OPTIMALITY AND OPTIMIZING DURING EXPERIMENTAL EVOLUTION

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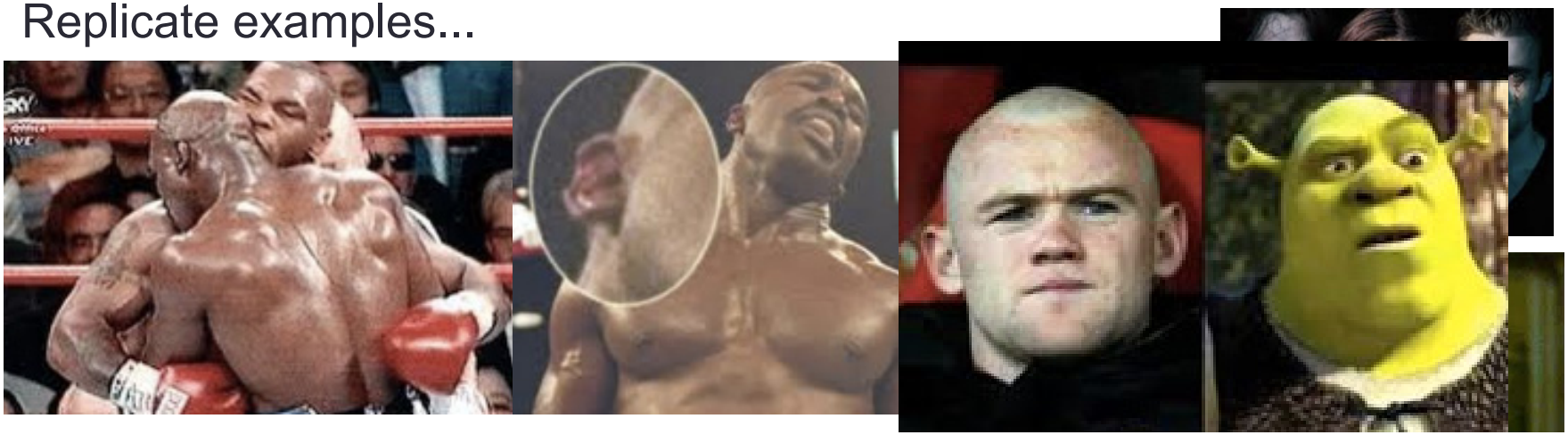
Cases of known adaptation

Ancestral, primitive behavior

Refined, optimized behavior

Replicate examples...



Cases of known adaptation

Ancestral, primitive behavior

Refined, optimized behavior

Other transformations possible

Cellular economy at many levels

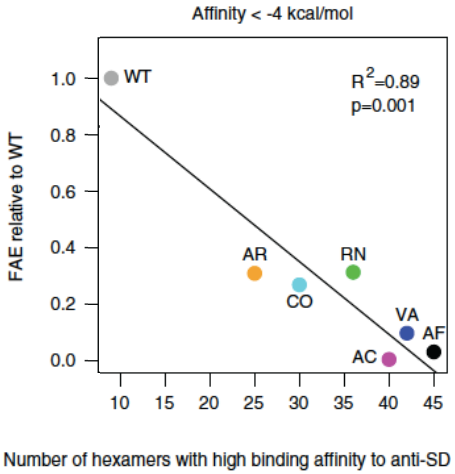
- Selection upon synonymous sites
- Selection to reduce the accessory genome
- Selection can occur with surprisingly little substrate specialization

- Optimizing economy of central metabolism
- Optimizing the use of foreign metabolic pathways
  - Large-scale transcriptome changes scaling with growth rate
  - Optimizing expression of enzymes of a foreign formaldehyde oxidation pathway

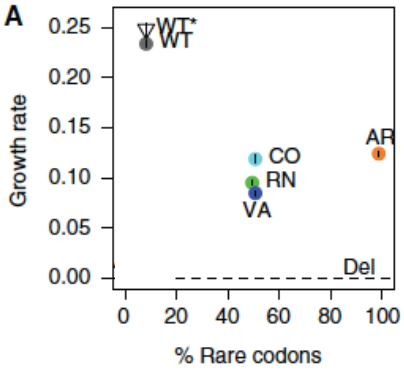
Selection upon synonymous sites

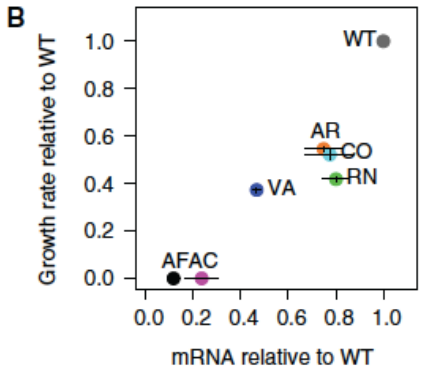
Table 1. Experimental Strains with Synonymous *fae* Alleles.

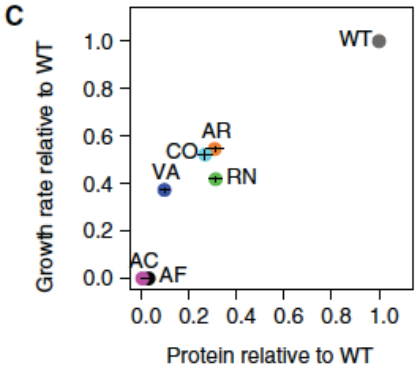
Strain	Codon Distribution: % Frequent	% Rare	Residues with Rare Codons	# Synonymous Mutations	5′ sequence: M A K I T K V Q V G E A L V
WT	71.9	8	Wild type	–	ATGGCAAAAATCACCAAGGTTCAGGTCGGCGAGGCCCTCGTC
AF	100	0	None	46	ATG..C..G.....G.....G.....G..G
AC	89	11	Active	52	ATG..C..G.....G.....G.....T.G..G
RN	49.4	49.4	Random	92	ATG.....A.....G..A..A.....AT.G..G
VA	49.4	50.6	Variable	102	ATG..C..G.....G.....G..A..A...T.G..A
CO	49.4	50.6	Conserved	94	ATG.....A..A..A..A..A.....A..G..G
AR	0	98.8	All	150	ATG.....A..A..A..A..A..A..A..AT.G..A

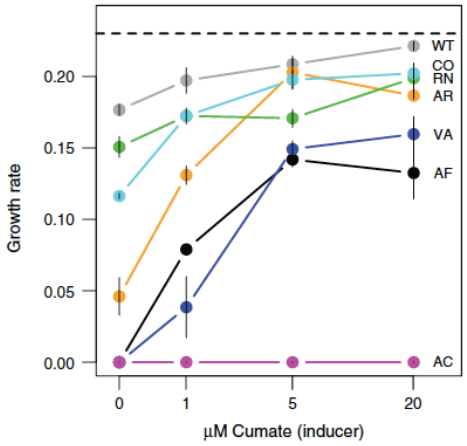


- Synthesized synonymous versions of key enzyme
- Main cause: anti-SDs









- Massive fitness effects; due to low expression

(Agashe et al., 2013. *Molecular Biology & Evolution*; Agashe et al., in prep)

Selection upon synonymous sites

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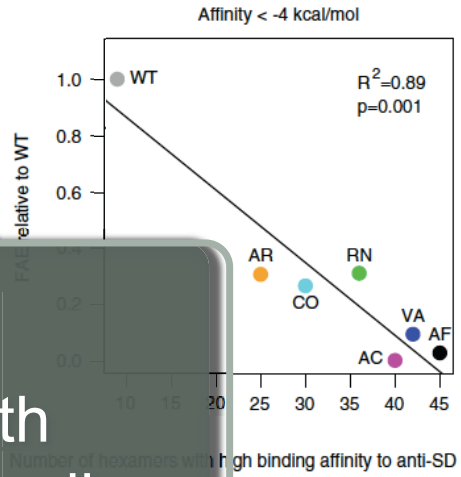
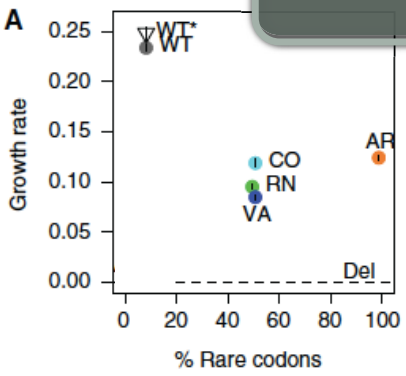
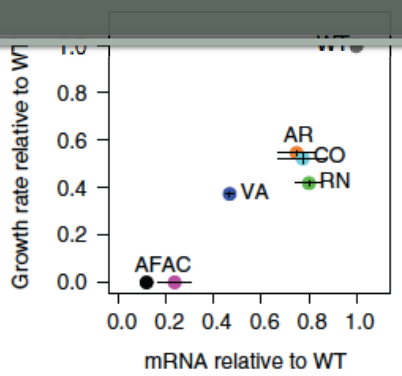


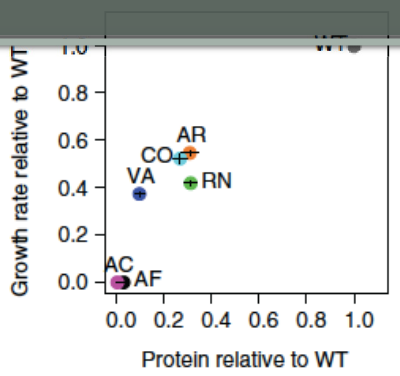
Subsequent adaptation:

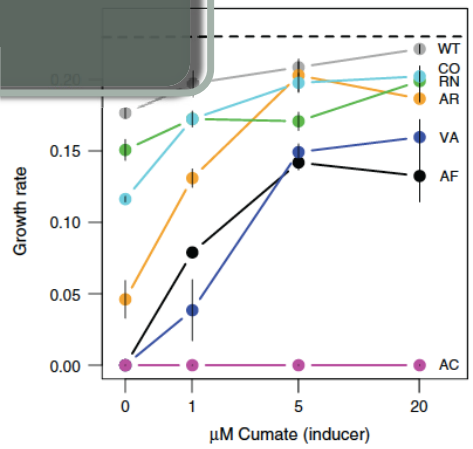
- Single mutations recover WT growth
- Sometimes promoter; sometimes coding
- 5’ end preventing anti-SDs

- Synthesiz[illegible] synonymous versions of key enzyme [illegible] se: anti-SDs





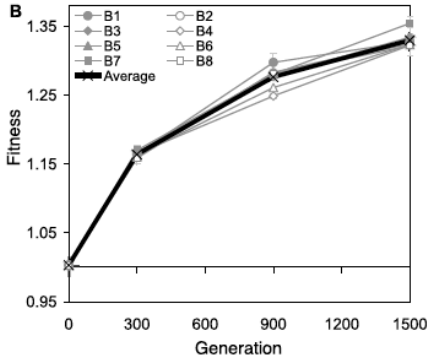


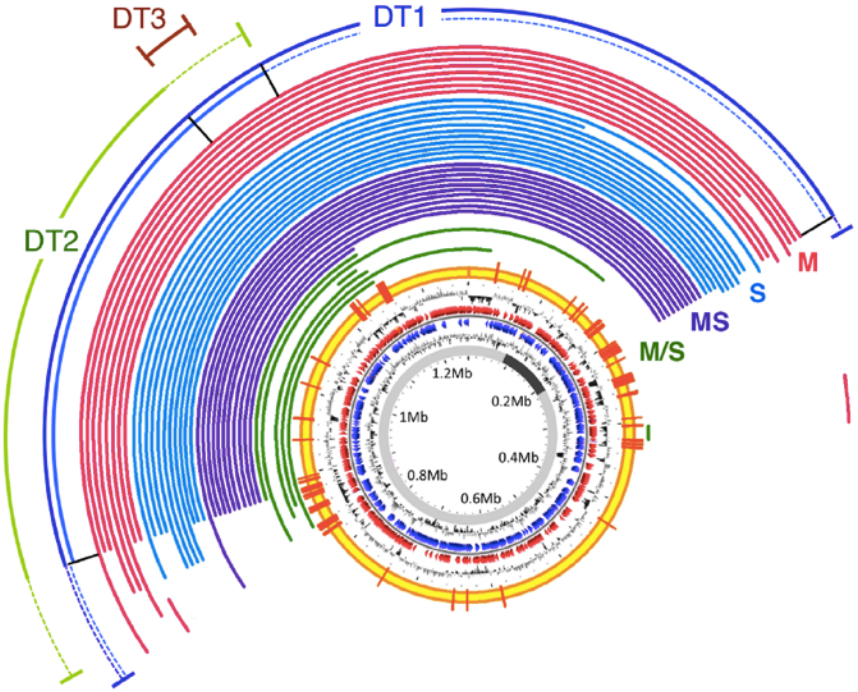


- Massive fitness effects; due to low expression

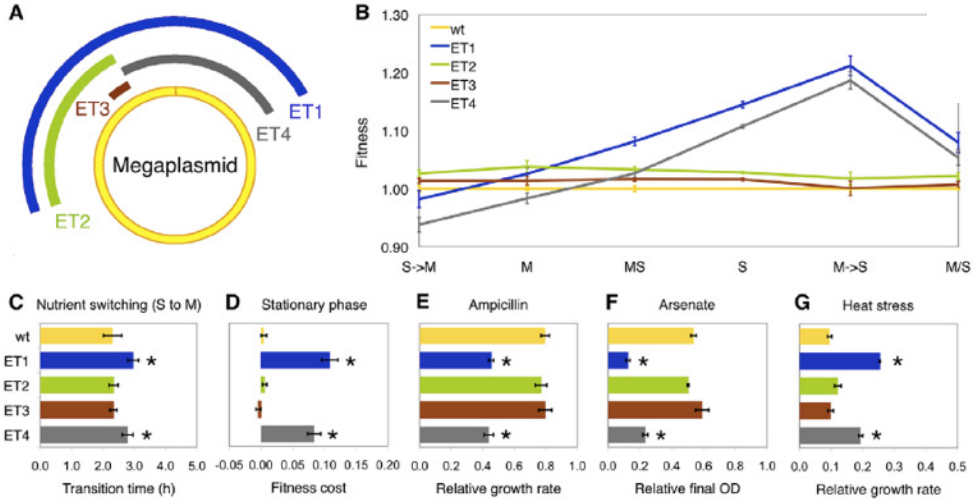
(Agashe et al., 2013. *Molecular Biology & Evolution*; Agashe et al., in prep)

