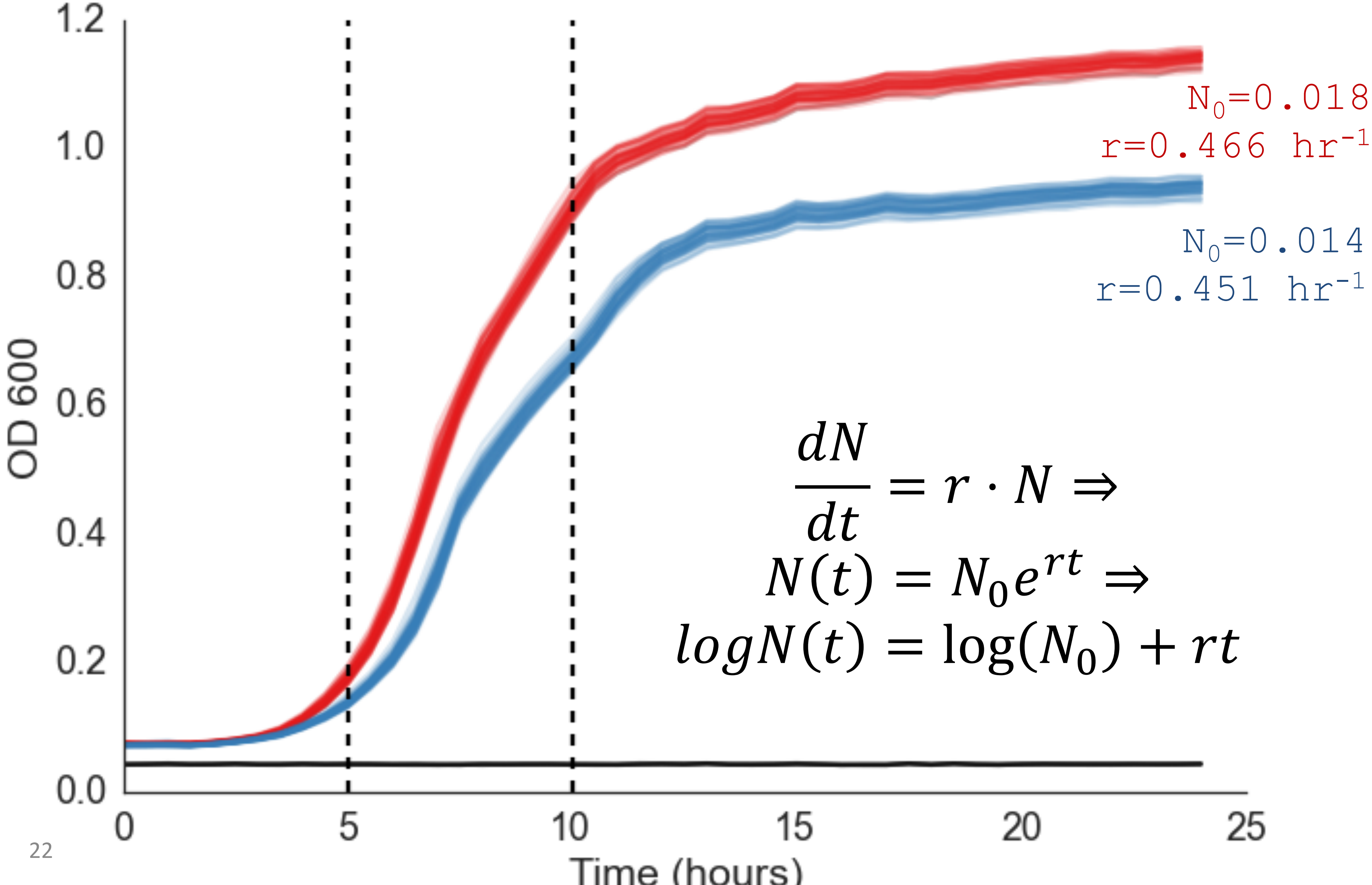




Growth models

Death to the Stock Photo

Exponential model



$$\frac{dN}{dt} = r \cdot N \Rightarrow$$

$$N(t) = N_0 e^{rt} \Rightarrow$$

$$logN(t) = \log(N_0) + rt$$

Exponential model

$$\frac{dN}{dt} = r \cdot N \Rightarrow N(t) = N_0 e^{rt} \Rightarrow logN(t) = \log(N_0) + rt$$

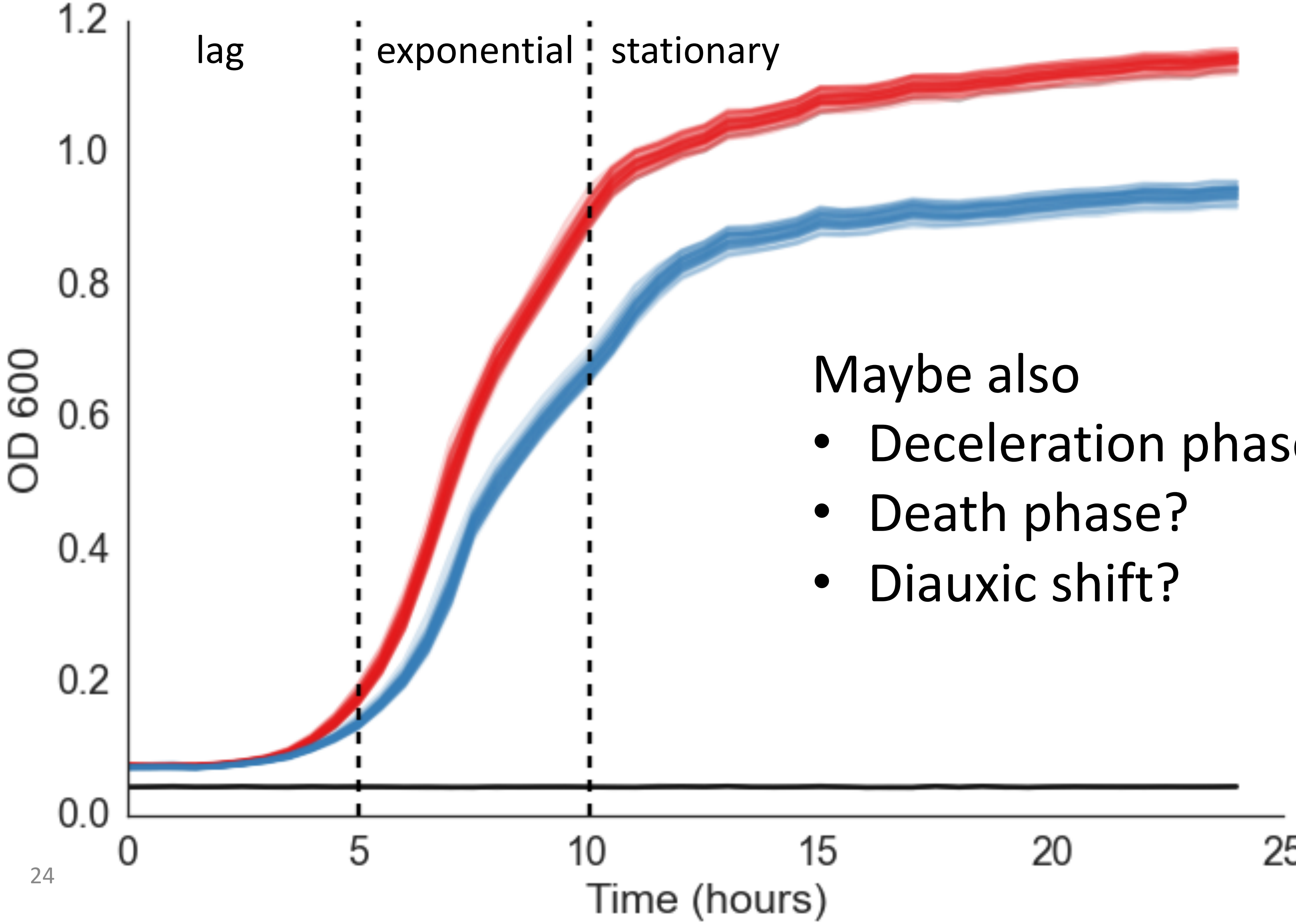
Traditional method says: $s = r - r = 0.015\ hr^{-1}$

