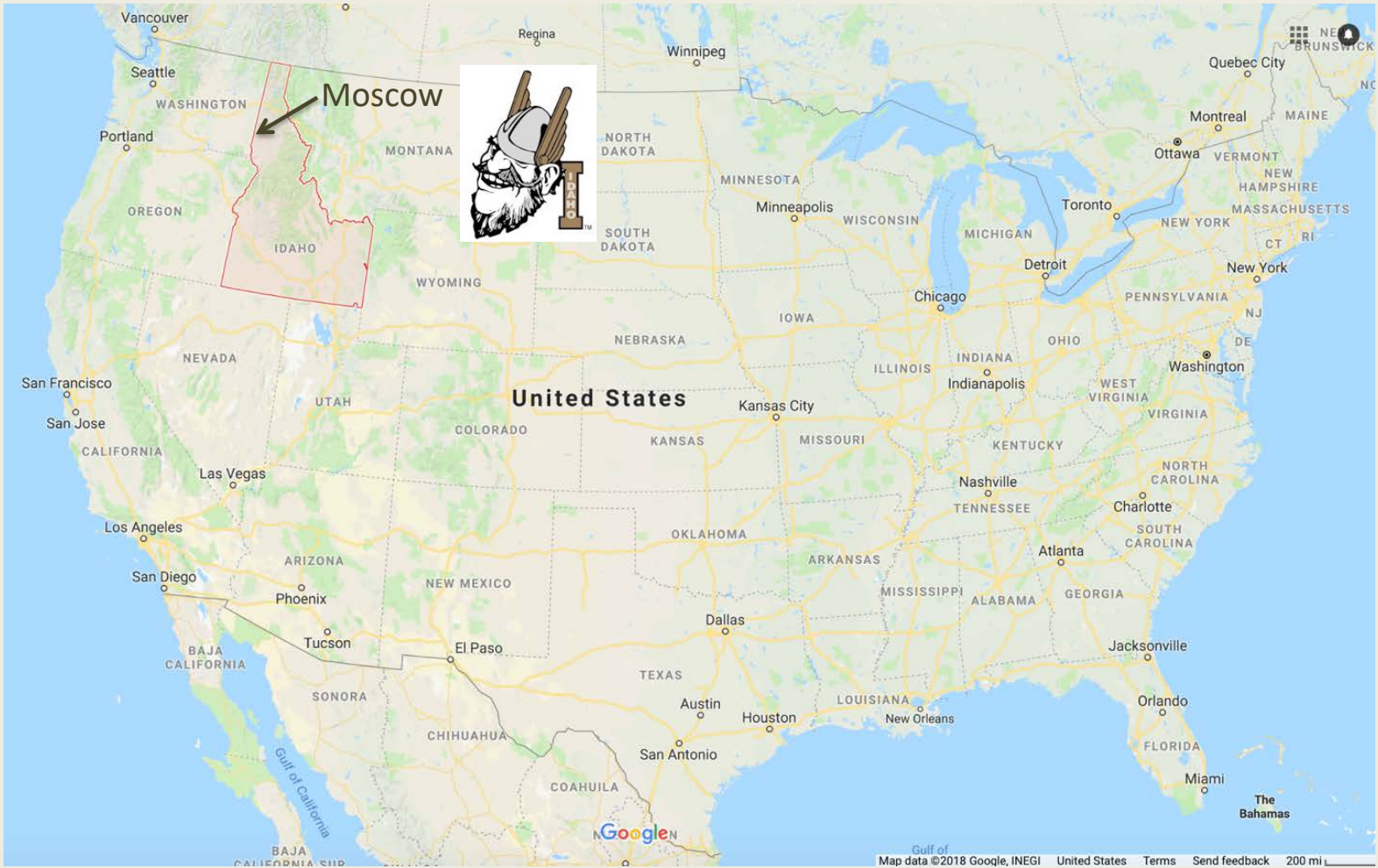
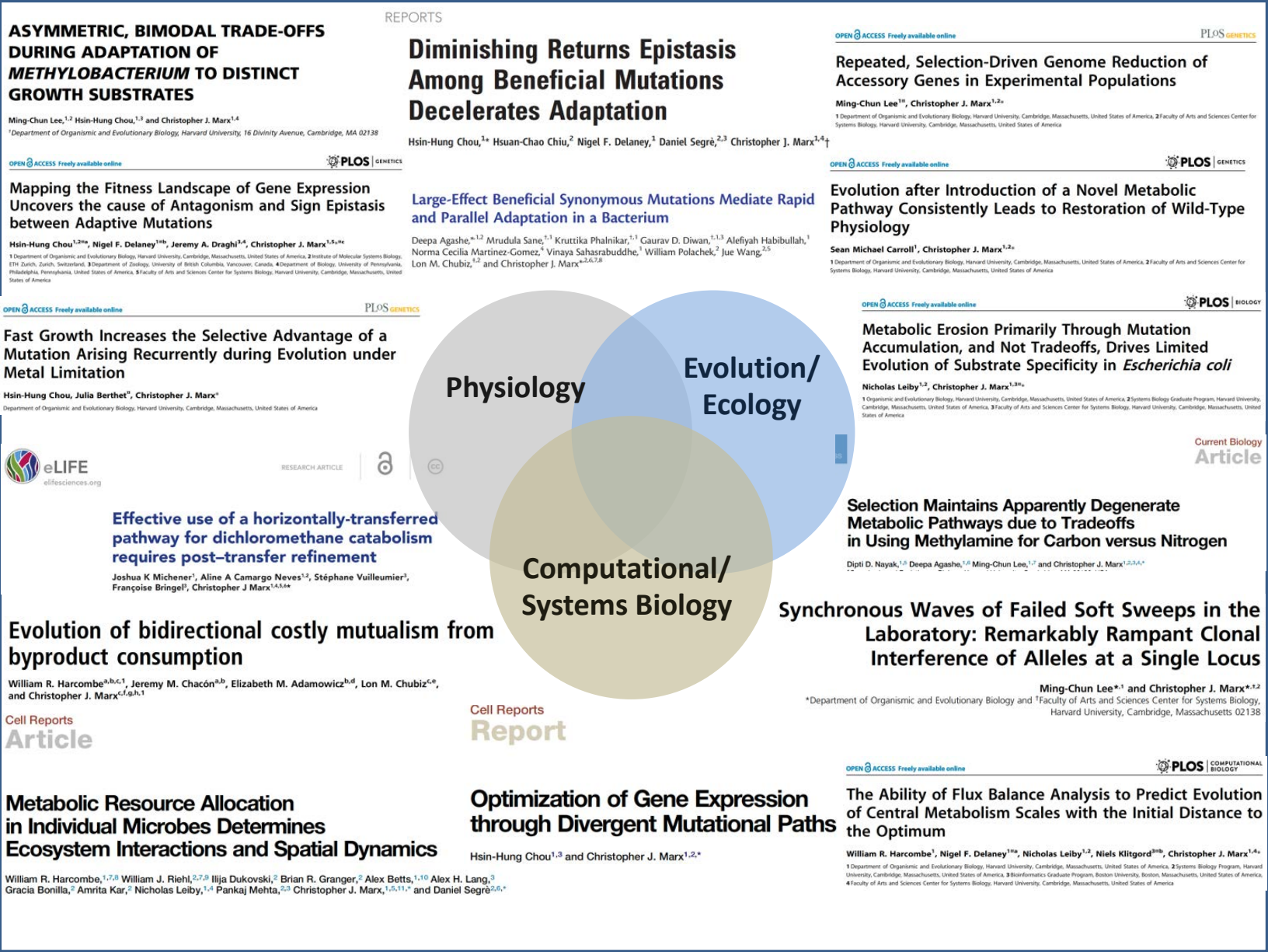
Where I'm visiting from...