Selection to remove up to 10% genome





- Parallel deletions of >600 kb of megaplasmid in 1500 gen. adaptation by *Methylobacterium*

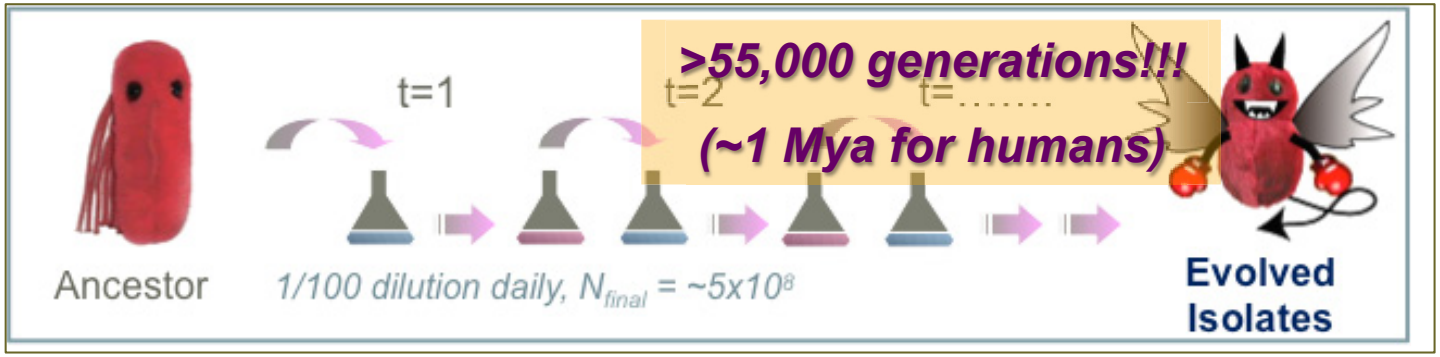


- Benefit due to genes removed, not just DNA
- Caused tradeoffs in other environments

(Lee et al., 2009. *Evolution*; Lee and Marx, 2012. *PLoS Genetics*)

Longest evolution: Lenski long-term lines

- On February 24th, 1988, Rich Lenski started 12 populations of *E. coli* B in minimal glucose medium

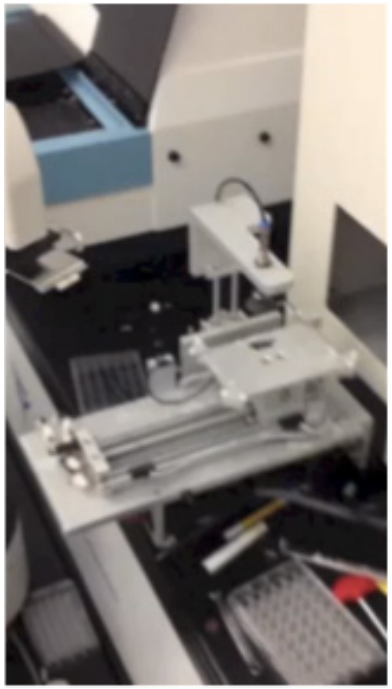


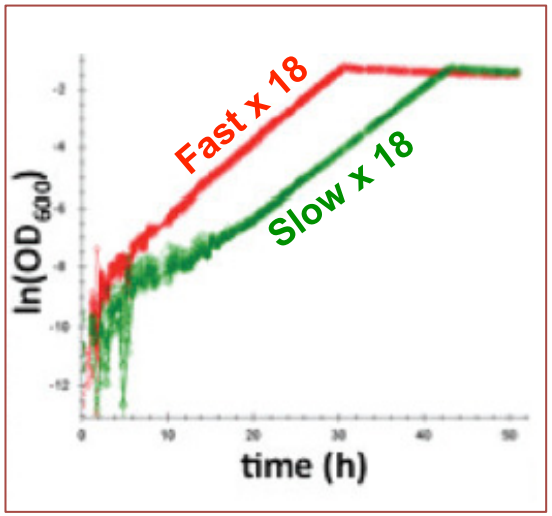
- USA popular culture in February, 1988:

#1 song: "Seasons Change" by Exposé

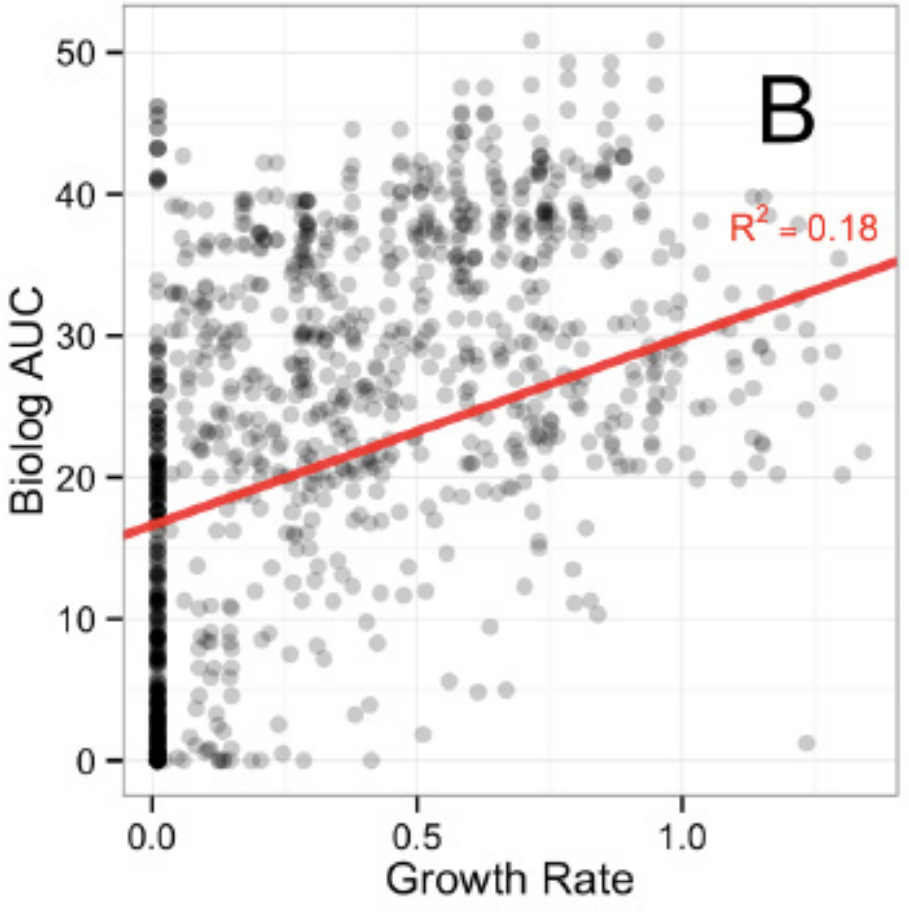
#1 album: Faith, George Michael

Little specialization over 50,000 gen.





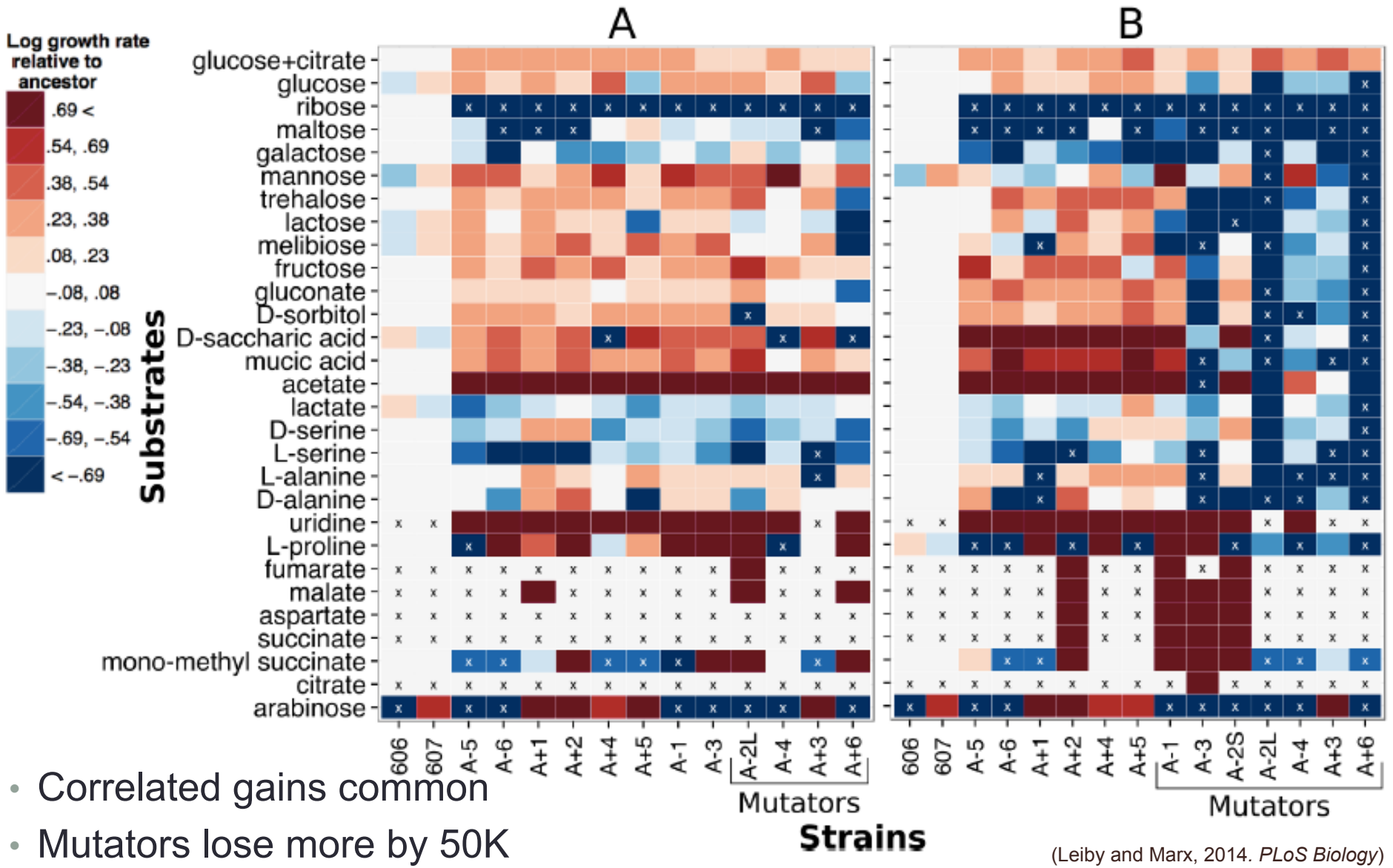
- Automated, robotic growth system



- Biolog ≠ growth

(Delaney et al., 2013. *J. Lab. Automation*; Delaney et al., 2013. *PLoS One*; Leiby and Marx, 2014. *PLoS Biology*)

Little specialization over 50,000 gen.

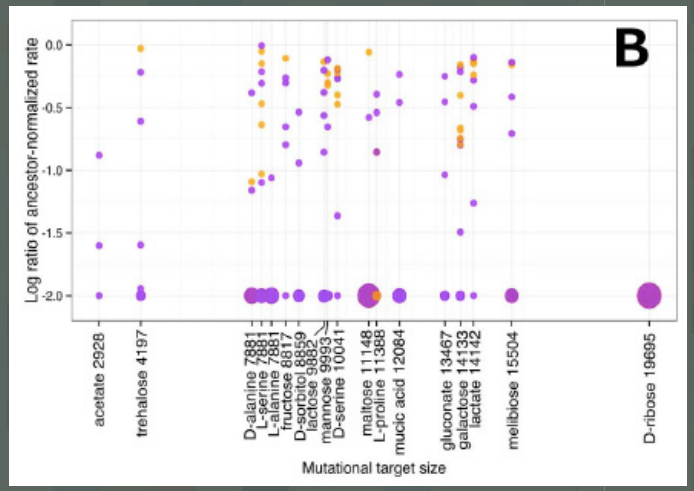


- Correlated gains common
- Mutators lose more by 50K

(Leiby and Marx, 2014. *PLoS Biology*)

Little specialization over 50,000 gen.