Can be converted to generations: $s_T = \frac{r - r}{r} \ln(2)\ \ gen^{-1}$

Chevin 2011

This analysis only captures one growth parameter -> one growth phase

Three growth phases?

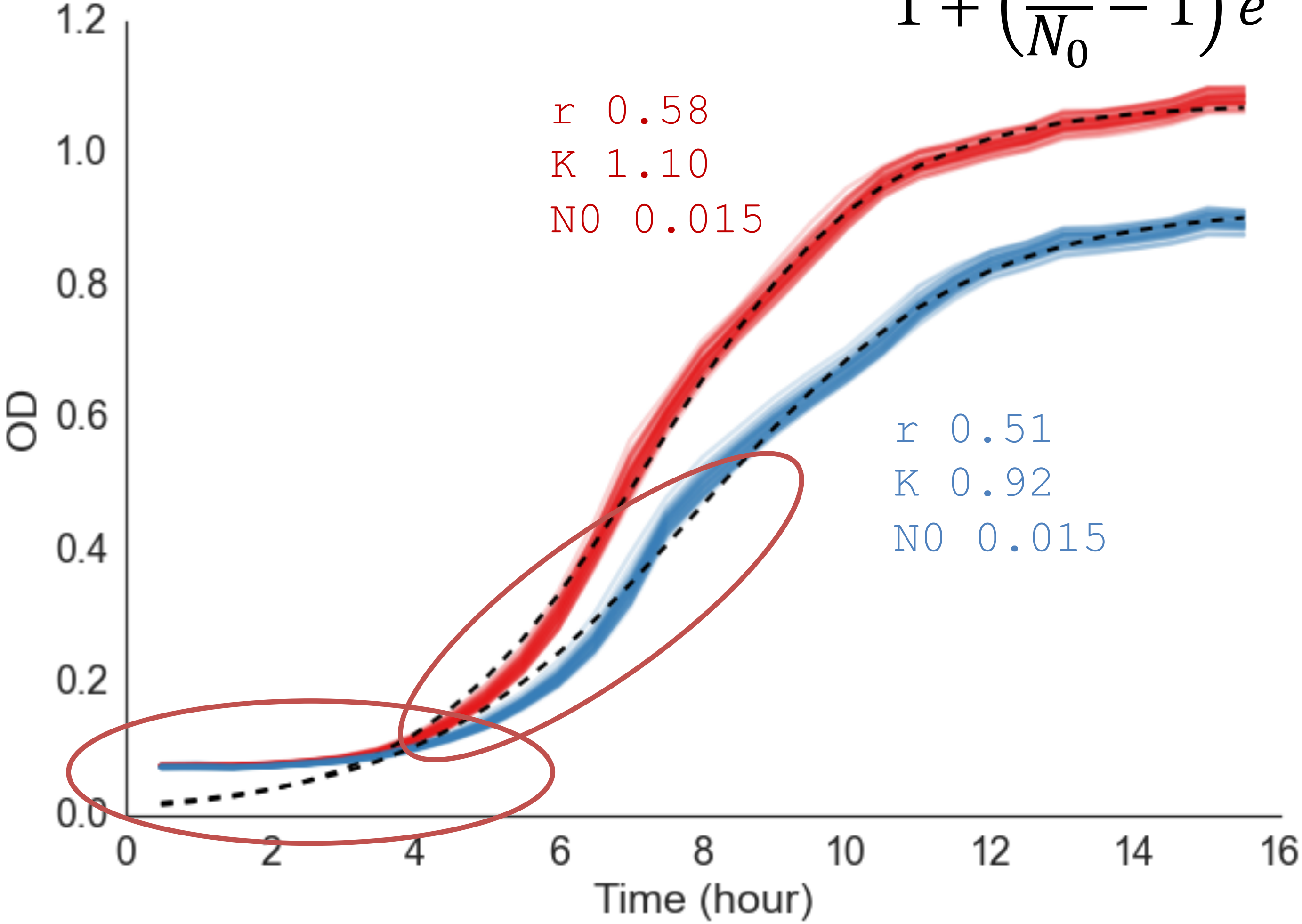


Maybe also

- Deceleration phase?
- Death phase?
- Diauxic shift?

Logistic model

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right) \Rightarrow N(t) = \frac{K}{1 + \left(\frac{K}{N_0} - 1\right)e^{-rt}}$$



Logistic model: why?

Can be derived from resource consumption models.

Consumer growth:

$$\frac{dN}{dt} = \epsilon \mathrm{aRN}$$

Abiotic resource with logistic growth:

$$\frac{dR}{dt} = -\mathrm{aRN}$$

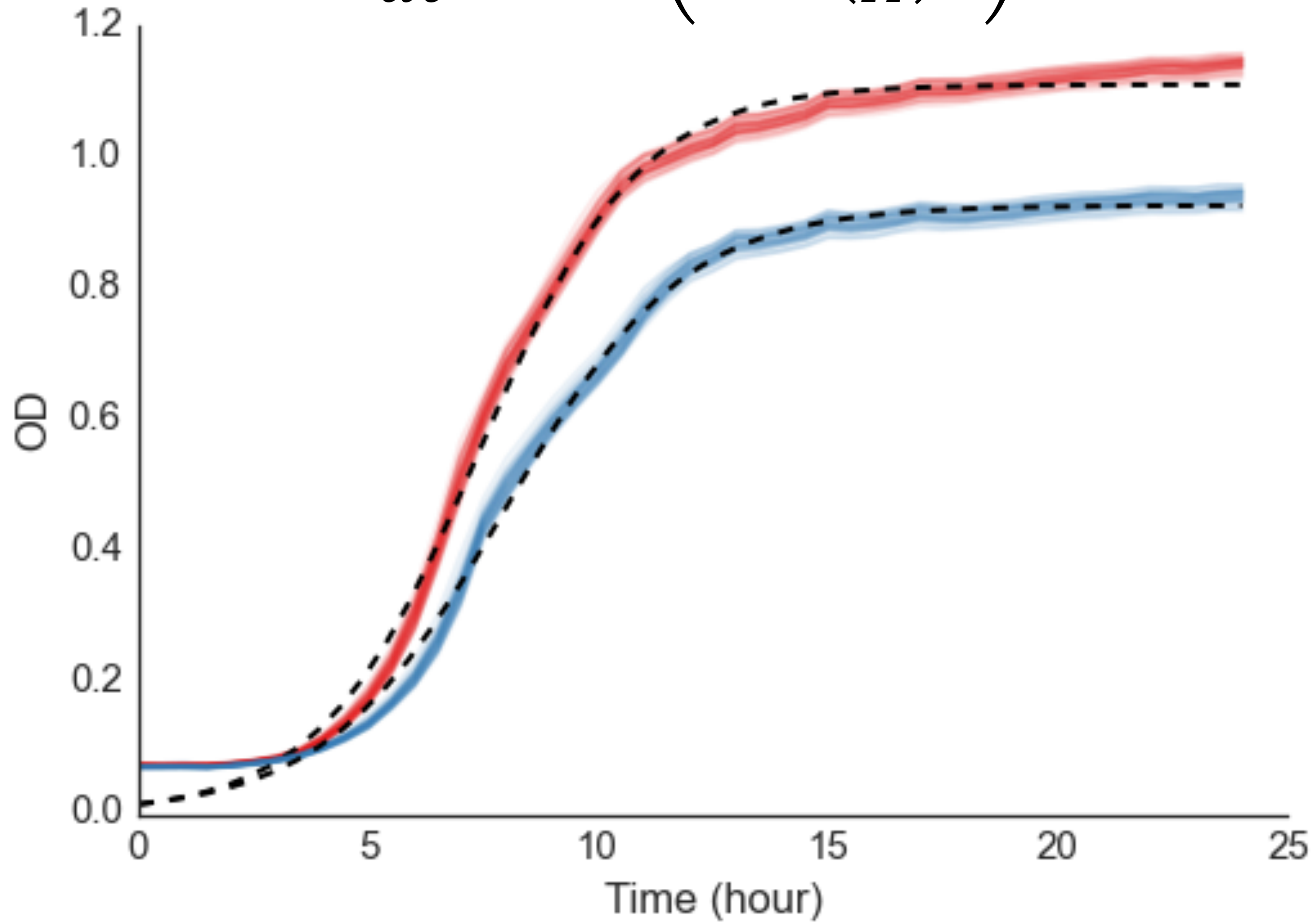
Biotic resource with logistic growth:

$$\frac{dR}{dt} = \mathrm{bR}\left(1 - \frac{\mathrm{R}}{\mathrm{K}}\right) - \mathrm{aRN}$$

http://blog.yoavram.com/growth-models/

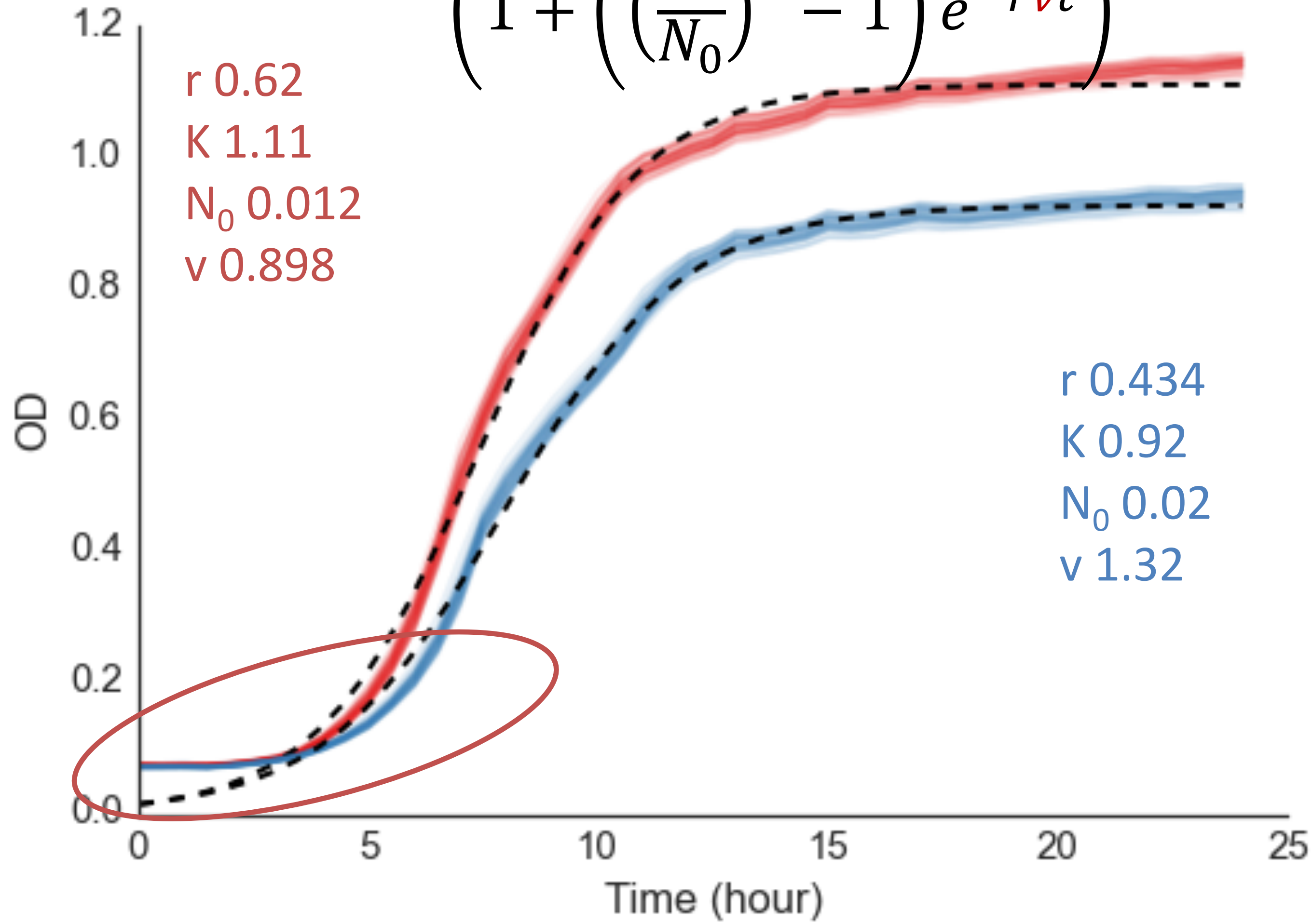
Generalized logistic (Richards) model

$$\frac{dN}{dt} = rN\left(1 - \left(\frac{N}{K}\right)^{\nu}\right)$$



Generalized logistic (Richards) model

$$N(t) = \frac{K}{\left(1 + \left(\left(\frac{K}{N_0}\right)^{\nu} - 1\right) e^{-r\nu t}\right)^{\frac{1}{\nu}}}$$