REPORTS
ASYMMETRIC, BIMODAL TRADE-OFFS DURING ADAPTATION OF METHYLOBACTERIUM TO DISTINCT GROWTH SUBSTRATES
Ming-Chun Lee,[1,2] Hsin-Hung Chou,[1,3] and Christopher J. Marx[1,4]
[†]Department of Organismic and Evolutionary Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138
Diminishing Returns Epistasis Among Beneficial Mutations Decelerates Adaptation
Hsin-Hung Chou,[1,*] Hsuan-Chao Chiu,[2] Nigel F. Delaney,[1] Daniel Segrè,[2,3] Christopher J. Marx[1,4,†]
OPEN ACCESS Freely available online
PLOS GENETICS
Repeated, Selection-Driven Genome Reduction of Accessory Genes in Experimental Populations
Ming-Chun Lee[1¤], Christopher J. Marx[1,2*]
1 Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, United States of America, 2 Faculty of Arts and Sciences Center for Systems Biology, Harvard University, Cambridge, Massachusetts, United States of America
OPEN ACCESS Freely available online
PLOS GENETICS
Mapping the Fitness Landscape of Gene Expression Uncovers the cause of Antagonism and Sign Epistasis between Adaptive Mutations
Hsin-Hung Chou[1,2¤a], Nigel F. Delaney[1¤b], Jeremy A. Draghi[3,4], Christopher J. Marx[1,5,¤c]
1 Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, United States of America, 2 Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland, 3 Department of Zoology, University of British Columbia, Vancouver, Canada, 4 Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania, United States of America, 5 Faculty of Arts and Sciences Center for Systems Biology, Harvard University, Cambridge, Massachusetts, United States of America
Large-Effect Beneficial Synonymous Mutations Mediate Rapid and Parallel Adaptation in a Bacterium
Deepa Agashe,[*,1,2] Mrudula Sane,[†,1] Kruttika Phalnikar,[†,1] Gaurav D. Diwan,[†,1,3] Alefiyah Habibullah,[1] Norma Cecilia Martinez-Gomez,[4] Vinaya Sahasrabuddhe,[1] William Polachek,[2] Jue Wang,[2,5] Lon M. Chubiz,[1,2] and Christopher J. Marx[*,2,6,7,8]
OPEN ACCESS Freely available online
PLOS GENETICS
Evolution after Introduction of a Novel Metabolic Pathway Consistently Leads to Restoration of Wild-Type Physiology
Sean Michael Carroll[1], Christopher J. Marx[1,2*]
1 Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, United States of America, 2 Faculty of Arts and Sciences Center for Systems Biology, Harvard University, Cambridge, Massachusetts, United States of America
OPEN ACCESS Freely available online
PLOS GENETICS
Fast Growth Increases the Selective Advantage of a Mutation Arising Recurrently during Evolution under Metal Limitation
Hsin-Hung Chou, Julia Berthet[¤], Christopher J. Marx*
Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, United States of America
Physiology
Evolution/ Ecology
Computational/ Systems Biology
OPEN ACCESS Freely available online
PLOS BIOLOGY
Metabolic Erosion Primarily Through Mutation Accumulation, and Not Tradeoffs, Drives Limited Evolution of Substrate Specificity in Escherichia coli
Nicholas Leiby[1,2], Christopher J. Marx[1,3*]
1 Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, United States of America, 2 Systems Biology Graduate Program, Harvard University, Cambridge, Massachusetts, United States of America, 3 Faculty of Arts and Sciences Center for Systems Biology, Harvard University, Cambridge, Massachusetts, United States of America
eLIFE elifesciences.org
RESEARCH ARTICLE
Effective use of a horizontally-transferred pathway for dichloromethane catabolism requires post–transfer refinement
Joshua K Michener[1], Aline A Camargo Neves[1,2], Stéphane Vuilleumier[3], Françoise Bringel[3], Christopher J Marx[1,4,5,6*]
Current Biology
Article
Selection Maintains Apparently Degenerate Metabolic Pathways due to Tradeoffs in Using Methylamine for Carbon versus Nitrogen
Dipti D. Nayak,[1,2] Deepa Agashe,[1,3] Ming-Chun Lee,[1,7] and Christopher J. Marx[1,2,3,4,*]
Evolution of bidirectional costly mutualism from byproduct consumption
William R. Harcombe[a,b,c,1], Jeremy M. Chacón[a,b], Elizabeth M. Adamowicz[b,d], Lon M. Chubiz[c,e], and Christopher J. Marx[c,f,g,h,1]
Cell Reports
Article
Synchronous Waves of Failed Soft Sweeps in the Laboratory: Remarkably Rampant Clonal Interference of Alleles at a Single Locus
Ming-Chun Lee*[,1] and Christopher J. Marx*[,†,2]
*Department of Organismic and Evolutionary Biology and [†]Faculty of Arts and Sciences Center for Systems Biology, Harvard University, Cambridge, Massachusetts 02138
Cell Reports
Report
Metabolic Resource Allocation in Individual Microbes Determines Ecosystem Interactions and Spatial Dynamics
William R. Harcombe,[1,7,8] William J. Riehl,[2,7,9] Ilija Dukovski,[2] Brian R. Granger,[2] Alex Betts,[1,10] Alex H. Lang,[3] Gracia Bonilla,[2] Amrita Kar,[2] Nicholas Leiby,[1,4] Pankaj Mehta,[2,3] Christopher J. Marx,[1,5,11,*] and Daniel Segrè[2,6,*]
Optimization of Gene Expression through Divergent Mutational Paths
Hsin-Hung Chou[1,3] and Christopher J. Marx[1,2,*]
OPEN ACCESS Freely available online
PLOS COMPUTATIONAL BIOLOGY
The Ability of Flux Balance Analysis to Predict Evolution of Central Metabolism Scales with the Initial Distance to the Optimum
William R. Harcombe[1], Nigel F. Delaney[1¤a], Nicholas Leiby[1,2], Niels Klitgord[3¤b], Christopher J. Marx[1,4*]
1 Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, United States of America, 2 Systems Biology Program, Harvard University, Cambridge, Massachusetts, United States of America, 3 Bioinformatics Graduate Program, Boston University, Boston, Massachusetts, United States of America, 4 Faculty of Arts and Sciences Center for Systems Biology, Harvard University, Cambridge, Massachusetts, United States of America