Substrates experiencing decline not correlated with similarity to glucose



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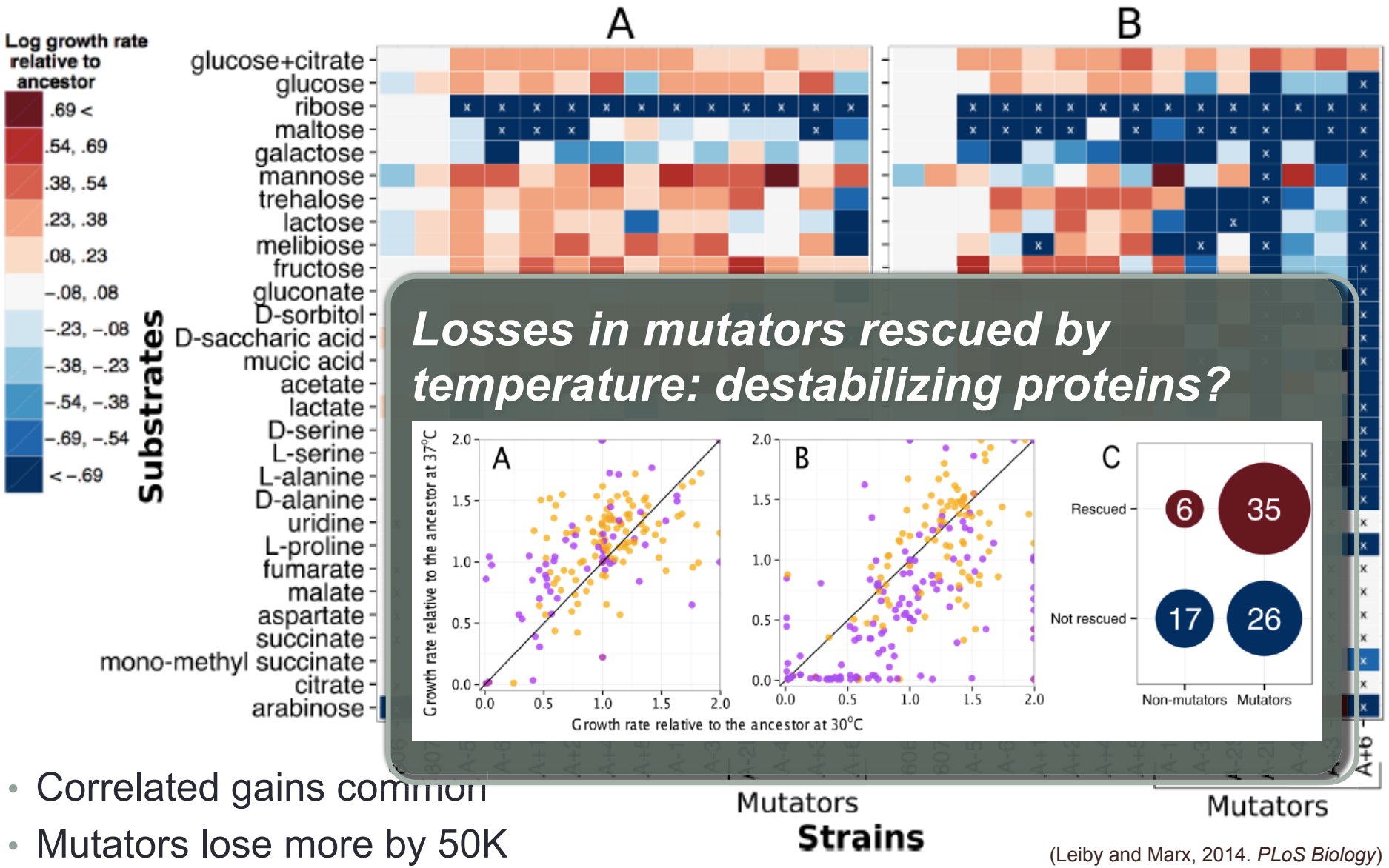

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Metabolism: connect phenotype to fitness at two scales

Metabolic control (MCA)

"Changes to which parts would most optimize fitness?"

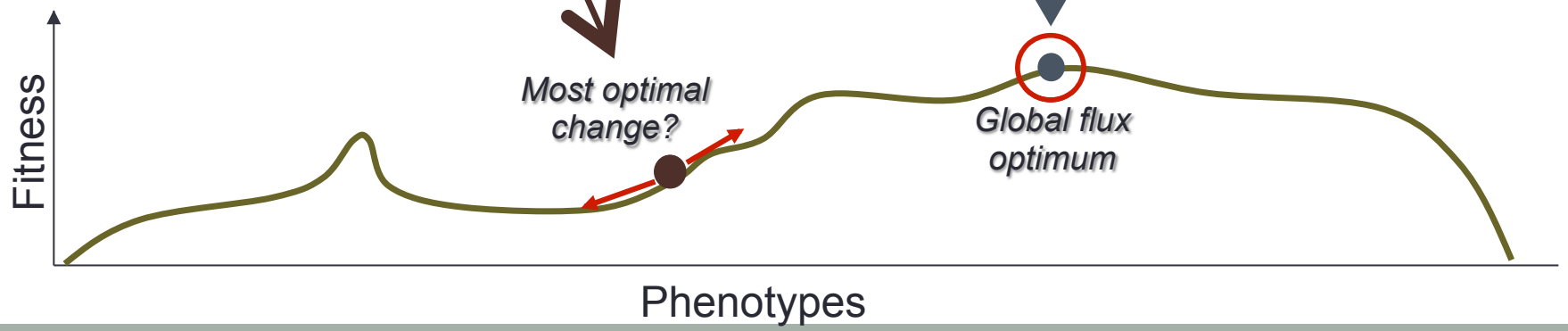
- **Kinetic model of chemical species through time (Michaelis-Menten)**
- **Need to know relevant parameters**
- **Sensitivity of flux to parameter changes ('control' coefficient, c_i)**
- **Most enzymes exert very little control on flux**
- **Assumption: flux α fitness**

Flux balance analysis (FBA)

"What is the optimal flux phenotype?"

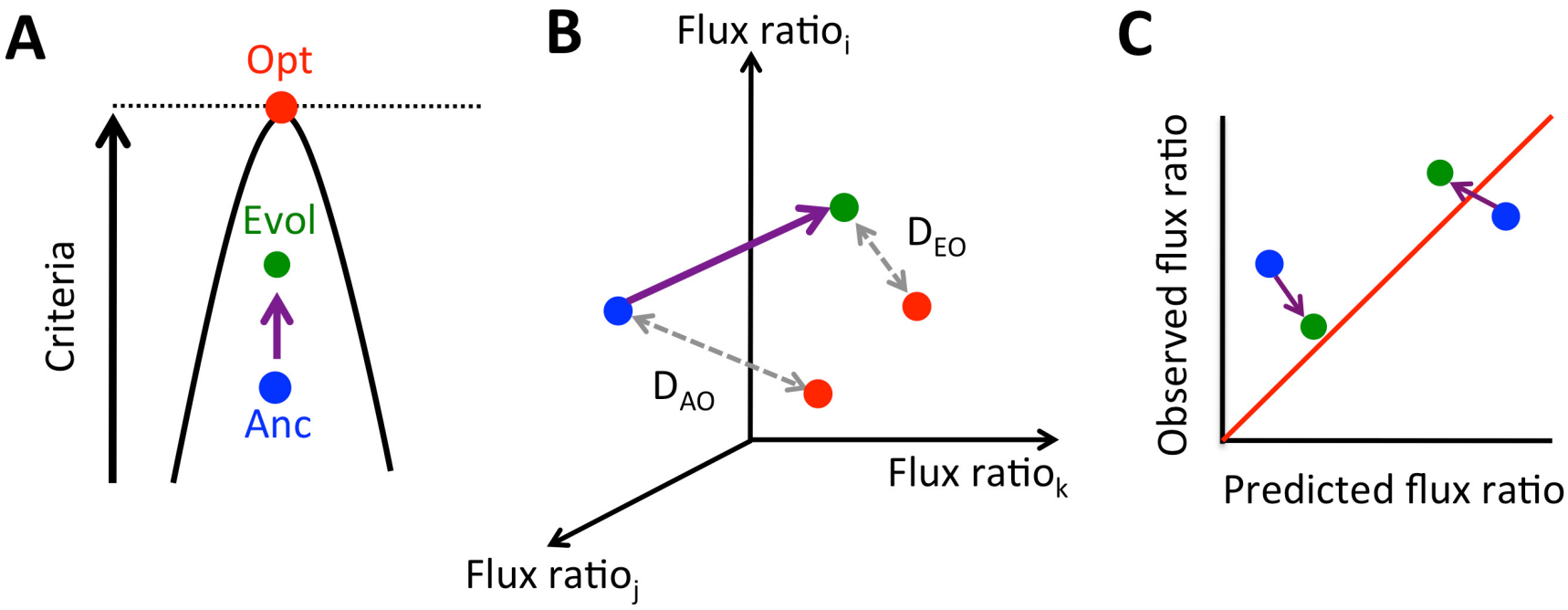
- **Describes network topology via a complete stoichiometric matrix**
- **Defines set of all possible balanced steady-states (w/in constraints)**
- **Choose flux state that is "optimal"**
- **Key assumption: past selection to become perfect for chosen criterion**

Adaptive landscape:



What does it mean to be optimal?

- No previous test if **internal fluxes** evolve to be FBA optimal
- Can compare predictions to measured fluxes in three ways:

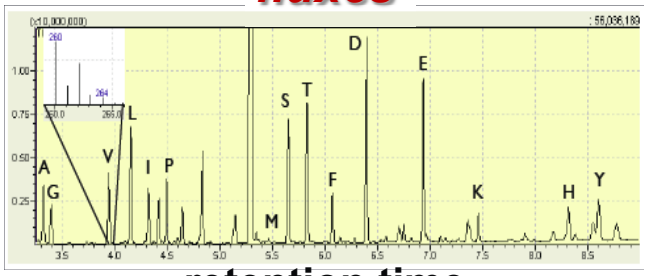


- What should be optimized? Normal = BM/S = **C yield**
- What is selected for in batch culture? Mainly growth **rate**

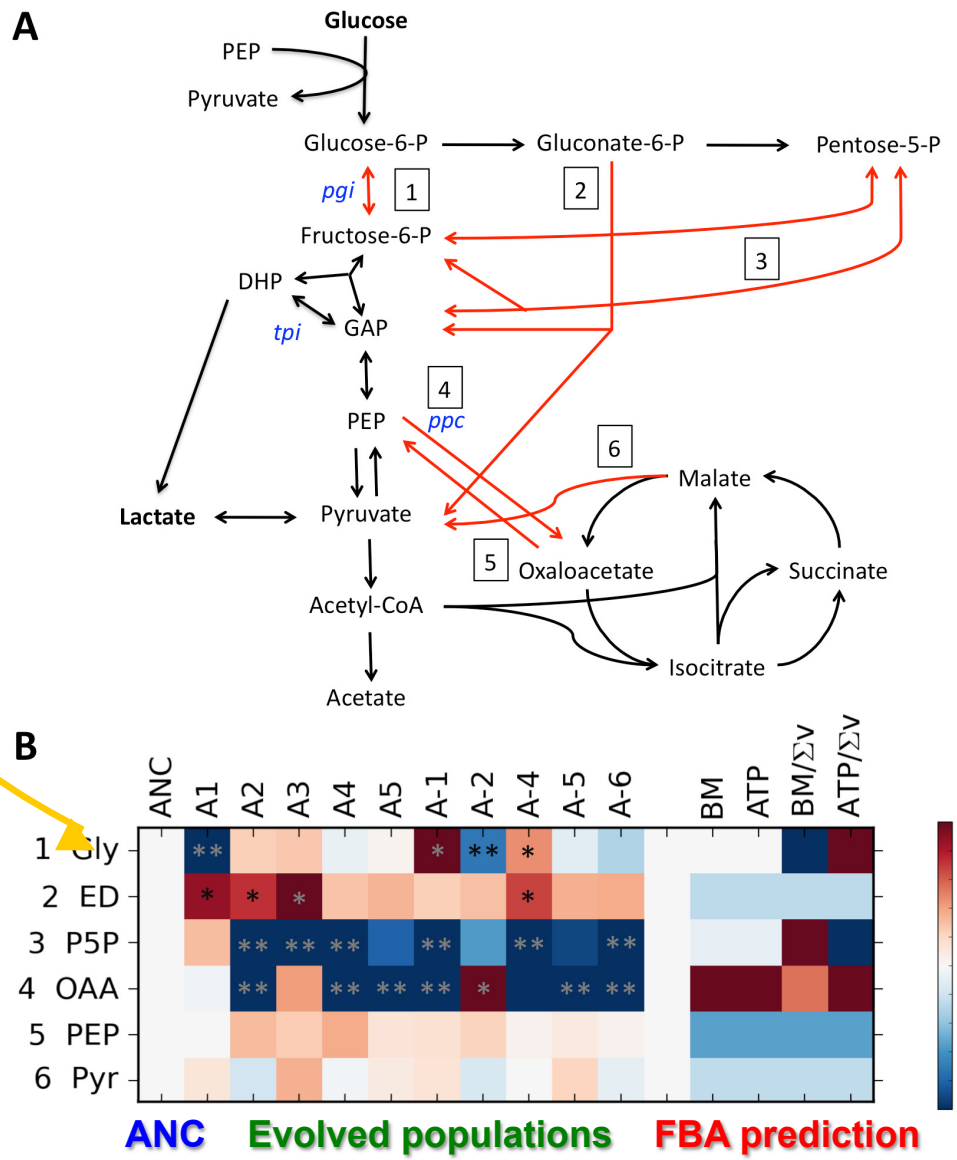
(Harcombe et al., 2013. *PLoS Computational Biology*)

Do fluxes evolve to FBA-predicted optimum?