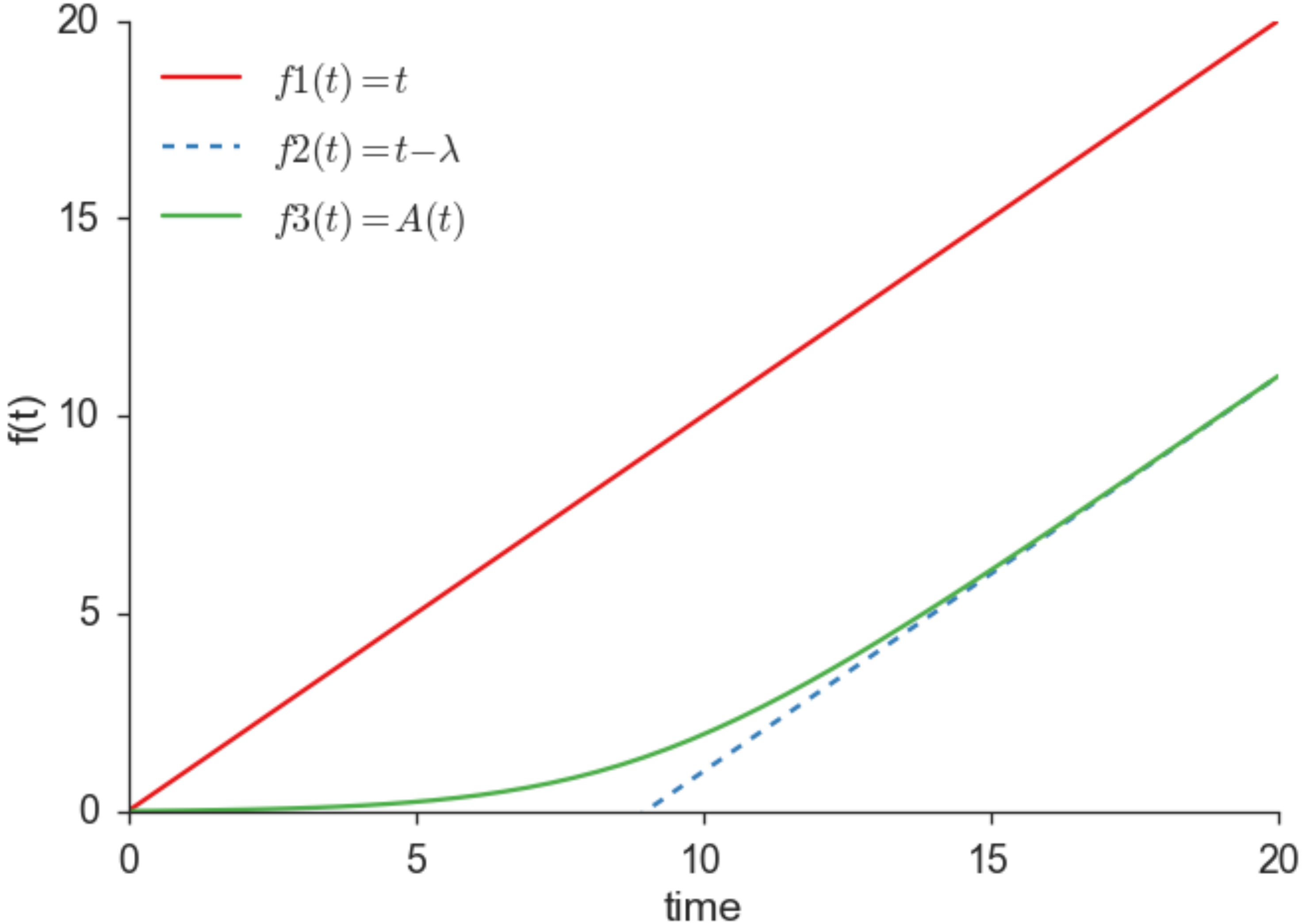
Baranyi-Roberts model

$$\frac{dN}{dt} = r\alpha(t)N\left(1 - \left(\frac{N}{K}\right)^{\nu}\right) \Rightarrow$$

$$N(t) = \frac{K}{\left(1 + \left(\left(\frac{K}{N_0}\right)^{\nu} - 1\right)e^{-r\nu\boldsymbol{A(t)}}\right)^{\frac{1}{\nu}}}$$

$$A(t) = \int_0^t \alpha(s)ds = t + \frac{1}{m}\log\left(\frac{e^{-mt} + q_0}{1 + q_0}\right)$$

Lag phase



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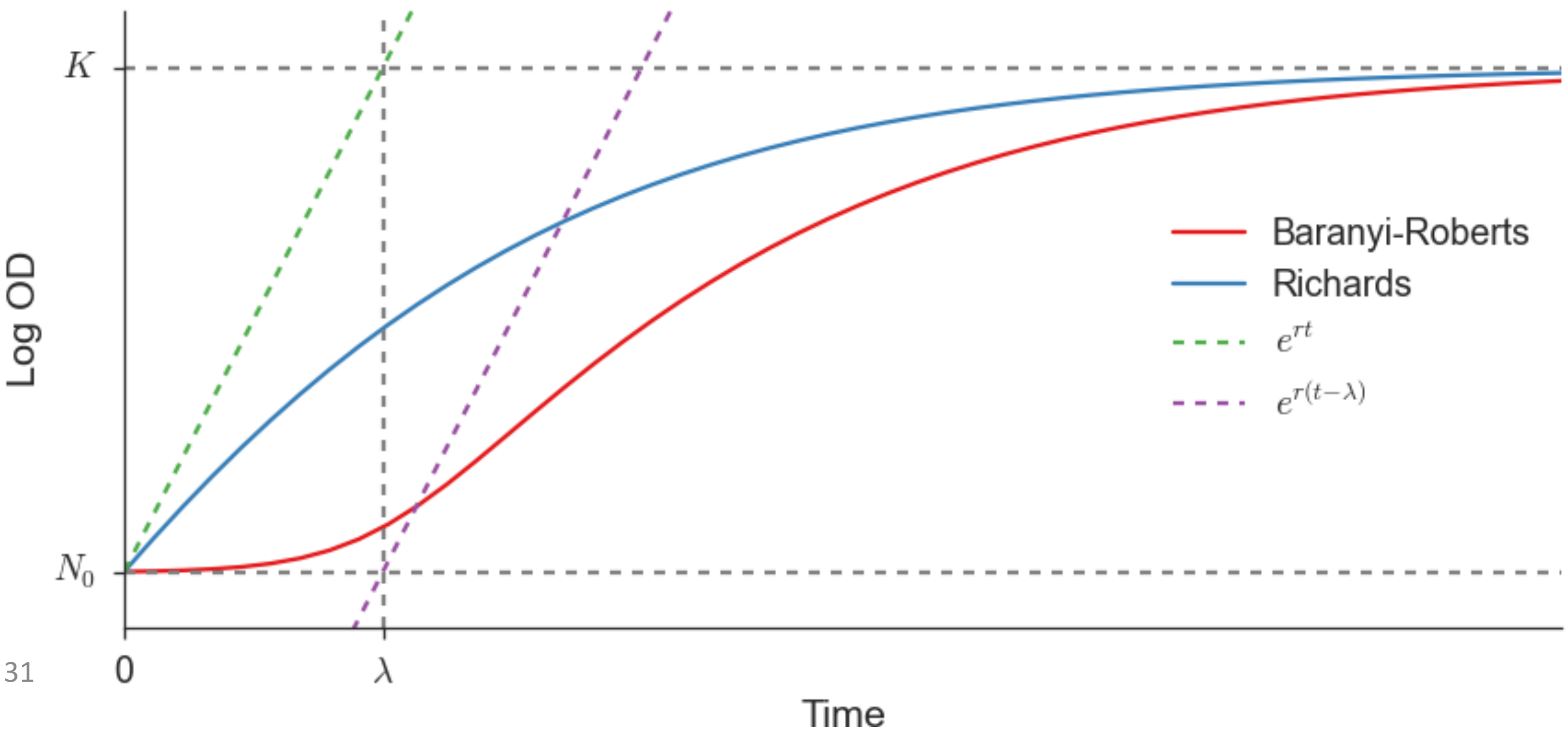