Experimental Evolution of *Methylobacterium*: 15 Years of Planned Experiments and Surprise Findings

15

Christopher J. Marx*

Department of Biological Sciences, Institute for Bioinformatics and Evolutionary Studies, Center for Modeling Complex Interactions, University of Idaho, Moscow, ID, USA.

*Correspondence: cmarx@uidaho.edu

https://doi.org/10.21775/9781912530045.15

Abstract

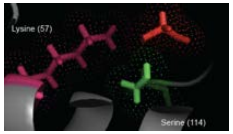
Experimental evolution has become an increasingly common approach for studying evolutionary phenomena, as well as uncovering physiological connections in a manner complementary to traditional genetics. Here I describe the development of *Methylobacterium* as a model system for using experimental evolution to study questions at the intersection of metabolism and evolution. Each experiment was initiated to address a particular question inspired by patterns in natural methylotrophs, such as trade-offs between single-carbon and multi-carbon growth, or the challenges involved in incorporating novel metabolic pathways or genes with poor codon usage that are acquired via horizontal gene transfer. What I could not have appreciated initially, however, was just how many fortuitous, surprise findings would emerge. These have ranged from the repeatability of evolution, complex dynamics within populations, epistasis between beneficial mutations, and even the ability to use simple mathematical models to generate testable, quantitative hypotheses about the fitness landscape.