^{13}C-method to assay fluxes



1. Harvest total protein
2. Hydrolyze amino acids
3. Derivatize
4. GC-MS for mass dist.
5. Analyze via network



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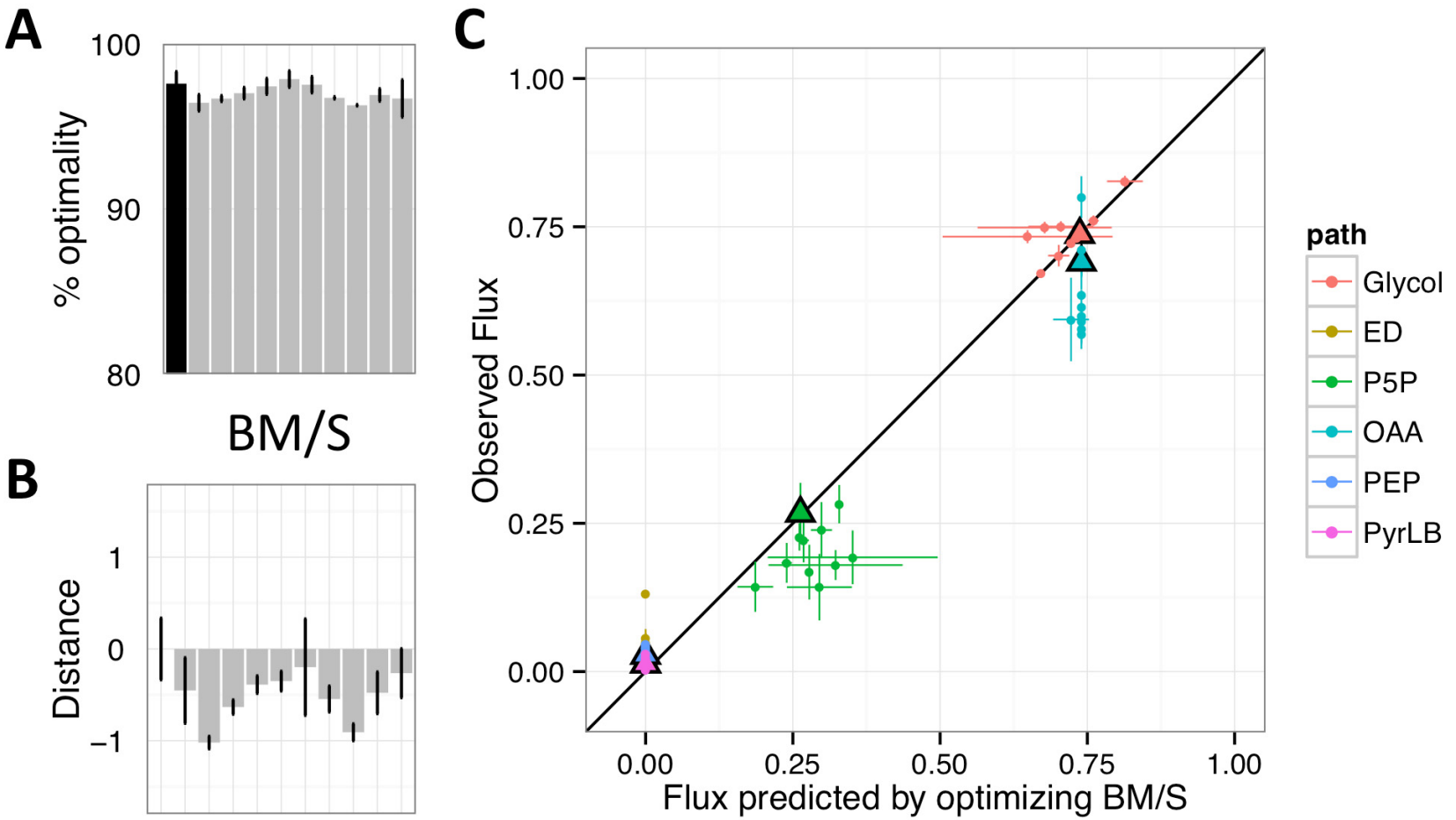
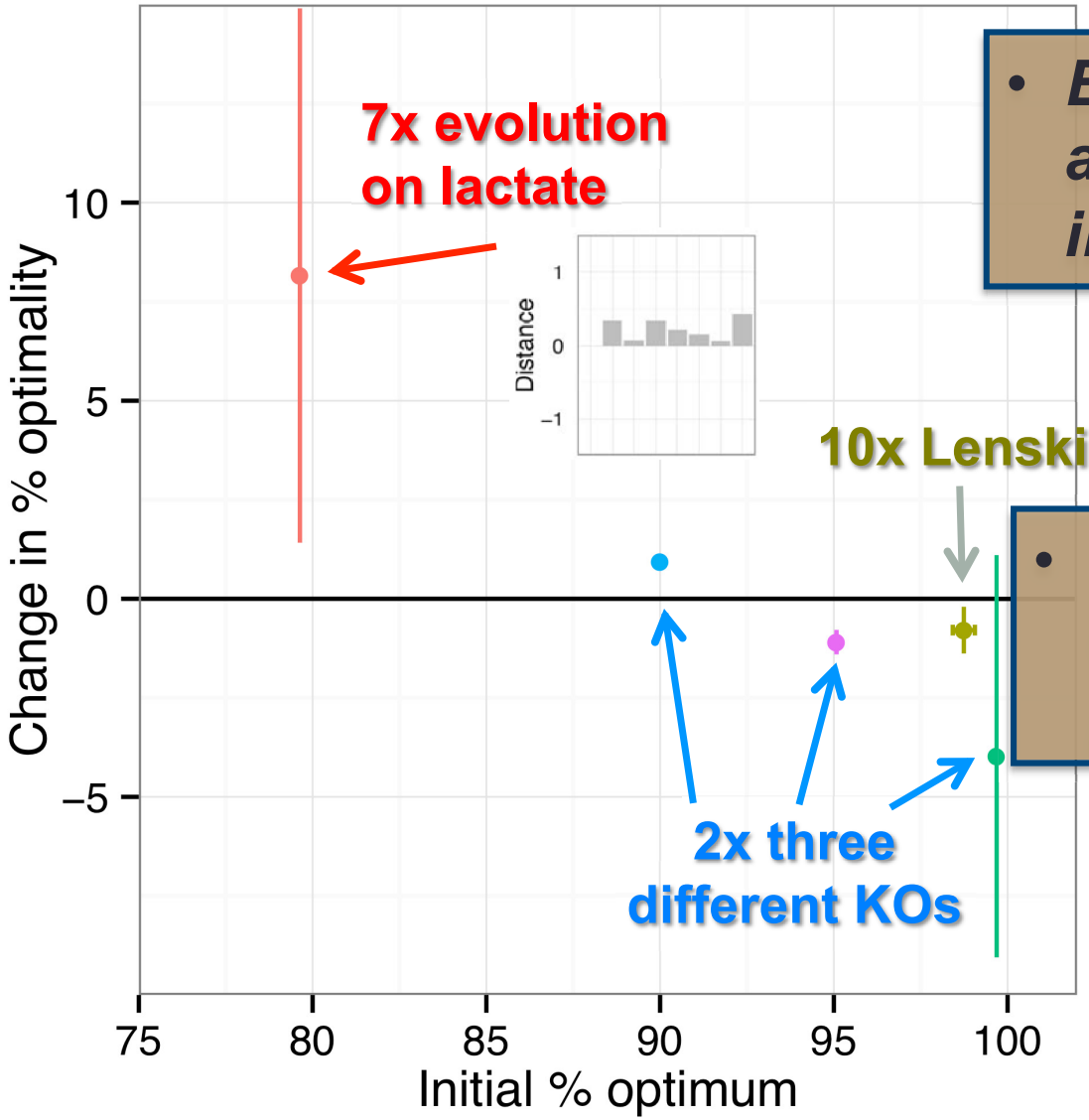
Do fluxes evolve to FBA-predicted optimum?



- ***Yield (BM/S) decreased slightly; fluxes consistently moved a bit away from optimum***

(Harcombe et al., 2013. *PLoS Computational Biology*)

Predictability depends on distance to optimum



- ***Either rate increased and slight drop, or both increased together***

- ***Take home: FBA optimizing for BM/S not perfect, but not bad...***

(Harcombe et al., 2013. *PLoS Computational Biology*)

Model system: C_1 in *Methylobacterium*

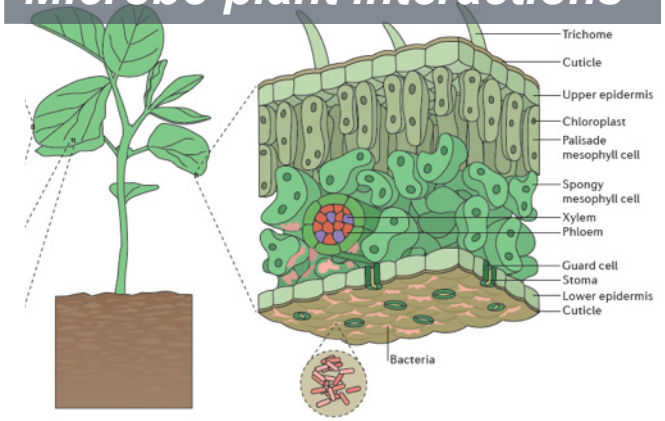
- *M. extorquens* is a plant epiphyte
- Model for C_1 metabolism (>50 yrs)

Leaf print on methanol plate.

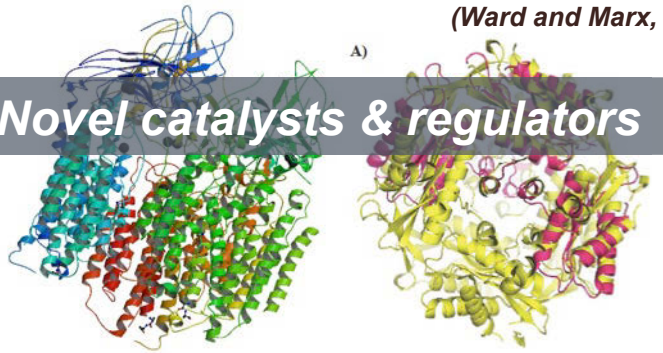
Plants release methanol during cell wall growth…

(Ward and Marx, unpublished)

Microbe-plant interactions



Novel catalysts & regulators



Soil ecology & global warming

Methanol-based biotechnology

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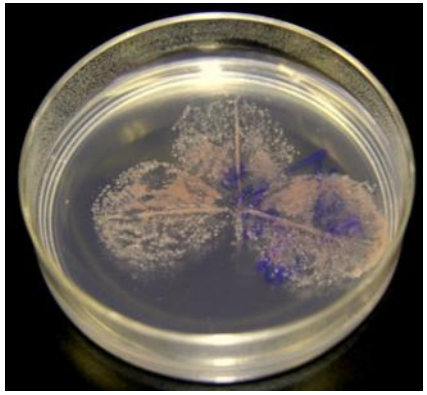

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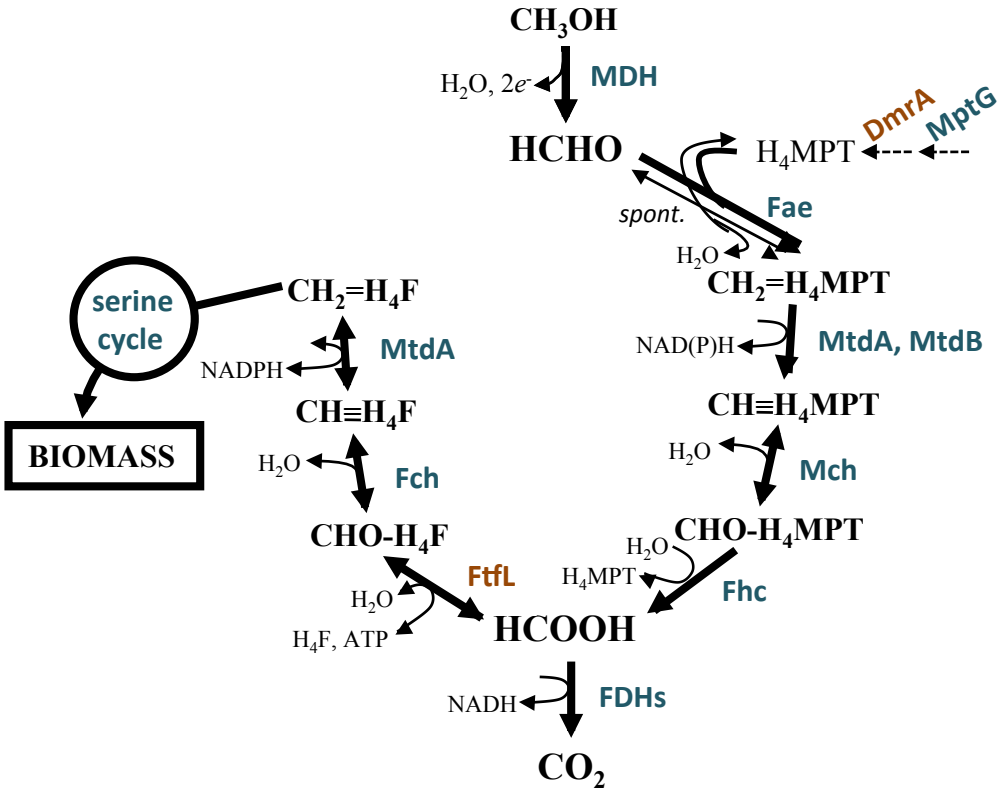
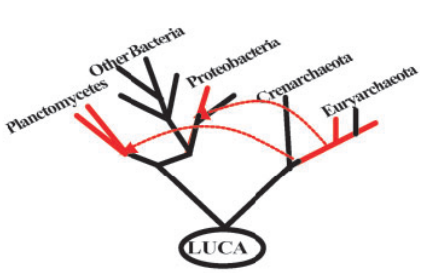
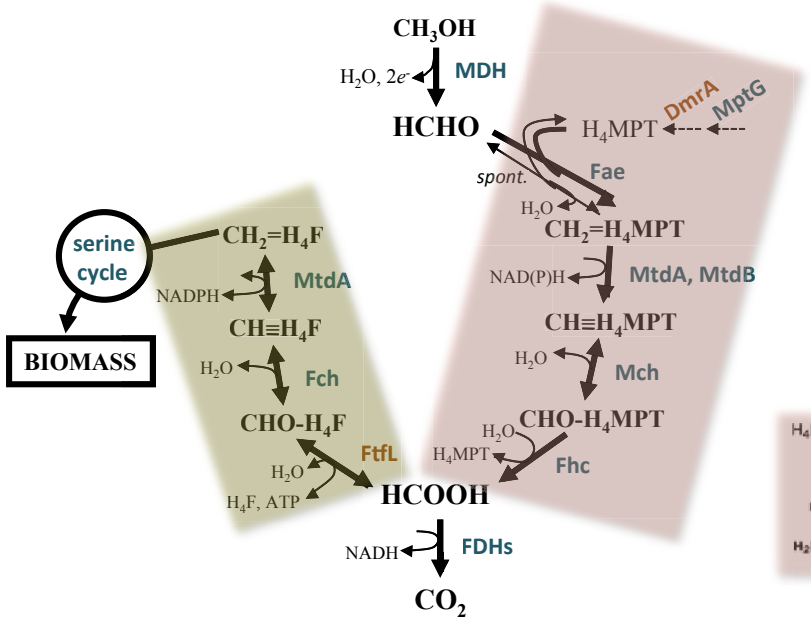
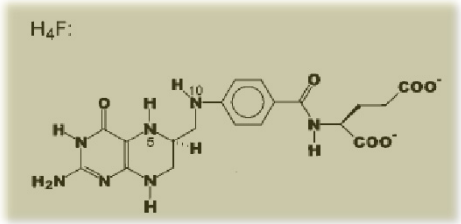