Competition model

We extend the Baranyi-Roberts model for two strains:

$$\frac{dN_1}{dt} = r_1 \cdot \alpha_1(t) \cdot N_1 \left(1 - \frac{\boldsymbol{N_1^{\nu_1} + a_2 N_2^{\nu_2}}}{\boldsymbol{K_1^{\nu_1}}}\right)$$

$$\frac{dN_2}{dt} = r_2 \cdot \alpha_2(t) \cdot N_2 \left(1 - \frac{\boldsymbol{a_1 N_1^{\nu_1} + N_2^{\nu_2}}}{\boldsymbol{K_2^{\nu_2}}}\right)$$

- Focus on **resource competition**
- No direct interactions

Competitions model

$$\frac{dN_1}{dt} = r_1 \cdot \alpha_1(t) \cdot N_1 \left(1 - \frac{\boldsymbol{N_1^{\nu_1} + a_2 N_2^{\nu_2}}}{\boldsymbol{K_1^{\nu_1}}}\right)$$

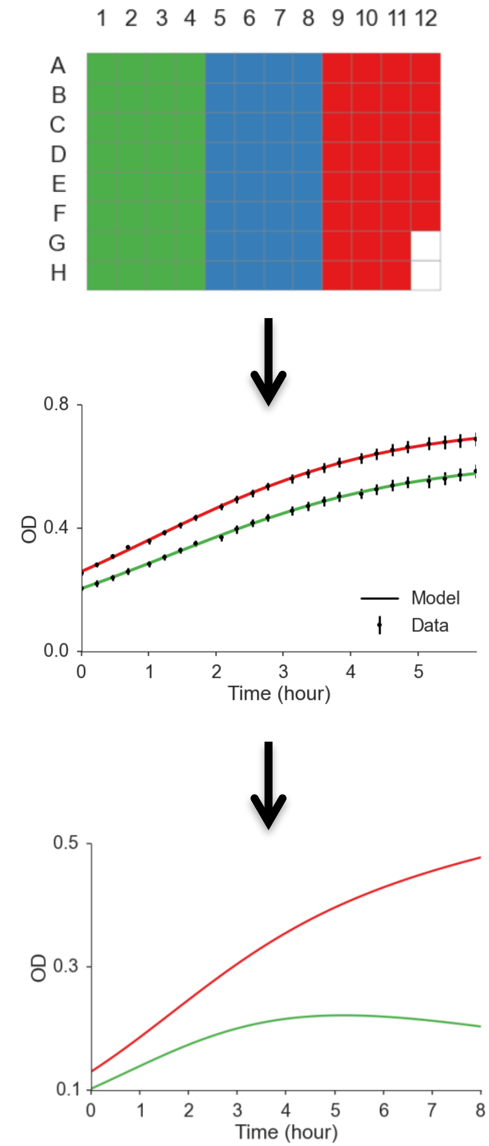
$$\frac{dN_2}{dt} = r_2 \cdot \alpha_2(t) \cdot N_2 \left(1 - \frac{\boldsymbol{a_1 N_1^{\nu_1} + N_2^{\nu_2}}}{\boldsymbol{K_2^{\nu_2}}}\right)$$

- Estimate a_1 and a_2 by fitting $N_1 + N_2$ total OD in mixed culture
- Infer N_1 and N_2 by solving model

Testing the predictive framework

Testing the predictive framework

- Two *E. coli* strains with fluorescent proteins (GFP, RFP)
- Growth in mono- and mixed culture
- Measure OD over time
- Fit growth model
- Predict competition results from fitted growth models
- Validate predictions using pairwise competition experiments
- Use fluorescence to measure frequencies over time



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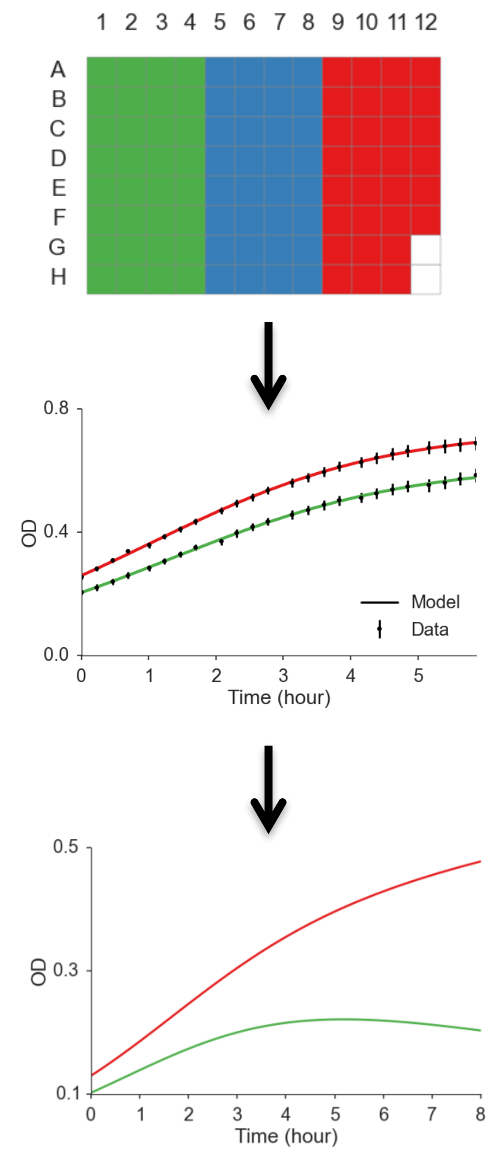

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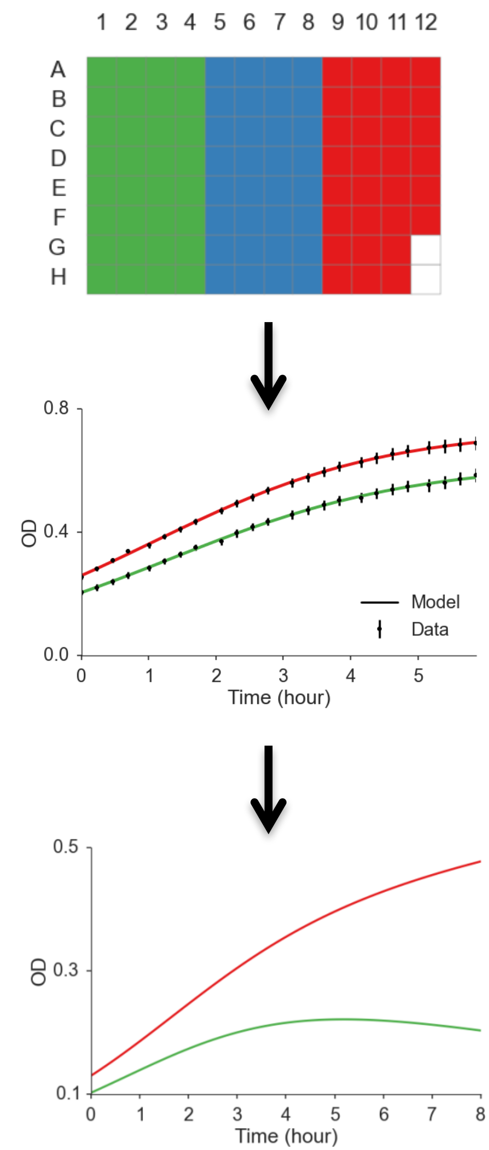