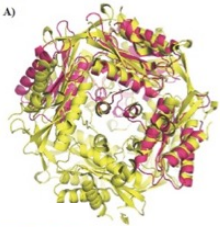
Introduction

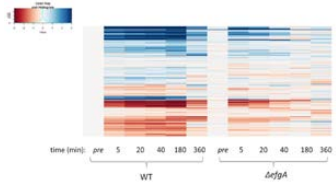
Experimental evolution of populations in the laboratory allows a researcher to simultaneously address evolutionary and physiological questions. The great advantage from an evolutionary perspective is that – in a typical experimental design – replicate populations initially have no within- or between-population genetic variation, and the selective conditions are under the control of the experimenter. This allows for a 'reductionist' approach to evolutionary questions, whereby the influence of one or a few individual factors upon the outcome can be ascertained (reviewed in Lenski, 2017). From a physiological perspective, experimental evolution is simply a patient version of a genetic selection experiment (reviewed in Marx, 2011). Rather than requiring a discrete change in phenotype to be immediately apparent upon plating, the continued transfers of the experiment permit mutations of 'modest' effect – such as a 10% increase in growth rate – to occur, escape drift, and rise towards fixation. Furthermore, the advent of high-throughput sequencing has revolutionized the ability to address both the evolutionary and physiological questions (reviewed in Bruger and Marx, 2018).

The kind invitation I received to write this chapter was a request to specifically describe the work in my laboratory where we have repeatedly used experimental evolution with *Methylobacterium extorquens* to address both evolutionary and physiological questions. Given that charge, I will shamelessly describe themes arising from our own work, but my primary goal is to highlight two broader messages. The first message is that experimental evolution can

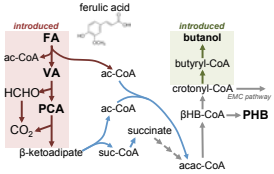
Novel regulator linking toxicity to translation in formaldehyde stress response

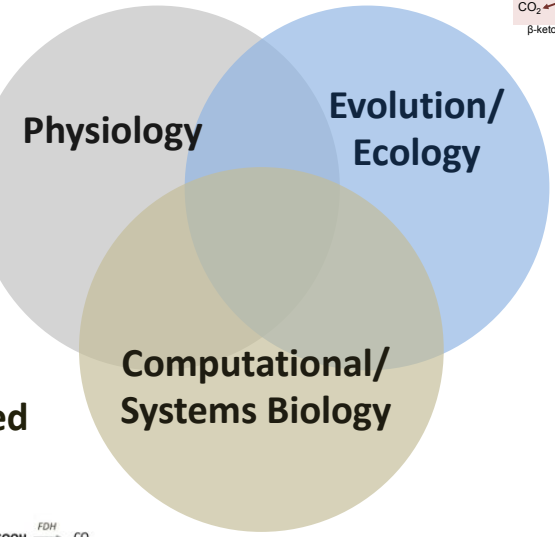




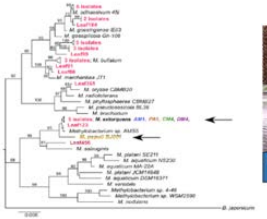


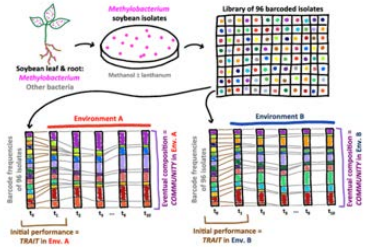
Phenotypic heterogeneity in metabolism converting plant-derived aromatics to bioproducts



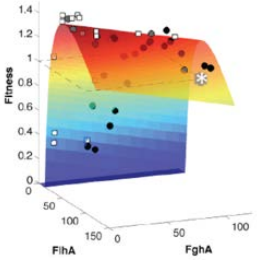


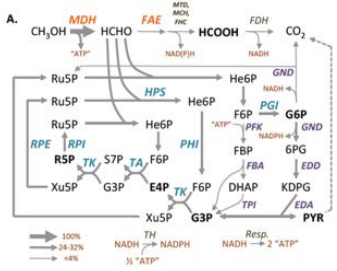
Within-genus diversity on plants: connecting traits, genomes, phylogeny





Fitness landscapes and evolvability of engineered metabolism





Epistasis

DEF. DIFFERENT EFFECT OF MUTATION UPON A PHENOTYPE DEPENDING UPON OTHER MUTATIONS PRESENT

↳ CAN BE ANY PHENOTYPE, HERE MAINLY FITNESS

IMAGINE TWO MUT., X & Y, EACH INDIVIDUALLY IMPROVE GROWTH BY 50%:

$W_{ANC} = 1.0$

$W_X = 1.5$

$W_Y = 1.5$

$W_{XY} =$ [ ] ?