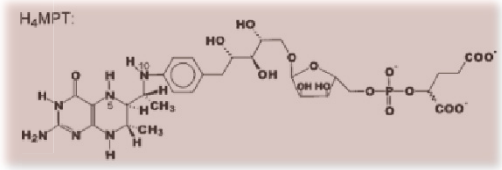
C_1 and HGT: new model for evolution

- C_1 genes transferred between bacteria; maybe from archaea
- **How are new functions incorporated?**
- Discovered one new gene (DmrA) that was *Methylobacterium*-specific innovation









DHFR

F → H_2F → H_4F

NADPH NADPH

DmrA

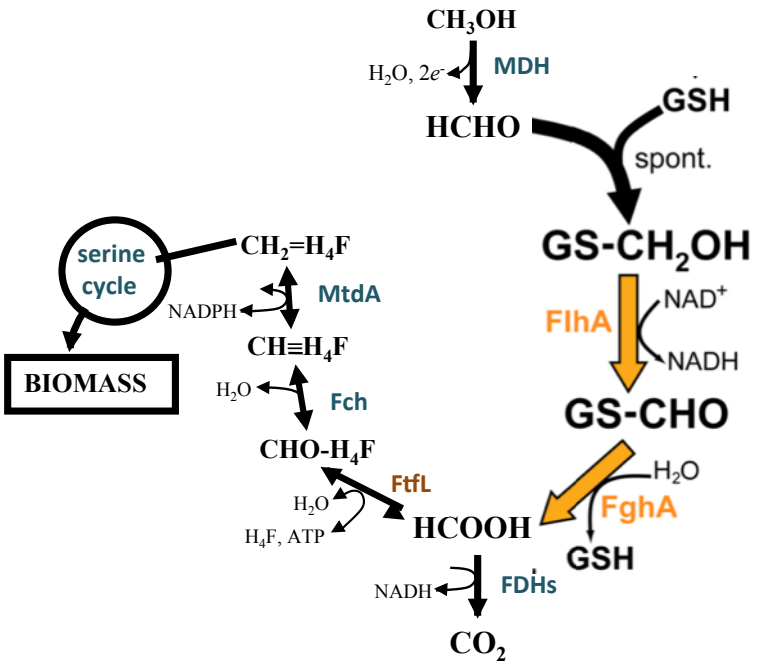
MPT → H_2MPT → H_4MPT

NADPH NADPH

(Marx et al., 2003a, 2003c. *J. Bacteriology*; Chistoserdova et al., 2004. *Mol. Biol. Evol.*; Kalyuzhnaya et al., 2005. *J. Bacteriology*)

Innovative combos not always initially fit...

C_1 and HGT: new model for evolution



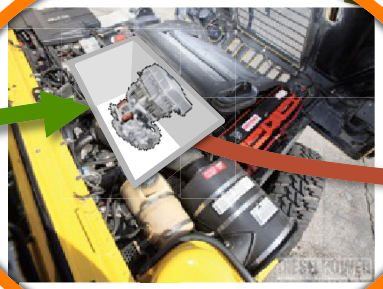
- Replaced existing formaldehyde oxidation pathway
  - Foreign GSH-dependent pathway from *Paracoccus denitrificans*
  - Single transcript, on plasmid, strong promoter
- ***Recovered growth, but 3x slower***

(Marx et al., 2003a, 2003b. *J. Bacteriology*; Chistoserdova et al., 2004. *Mol. Biol. Evol.*; Kalyuzhnaya et al., 2005. *J. Bacteriology*)

C_1 and HGT: new model for evolution

- An analogous scenario:

IN



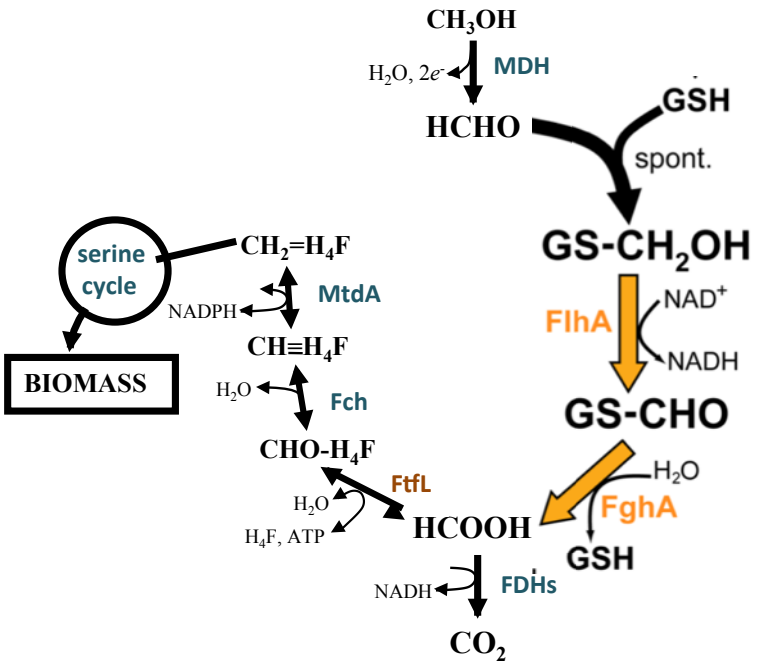
OUT

evolve…

+

???

C_1 and HGT: new model for evolution



- Replaced existing formaldehyde oxidation pathway
  - Foreign GSH-dependent pathway from *Paracoccus denitrificans*
  - Single transcript, on plasmid, strong promoter
- ***Recovered growth, but 3x slower***

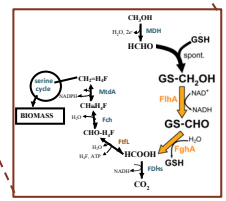
- ***Hypothesis, v.1:*** *Biggest benefit mutations directly involved with C_1 replacement*

- ***Hypothesis, v.2:*** *Metabolic model of C_1 catalysis can predict targets/interactions*

(Marx et al., 2003a, 2003b. *J. Bacteriology*; Chistoserdova et al., 2004. *Mol. Biol. Evol.*; Kalyuzhnaya et al., 2005. *J. Bacteriology*)

Evolution with engineered HGT

Engineered ancestor

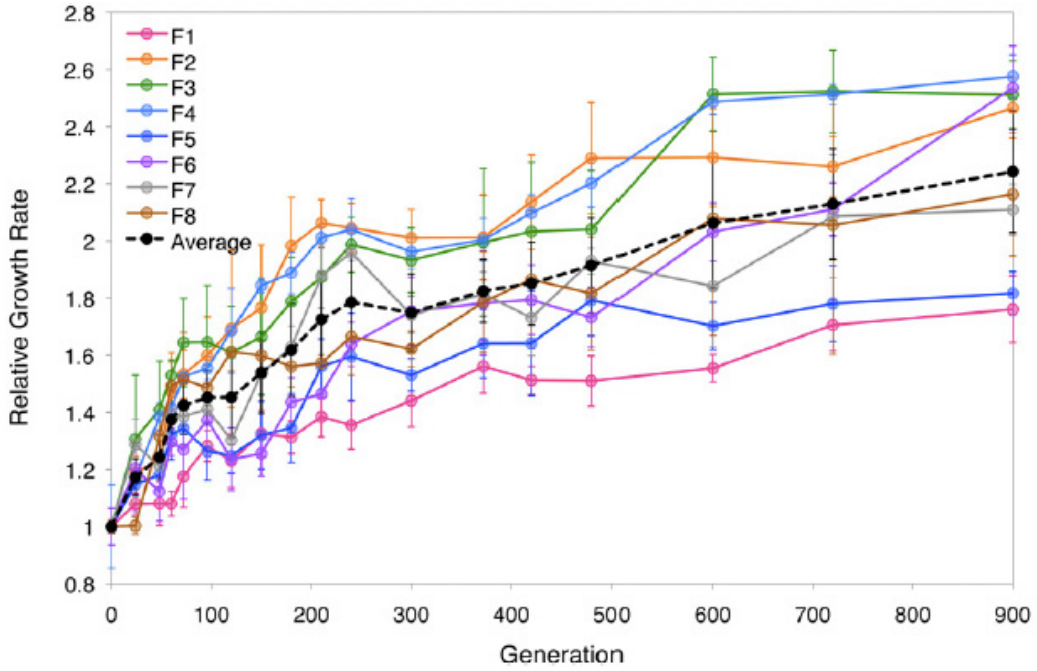


Evolved in methanol

900 gen.
$N_e \approx 10^8$

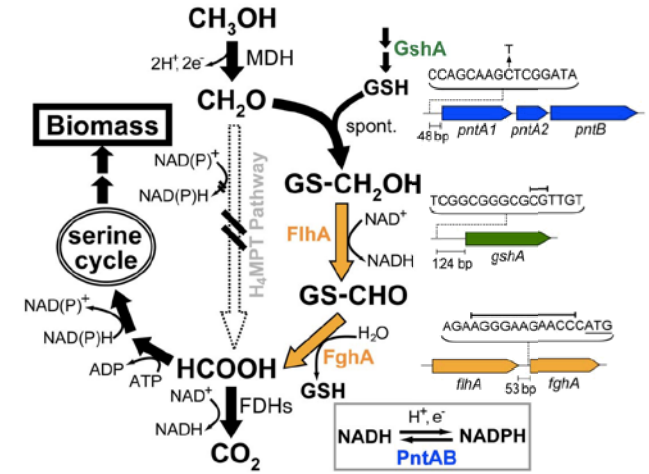
-80 °C

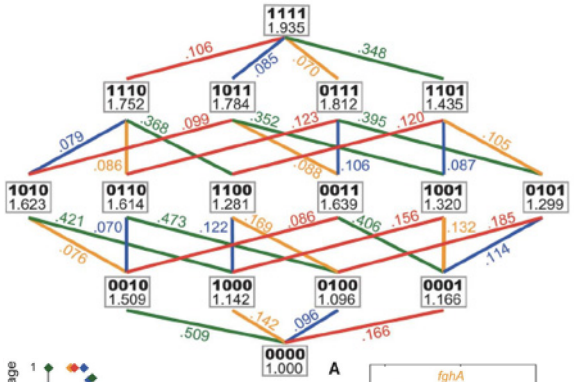
- Dramatic (>2X) but varied improvement
- Almost entirely specific to methanol
- *What changed?*

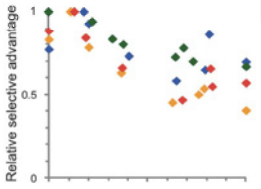


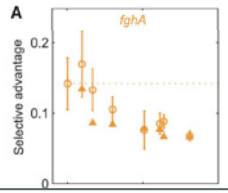
(Chou et al., 2011. *Science*; Lee and Marx, 2013. *Genetics*)

Beneficial mutations outside pathway



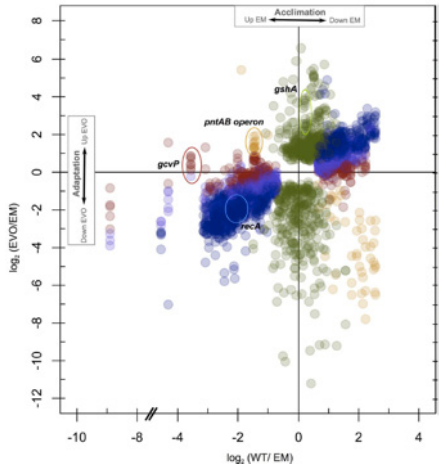


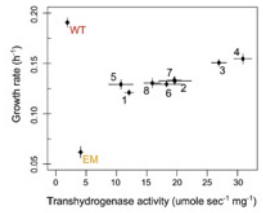


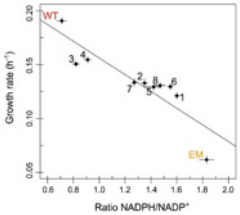


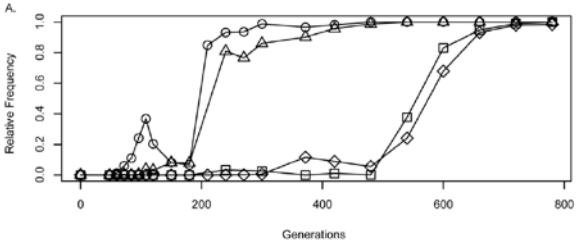
$$W_{\text{mutant}} = \prod_{i \in \text{Alleles}} \lambda_i b_0 - \prod_{i \in \text{Alleles}} \theta_i c_0$$