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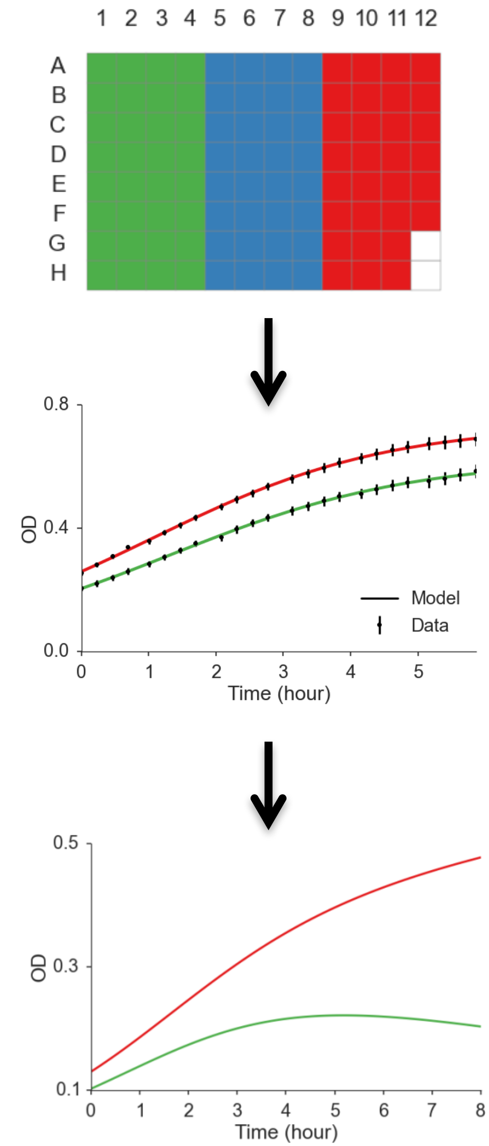
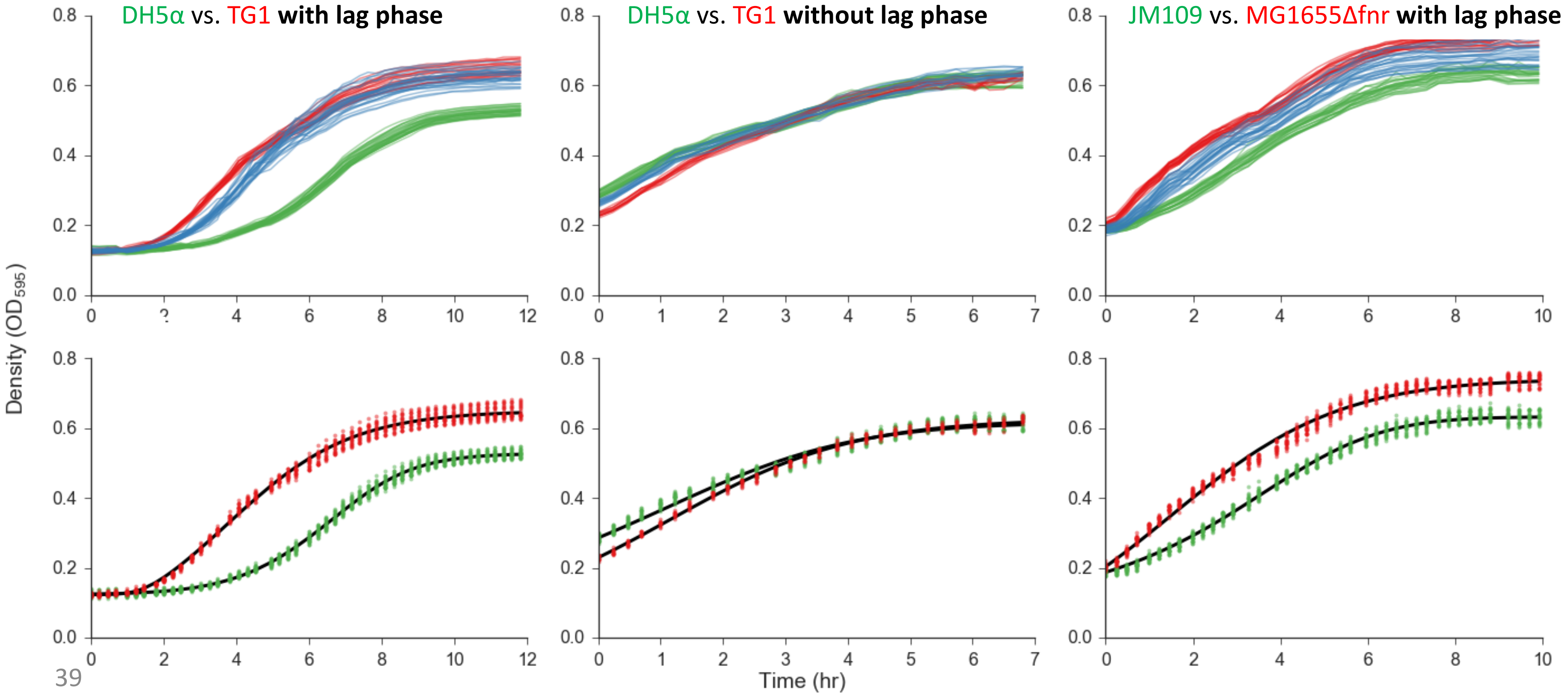