W = FITNESS

1.0 ← W_{ANC} $s_X - s_Y$

1.5 ← LARGEST SINGLE

2.0 ← $1 + s_X + s_Y$

2.25 ← $(1+s_X)\cdot(1+s_Y)$

3.0 ← $W_A + W_B$ $1+s_X+1+s_Y$

How calculate epistasis?

- WHICH NULL MODEL FOR "INDEPENDENCE" TO USE?
- FOR SMALL s $(1+s_x)(1+s_y) \approx 1+s_x+s_y$

- EPISTASIS IS DEVIATION FROM INDEPENDENCE (ε)

TWO THOUGHT EXAMPLES → MULTIPLICATIVE

1. SUCCESSFUL SEEDLINGS
   - # SEEDS
   - PROP. THAT GERMINATE

2. ENZYME ACTIVITY
   - EXPRESSION LEVEL [E]
   - ACTIVITY PER MOLECULE (K_{cat})

TOTAL GERMINATED:
(# SEEDS) x (PROP. THAT GERMINATE)

$1.5 \cdot 1.5 = 2.25$

TOTAL ACT = V_{max} = $[E] \cdot K_{cat}$

$1.5 \cdot 1.5 = 2.25$

Depict epistasis graphically

$W_{ANC} = 1.0$

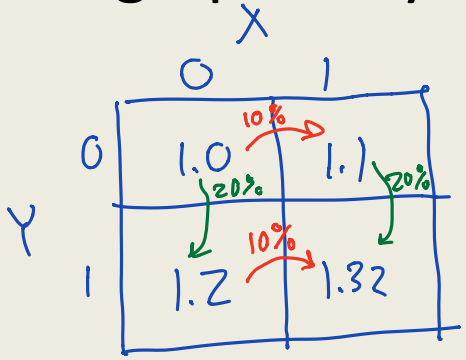
$W_X = 1.1$

$W_Y = 1.2$

$W_{XY} = \boxed{1.32}$ (NULL)