- Most mutations in or directly related to new C_1 pathway
- Uncovered general pattern of diminishing returns that decelerates adaptation
- Could predict epistasis at rough level
- Broad physiological disturbance and recovery (global mRNA; NAD(P)H levels)
- Uncovered order and dynamics of alleles









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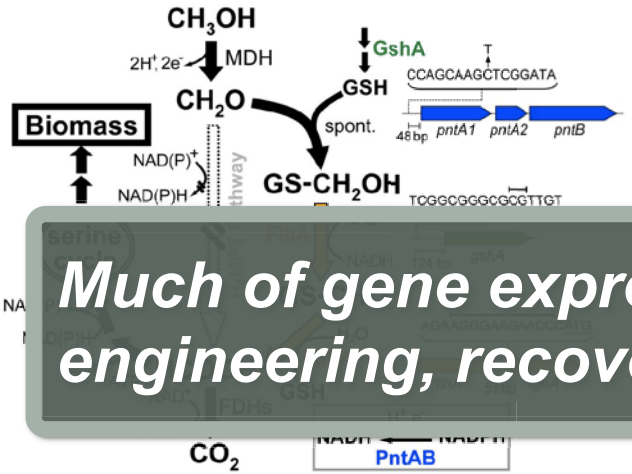
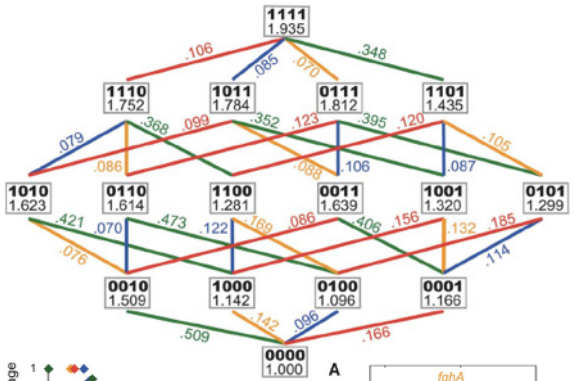
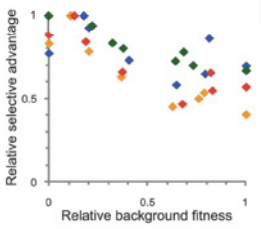
Beneficial mutations outside pathway

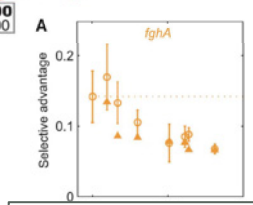


- Most mutations in or directly related to new C_1 pathway
- Uncovered general pattern of diminishing returns that decelerates adaptation
- Could predict mutations at gene level
- Broad physiological disturbance and recovery (global mRNA; NAD(P)H levels)
- Uncovered order and dynamics of alleles

Much of gene expression changed upon engineering, recovered in parallel with growth rate







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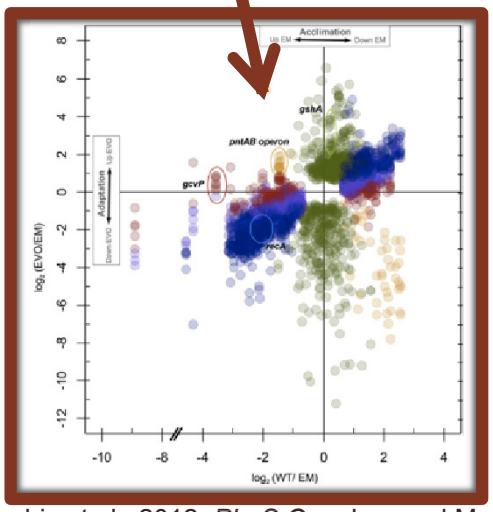
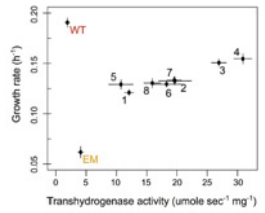
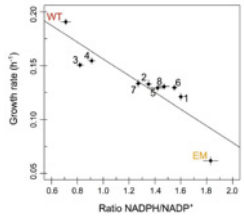
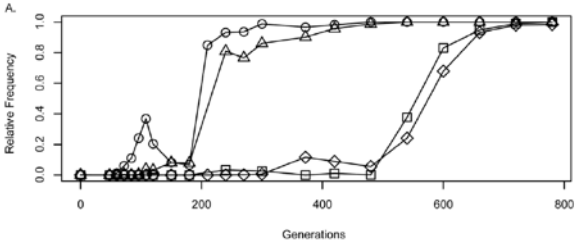




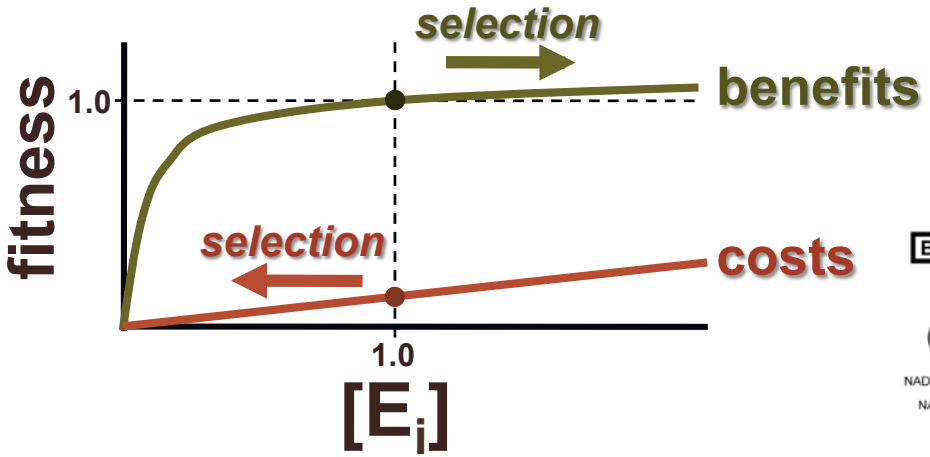


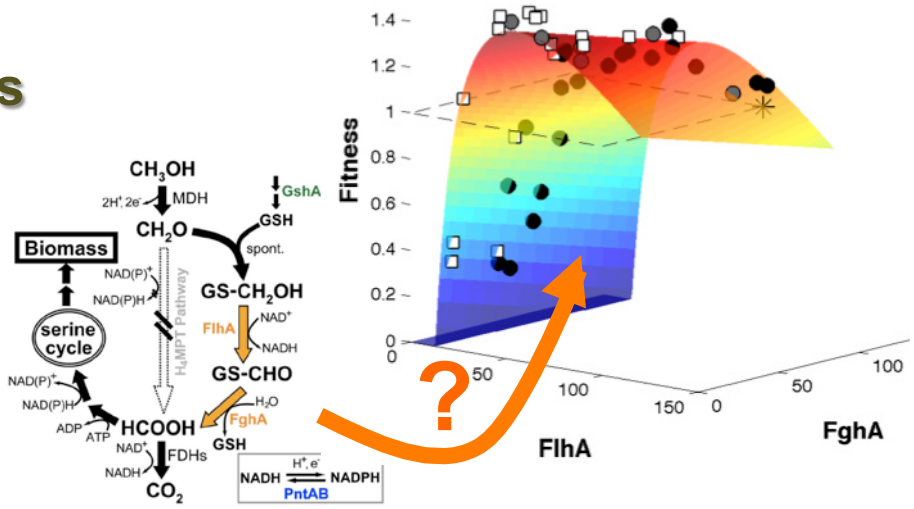


(Chou et al., 2011. *Science*; Chubiz et al., 2012. *PLoS One*; Lee and Marx, 2013. *Genetics*; Carroll and Marx, 2013. *PLoS Genetics*)

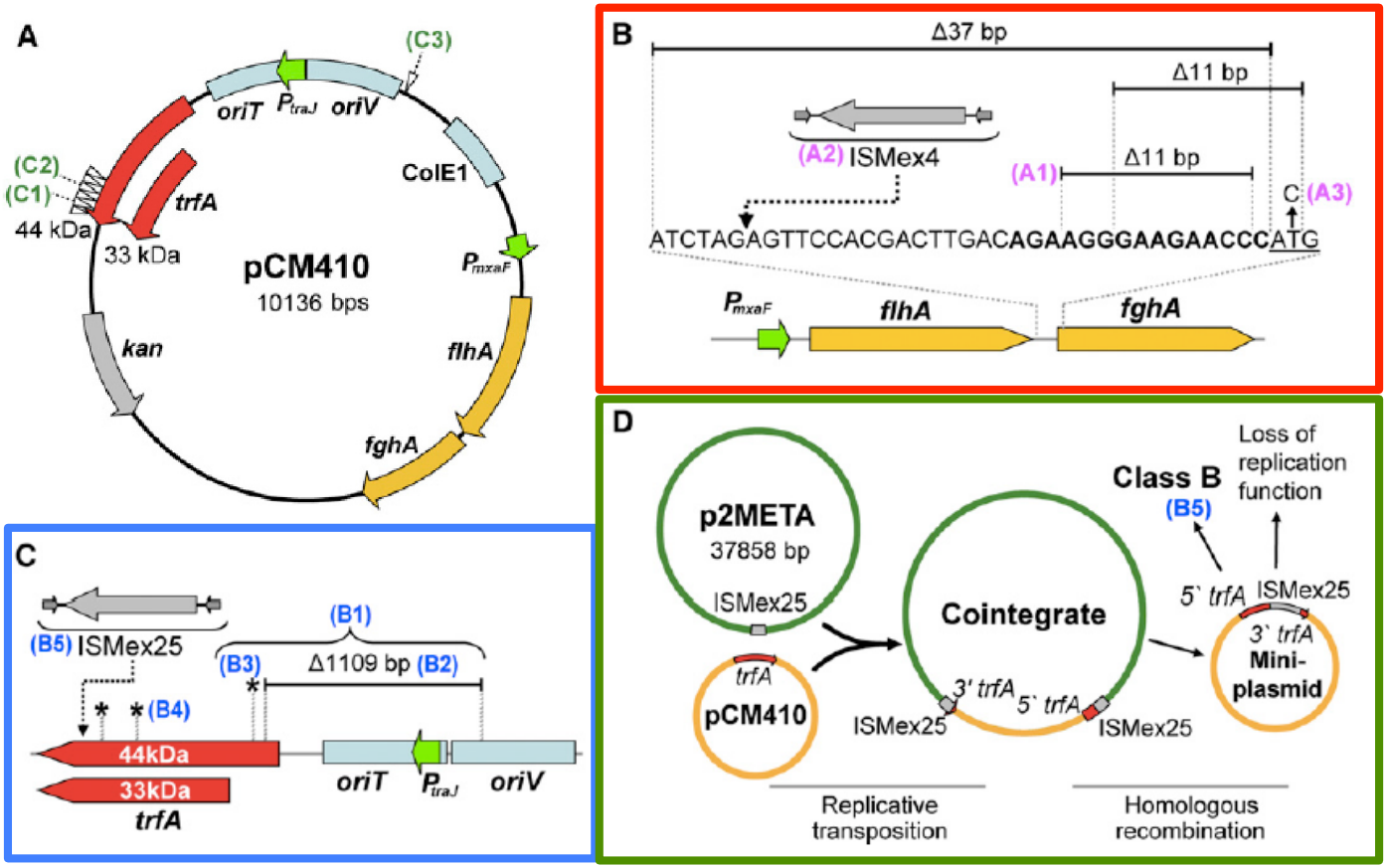
Optimize (benefits–costs) for pathway

- Need enzymes for catalysis, but they are costly to make.
- What is the optimal amount across a pathway?
- How do populations evolve toward this?
- How well do expression-altering mutations interact?
- *Predict this based upon mapping phenotypes to fitness?*





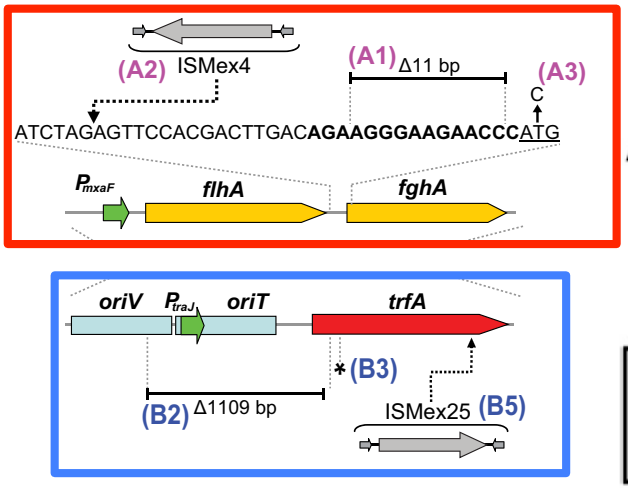
Beneficial mut. affecting pathway exp.