Growth curves data

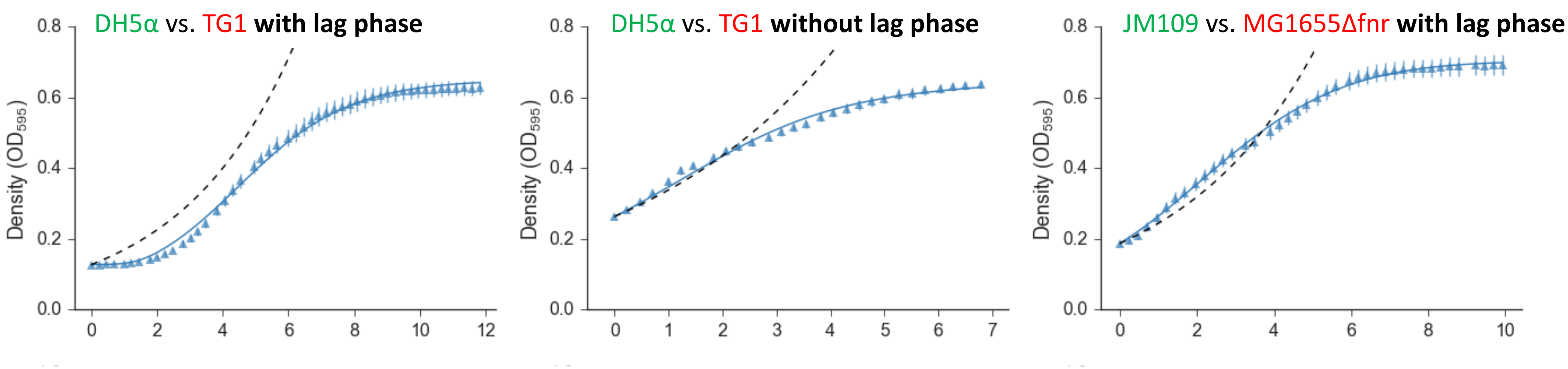
Top: Data from 3 experiments with *E. coli*

Bottom: Monoculture data with fitted growth models



Growth curves data

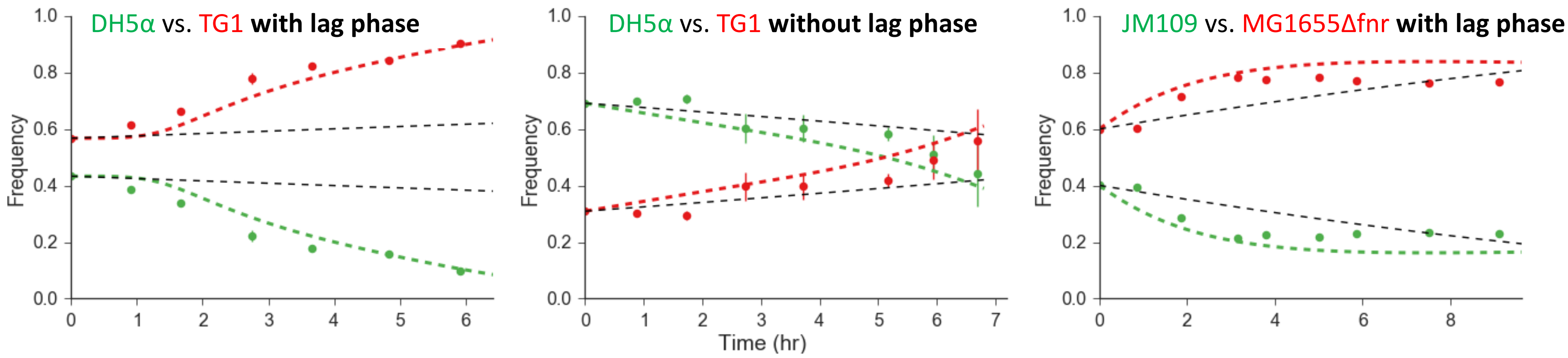
Mixed culture data with fitted competition models



*Dashed lines are fitted exponential models

Competition prediction

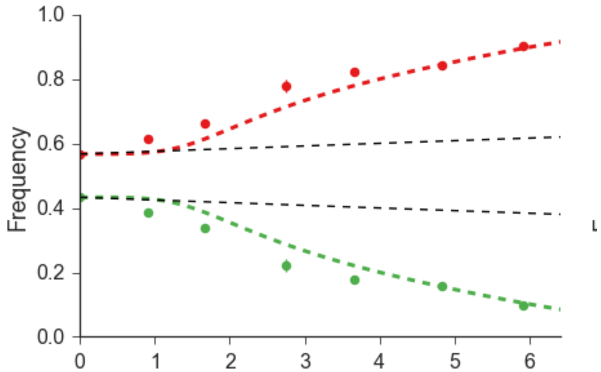
Dashed: competition model prediction
Markers: flow cytometry data



*Dashed lines are exponential models prediction

Summary

1. Fit growth models to growth curves
2. Predict competition results
3. Infer fitness



Collaborators:

Eynat Dellus-Gur, Maayan Bibi, Uri Obolski, Judith Berman, Lilach Hadany (Tel-Aviv University)

Preprint:

Ram et al. Predicting microbial relative growth in a mixed culture from growth curve data. bioRxiv, doi:10.1101/022640

Software website: curveball.yoavram.com

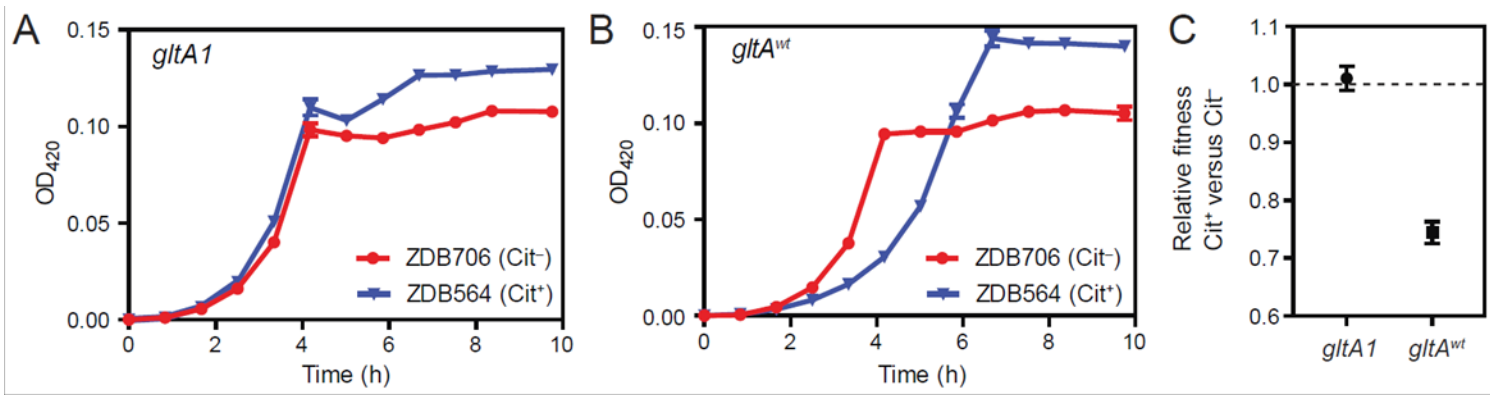
Test case: Citrate utilization in *E. coli*

Quandt et al., eLife 2015;10.7554/eLife.09696 (**Barrick lab**)

- mutations in the *gltA* gene - encodes citrate synthase (CS)
- before and after *E. coli* evolved to grow aerobically on citrate (Cit^+)
- first *gltA* mutation increases CS activity, beneficial for growth on acetate
- contributed to preserving the rudimentary Cit^+ trait from extinction
- Cit^+ then refined by more mutations
- The potentiating *gltA* mutation became deleterious to fitness
- beneficial *gltA* mutations evolved to reduced CS activity

Test case: Citrate utilization in *E. coli*

Figure 4:



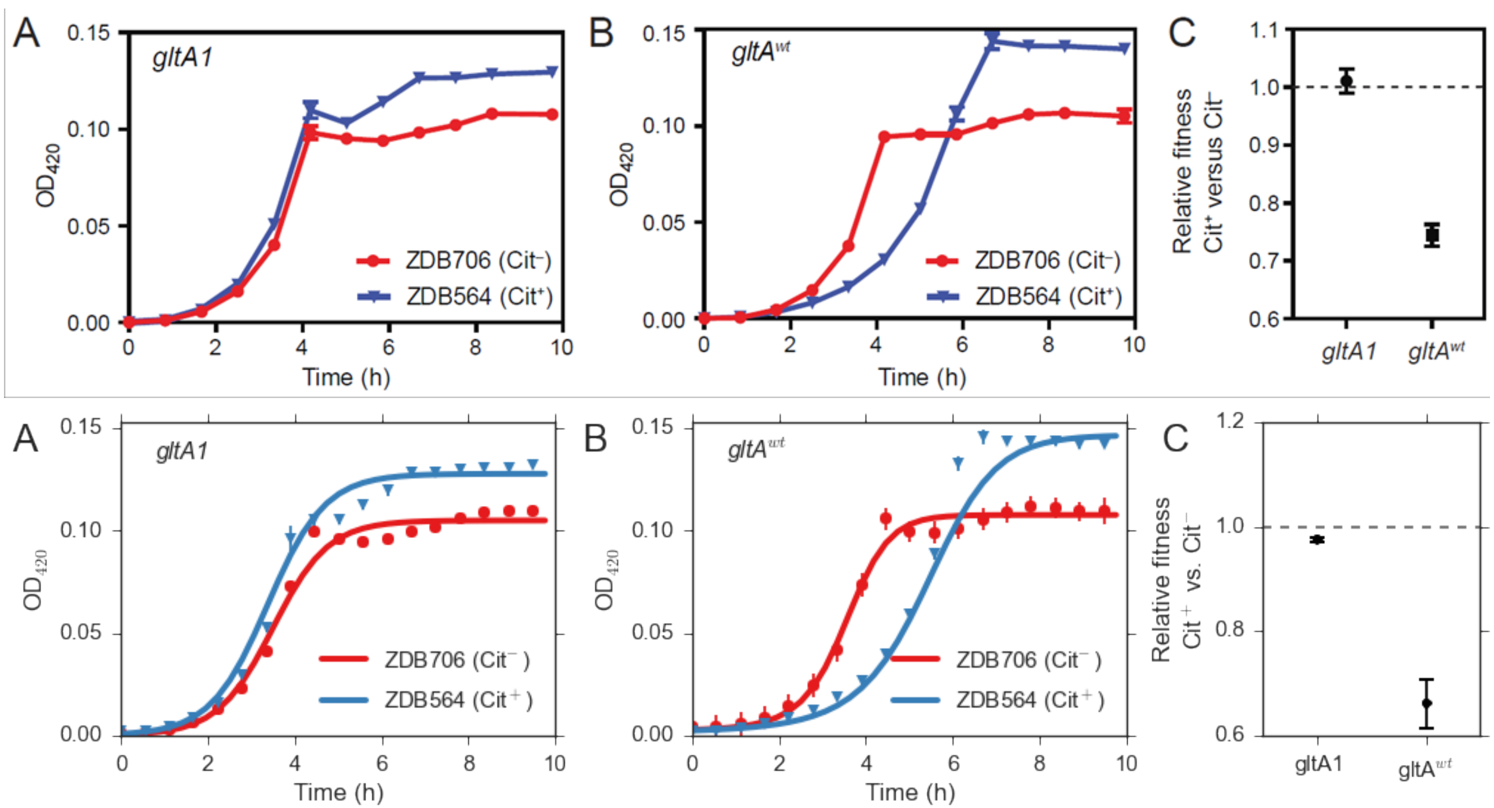
A) Early Cit+ and Cit- revertant with gltA1 mutation: Cit+ has higher K

B) Early Cit+ and Cit- revertant with wildtype gltA: Cit- has shorter lag and lower K

C) Relative fitness Cit+ vs. Cit- from competition assays

Test case: Citrate utilization in *E. coli*

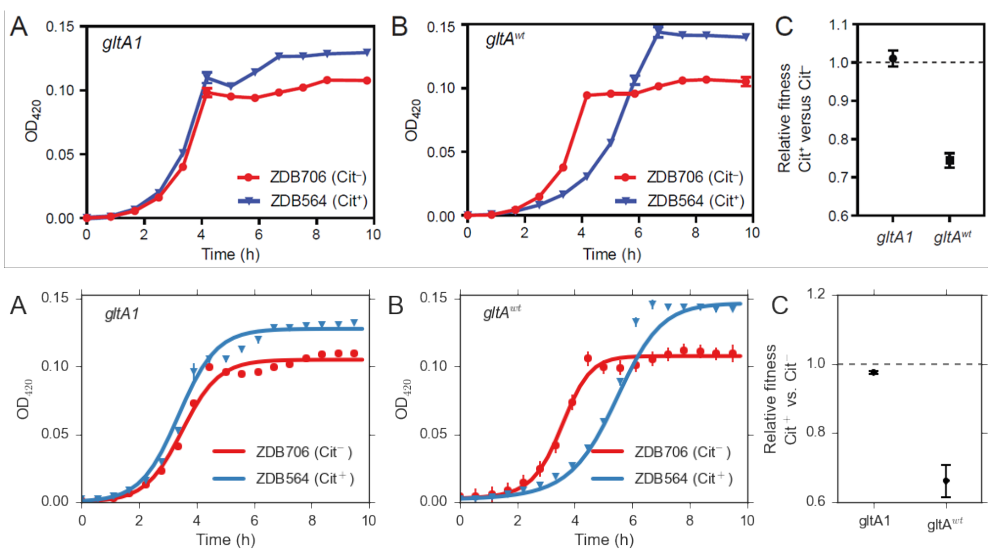
Fig. 4 from Quandt et al., 2015



Reproduce Fig. 4 with *Curveball* using <u>***only***</u> growth curves data

Test case: Citrate utilization in *E. coli*

Fig. 4 from Quandt et al., 2015



Reproduce Fig. 4 with **Curveball**

The discrepancy between panel **C** In the top and bottom figures can be explained by:

1. The model fits are not perfect, especially in Cit+ gltA^{wt}: there is a strange "bump" in the curves.
2. Competition and growth assays were done in different conditions: 100uL x 96 wells vs. 50mL flasks; x10 glucose concentration in growth assays.

Future directions

- Complex growth curves:
  - Bi-phasic growth:
    - Diauxic shift: glucose-ethanol, glucose-lactose etc.
    - Changing drug concentrations
  - Deep stationary phase
  - Cell death
- Null model for detection of direct interactions
- Predict adaptive evolution
- Interpret fitness differences

Death to the Stock Photo

Acknowledgments

Collaborators on Curveball:

Hadany Lab

Lilach Hadany

Uri Obolski

Eynat Dellus-Gur

Berman Lab

Judith Berman

Maayan Bibi

Israeli Ministry of Science & Technology

MARIE CURIE ACTIONS

Manna Center Program for Food Safety & Security Tel Aviv University

Lab Evolution

CEHG

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Thank You!

Presentation: **github.com/yoavram/EcoEvoLunch2017**

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Death to the Stock Photo