X	Y
0	0
1	0
0	1
1	1

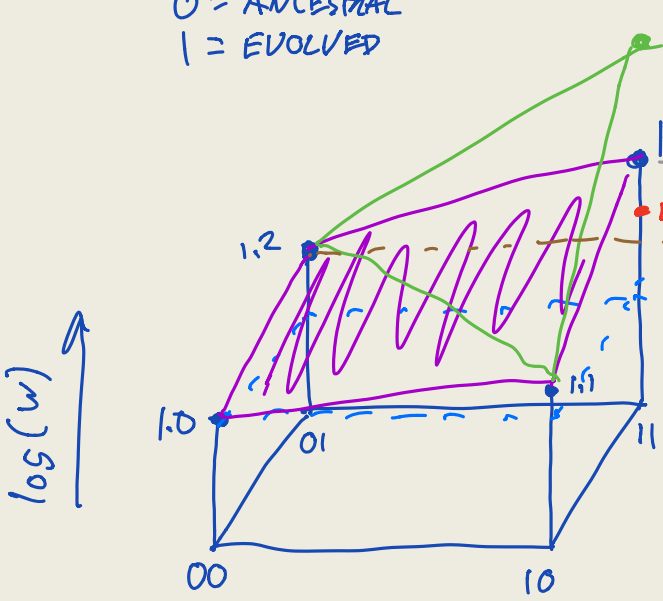
0 = ANCESTRAL
1 = EVOLVED



$\varepsilon = W_{DATA} - W_{NULL}$

$\varepsilon = W_{XY} - W_X \cdot W_Y$

"LOLLIPOP" DIAGRAM



POSITIVE EPISTASIS

← NULL

NEGATIVE EPISTASIS

- HIGHEST SINGLE

↳ IF DOUBLE IS ABOVE HIGHEST SINGLE

↳ BOTH ALLELES BENEFICIAL ON ALL TESTED BACKGROUNDS

"MAGNITUDE" EPISTASIS

Sign epistasis

DEF: ONE OR MORE ALLELES CHANGE FROM BEN. TO DELETERIOUS

Y \ X	0	1
0	1.0	1.1
1	1.2	0.8

SIGN EPISTASIS FOR BOTH

Y \ X	0	1
0	1.0	0.5
1	0.3	1.0

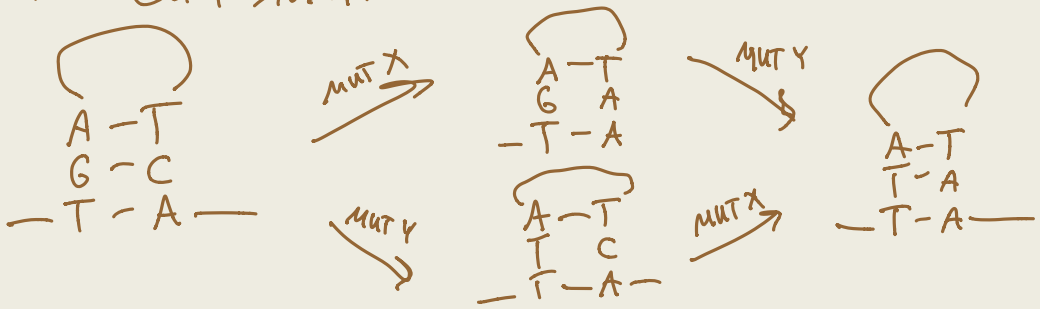
Y \ X	0	1
0	1.0	1.2
1	1.5	1.3

ONLY SIGN EPISTASIS FOR X

→ ALL COMBOS EXCEPT (11) ARE VIABLE, BUT (11) IS DEAD
↳ SYNTHETIC LETHAL

CROSSING A "FITNESS VALLEY"
SLOW CHANGES IN 16S rRNA

COMMON FOR "COMPENSATORY" MUTATIONS



Epistasis w/in proteins

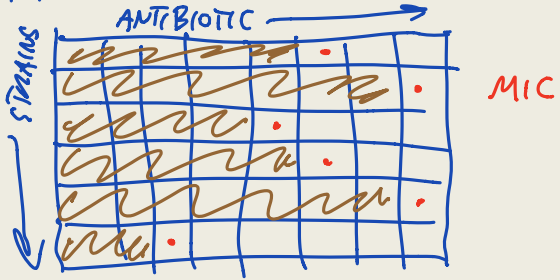
- EVOLVED ALLELES w/ SEVERAL MUTATIONS
  - HOW DO MUT. AFFECT EACH OTHER?
  - STRONG ENOUGH TO LIMIT PATHS OF ADAPTATION?

- EXAMPLE: TEM-1 BETALACTAMASE IN *E. coli*
  ↳ ENZYME THAT PROVIDES RESISTANCE TO PENICILLINS

WT —(5 MUT.)→ TEM* (HIGH RESISTANCE TO CEFOTAXIME)

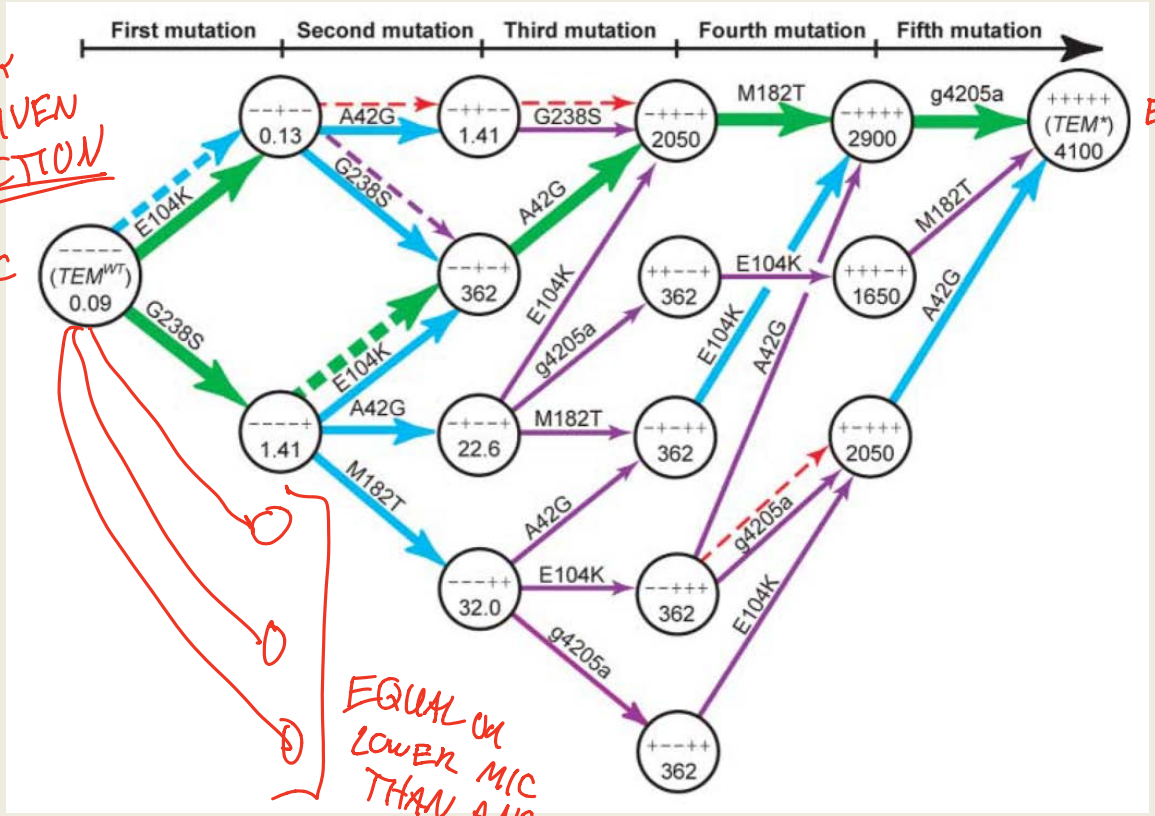
40,000-FOLD INCREASE

1. CONSTRUCTED ALL $2^5 = 32$ ALLELES (4 NON-SYNON., 1 PROMOTER)
2. ASSAY RESISTANCE: "MINIMUM INHIBITORY CONCENTRATION" (MIC)
   ↳ IN PARTICULAR COND. 37°C, LB, 96-WELL PLATE
   24 h LATER
   BELOW SOME THRESHOLD OF GROWTH