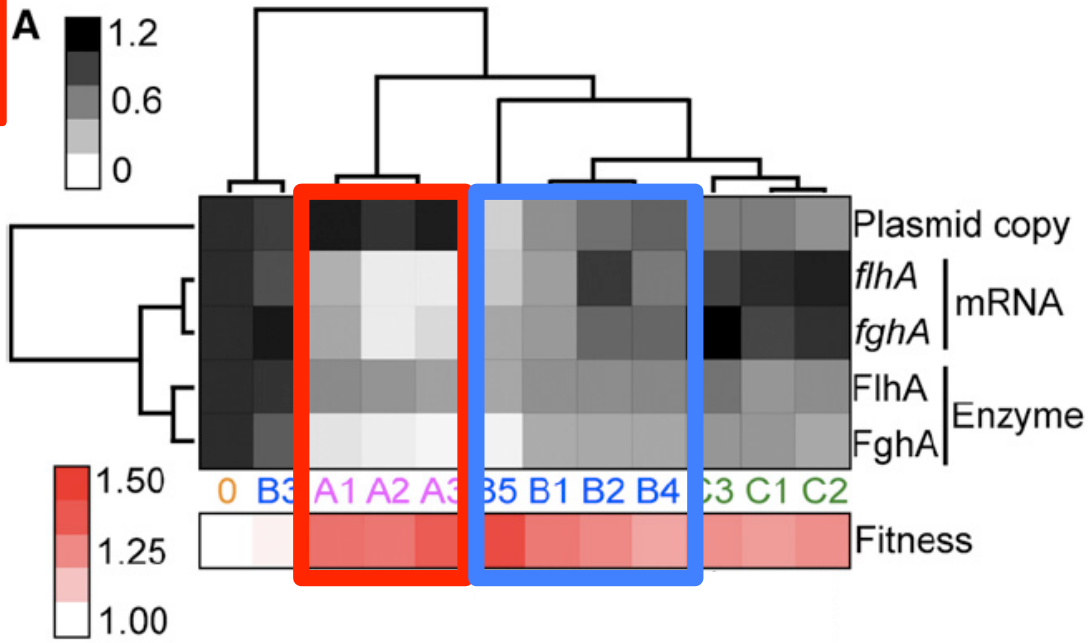


- Mutations of many types in different populations; 25-45% benefit; affect expression differently

(Chou and Marx, 2012. *Cell Reports*)

Expression per copy vs. copy#

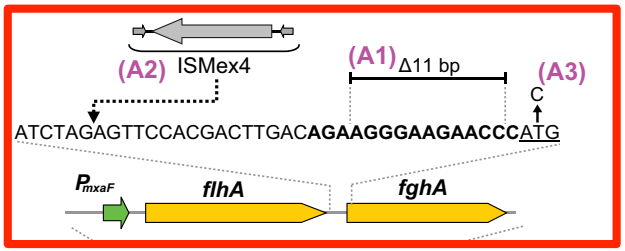


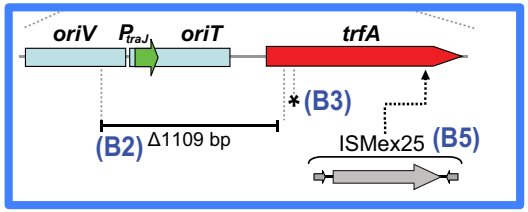


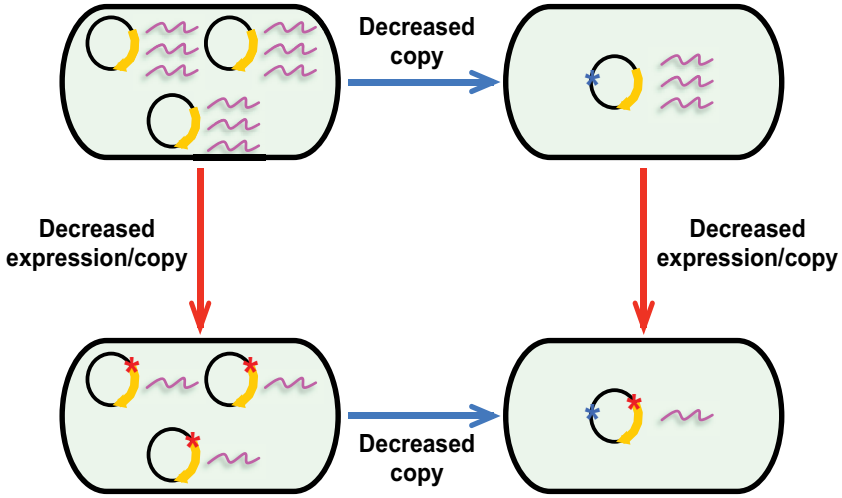
- Distinct, independent mechanisms to **reduce** expression of the GSH pathway enzymes
- But no promoter mutations?...

(Chou and Marx, 2012. *Cell Reports*)

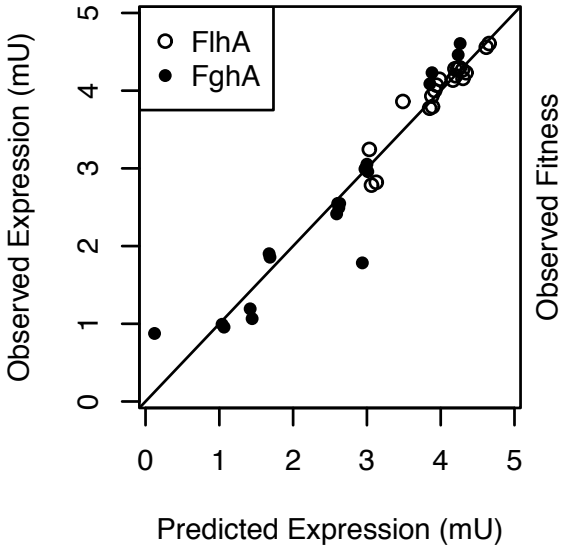
Independent effects upon expression

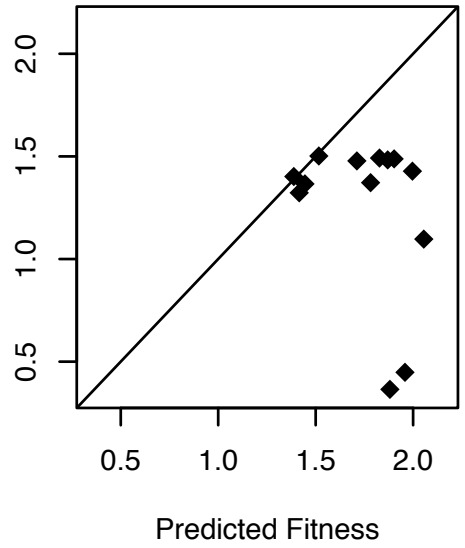






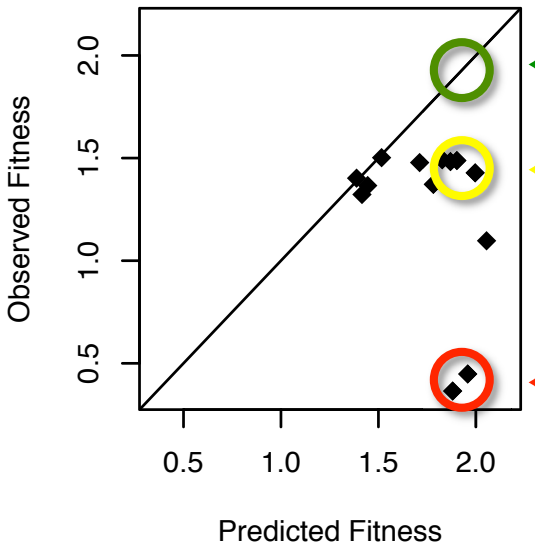
- Two classes should interact independently:
  - $E_{AB} = E_A \times E_B$
  - Yes
- Indep. upon fitness?
  - **No.**





(Chou et al., *PLoS Genetics*, 2014)

Why antagonism and sign epistasis?

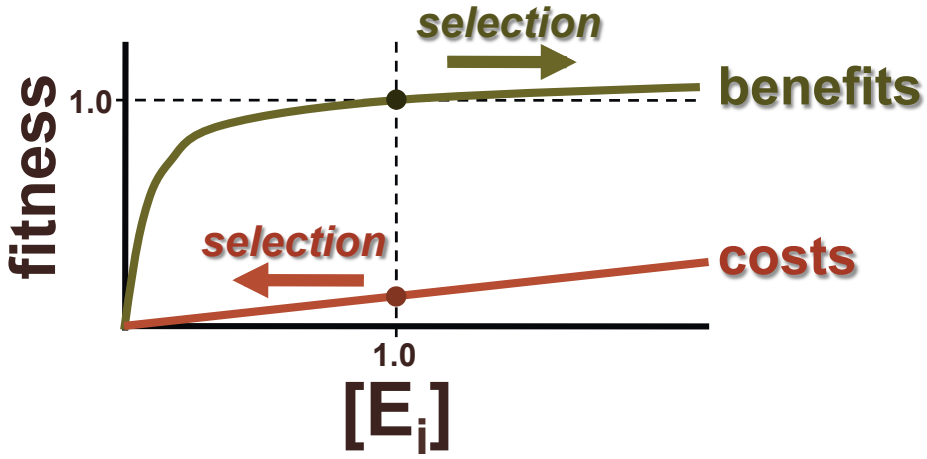


$W_{AB} = W_A \times W_B$; both provide benefit when together

$W_{AB} \approx \max(W_A, W_B)$; pair same as best individual

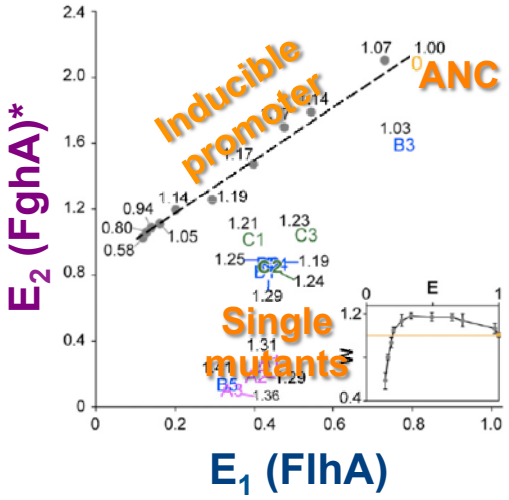
$W_{AB} < W_{ANC}$; pair much worse than individual mut.

- Caused by tension between benefits and costs?
  - Metabolic Control Analysis (MCA)



(Chou et al., *PLoS Genetics*, 2014)

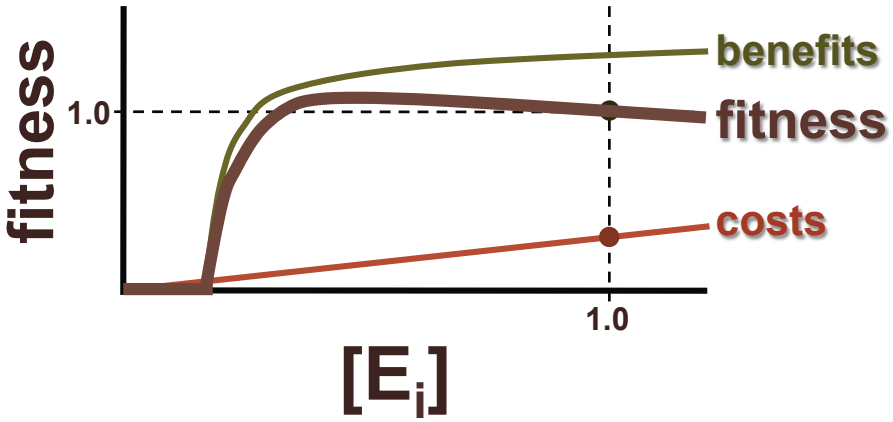
Map expression to fitness via model



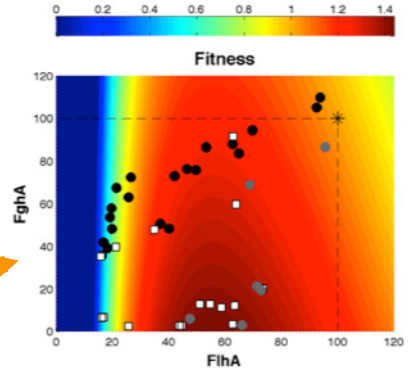
*FghA necessary for catalysis but not in benefit term because all data higher than threshold

$$W = \textit{Flux above threshold} - \textit{enzyme costs}$$

$$W = (v_{max} \times \mathbf{E_1}/(\mathbf{E_1} + E_{½\,max}) - v_T) - a \times \mathbf{E_1} - b \times \mathbf{E_2}$$

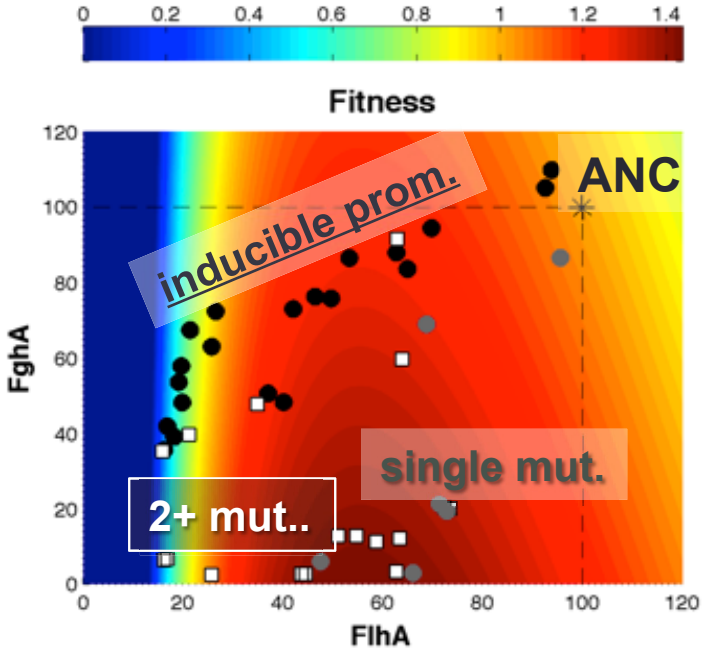


- ANC, single mutants, inducible promoter constructs (27 data points) to fit parameters
  - Try to predict 17 mutational combinations