(Weinreich et al., 2006. *Science*)

Epistasis w/in proteins



SHOWS ONLY STEPS DRIVEN BY SELECTION

ANC

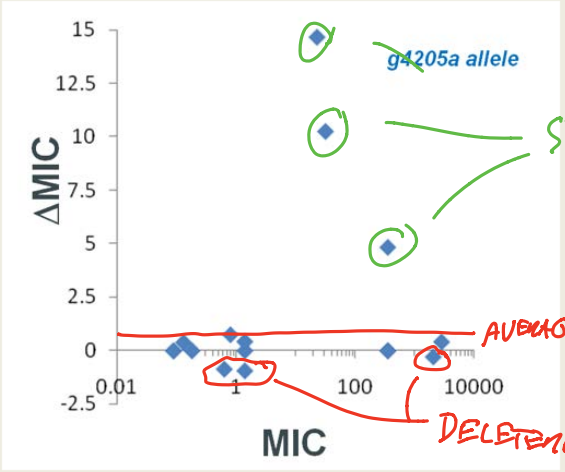
EVOL

- MANY COMBOS MISSING: SOME FLAT OR DOWNWARD STEPS
- SINGLE PEAK → NO INTERMEDIATES TO GET TRAPPED UPON
- RELATIVELY FEW PATHS POSSIBLE OF 120 POTENTIAL, SOME MORE LIKELY

(Weinreich et al., 2006. *Science*)

Patterns of epistasis btw/ genes?

- REPLOTTED TO SEE FULL SET OF DATA
  - ↳ ONE ALLELE ON ALL BACKGROUNDS TESTED



- SIGN EPISTASIS
- ε OFTEN $>> \bar{s}$
- IDIOSYNCRATIC w/ REGARD TO BACKGROUND MIC

→ GENERICALLY TRUE

(replotting Weinreich et al., 2006. *Science*)

Studying epistasis btw/ genes

- WILL SAME TRENDS HOLD FOR btw/ GENES

  w/in PROTEINS = BIOPHYSICS

  btw/ PROTEINS ...-> BIOPHYSICS (AT PROTEIN INTERFACES OR PROT-DNA INTERACTIONS)

  -> "INTERACT" THROUGH PHYSIOLOGY

- IMAGINE ANALOGOUS EXPT FOR EPISTASIS btw/ GENES

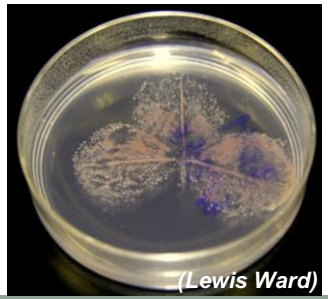
  WT ——> EVOLVED

  SEQ. TO FIND MUTATIONS

  -> MAKE COMBOS
  TEST FITNESS

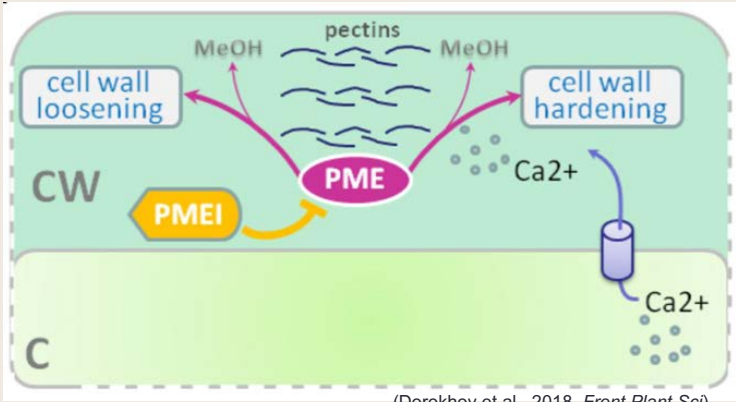
Methylobacterium model for methylotrophy

- *M. extorquens* main system for 60 years
- Dominant member of plant microbiome
- Facultative methylotroph that grows on limited multi-C substrates
- Uncovered majority of knowledge of C_1 dissimilation and assimilation