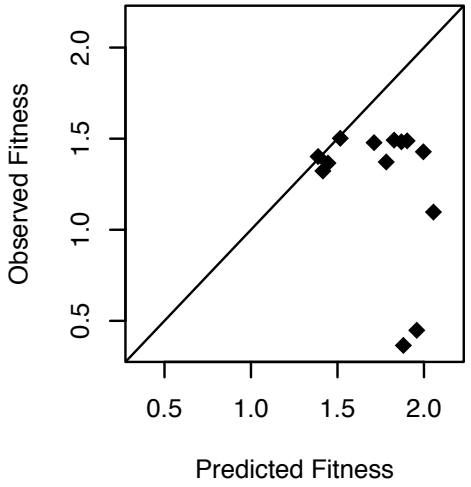


(Chou and Marx, 2012. *Cell Reports*; Chou et al., *PLoS Genetics*, 2014)

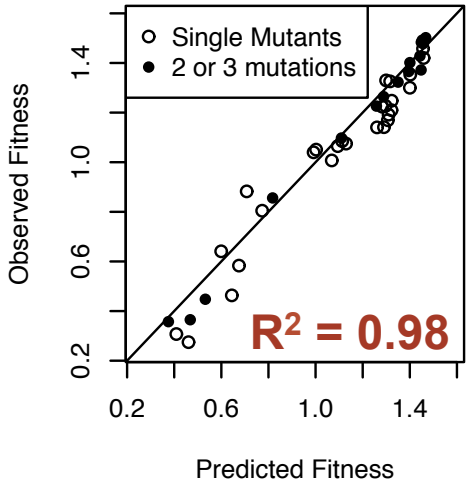
Model predicts mutational combos



$$W_{AB} = W_A \times W_B$$



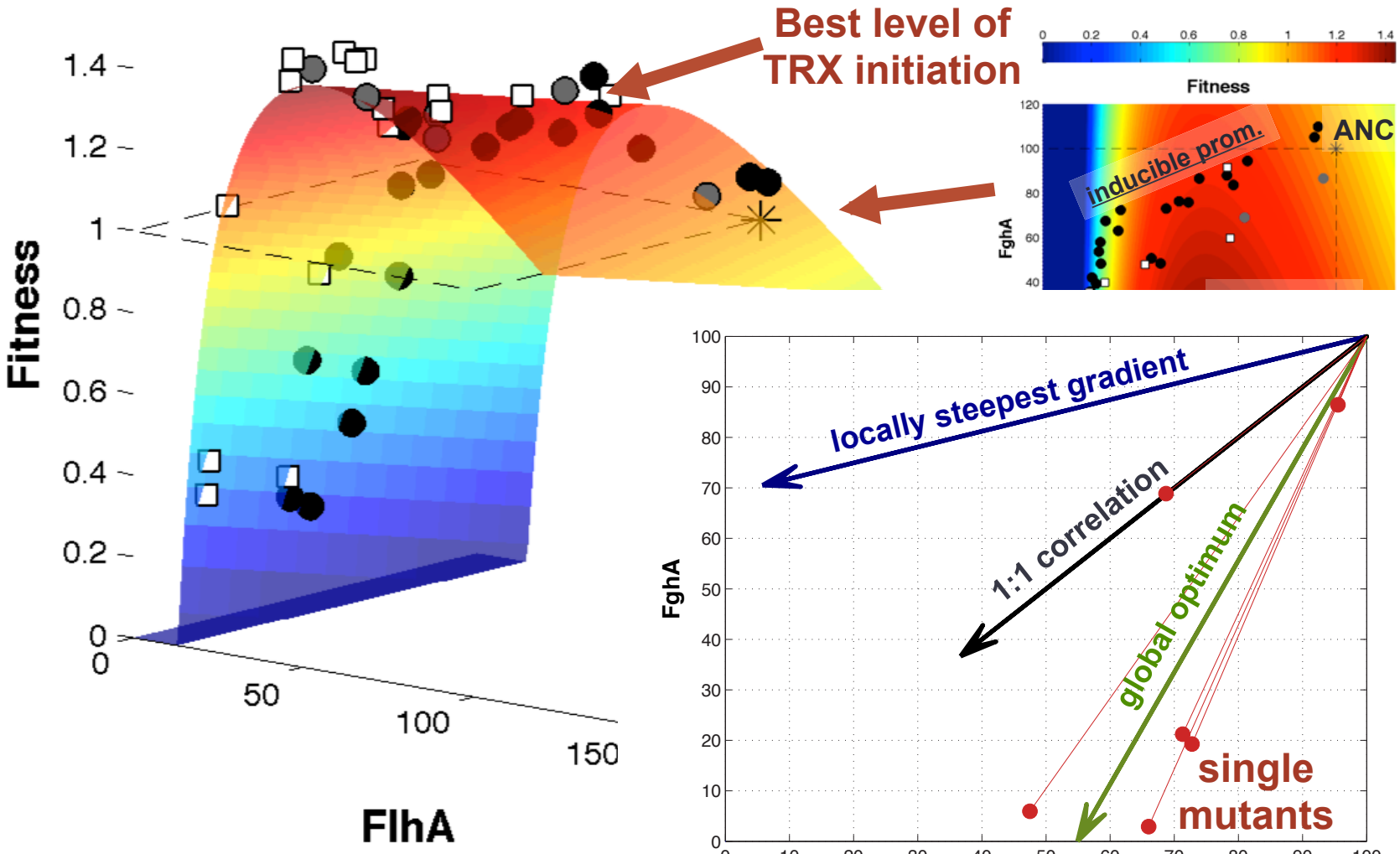
$$W = (v_{max} \times \mathbf{E_1}/(\mathbf{E_1} + E_{½\ max}) - v_T) - a \times \mathbf{E_1} - b \times \mathbf{E_2}$$



- Mechanistic model works quite well

(Chou et al., *PLoS Genetics*, 2014)

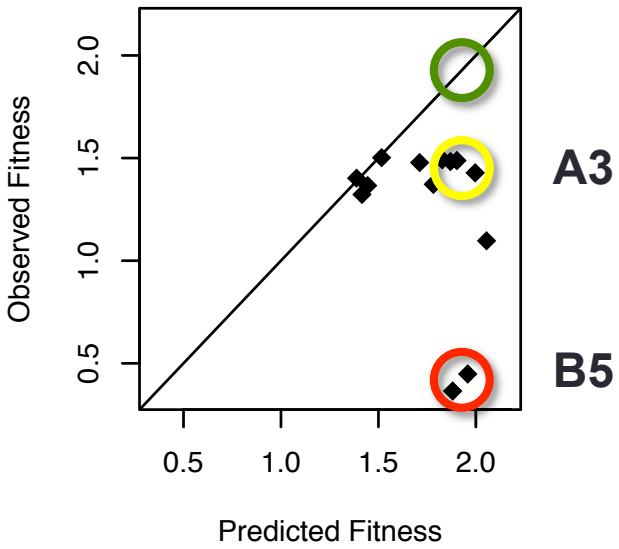
Interpret adaptation in light of model

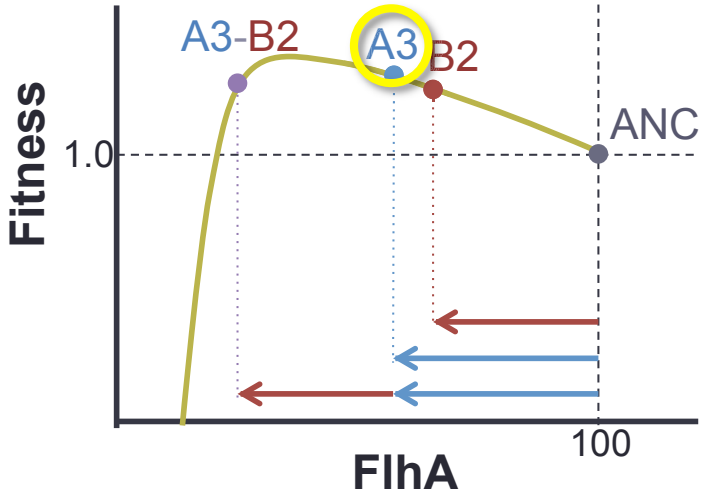


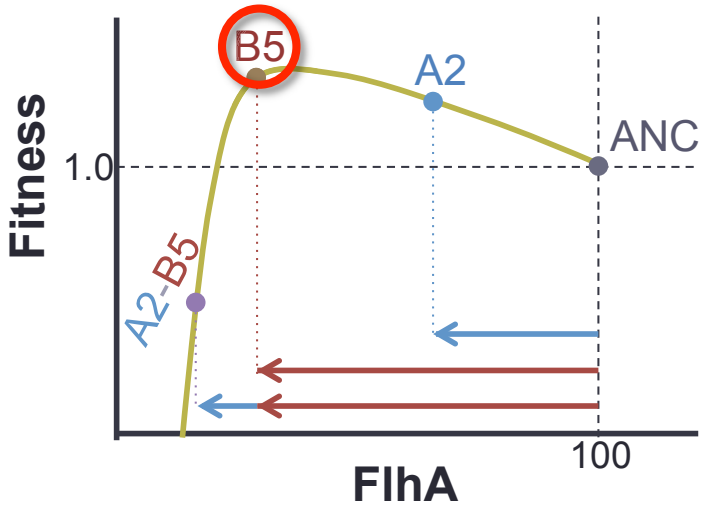
- *What direction was phenotypic change?*
- *How far to optimum was achieved?*

(Chou et al., *PLoS Genetics*, 2014)

Interpret interactions in light of model



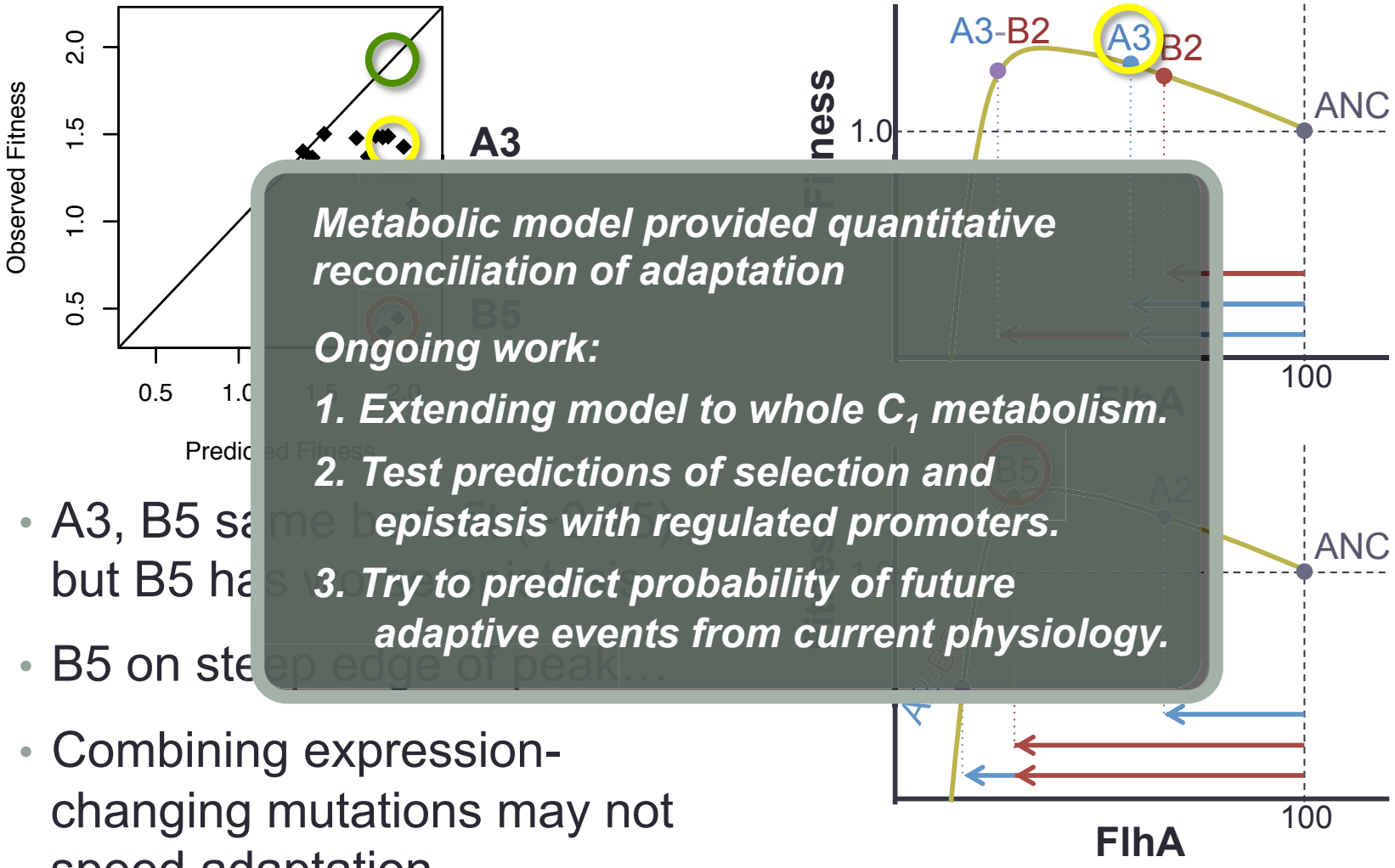




- A3, B5 same benefit (~0.45), but B5 has worse epistasis
- B5 on steep edge of peak…
- Combining expression-changing mutations may not speed adaptation

(Chou et al., *PLoS Genetics*, 2014)

Interpret interactions in light of model



Metabolic model provided quantitative reconciliation of adaptation

Ongoing work:

1. ***Extending model to whole C_1 metabolism.***
2. ***Test predictions of selection and epistasis with regulated promoters.***
3. ***Try to predict probability of future adaptive events from current physiology.***

- A3, B5 same benefit (~0.5), but B5 has worse epistasis
- B5 on steep edge of peak…
- Combining expression-changing mutations may not speed adaptation

(Chou et al., *PLoS Genetics*, 2014)

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- Daniel Segrè (Boston U.)
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Maryska Kaczmarek

View from our living room