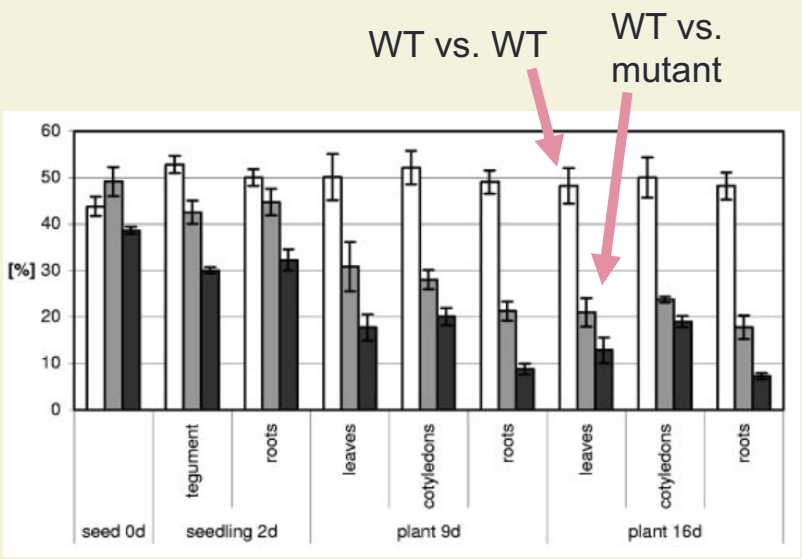
(Peyraud et al., 2011. *BMC Syst Biol*)

Methanol release as connection between plant microbiome niche and C_1 use



(Dorokhov et al., 2018. *Front Plant Sci*)

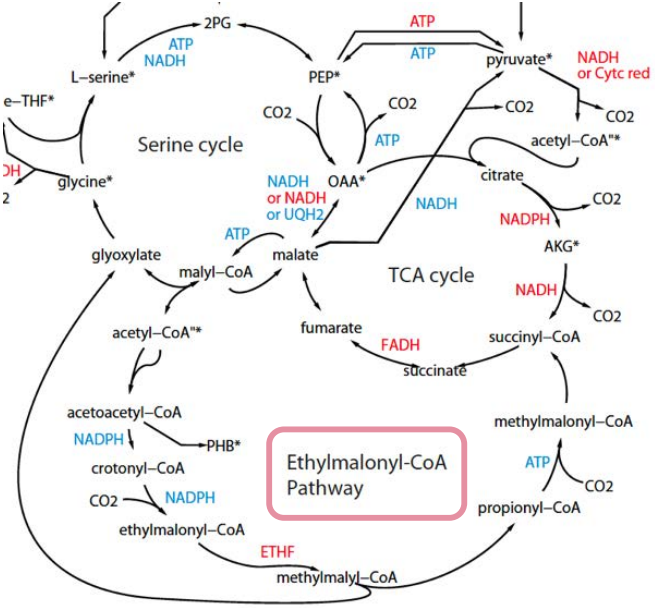
- Plants release 3-10% NPP as methanol
- Mainly from pectin methylesterases



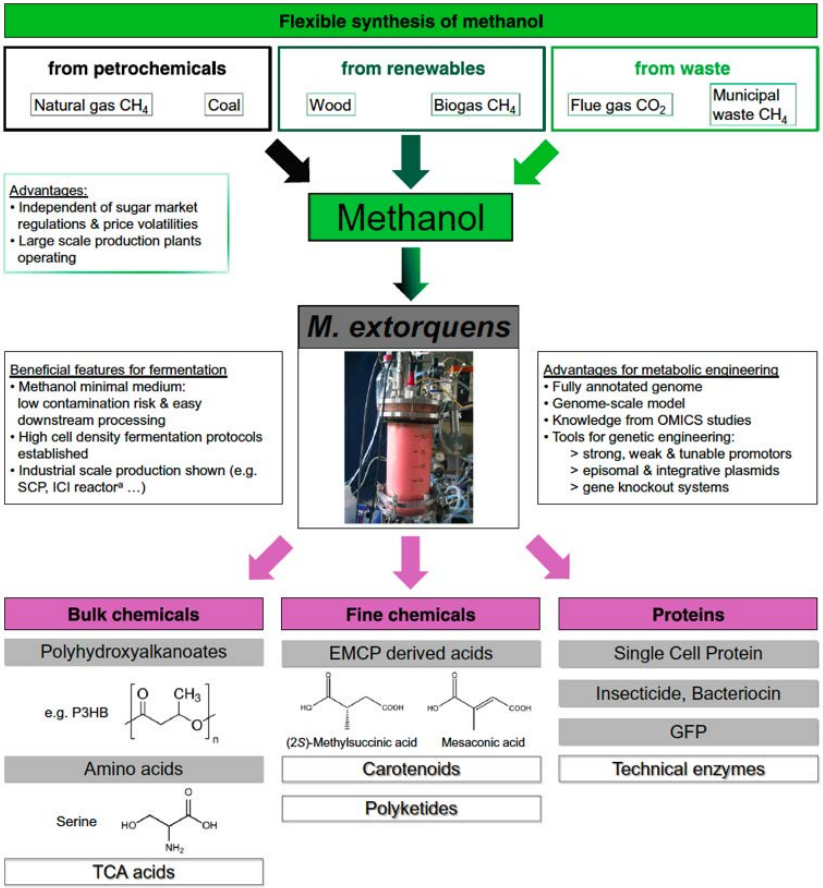
(Sy et al., 2005. *Appl Env Microbiol*)

- C_1 mutants (grey, black) compromised on plants

Methylobacterium and biotechnology

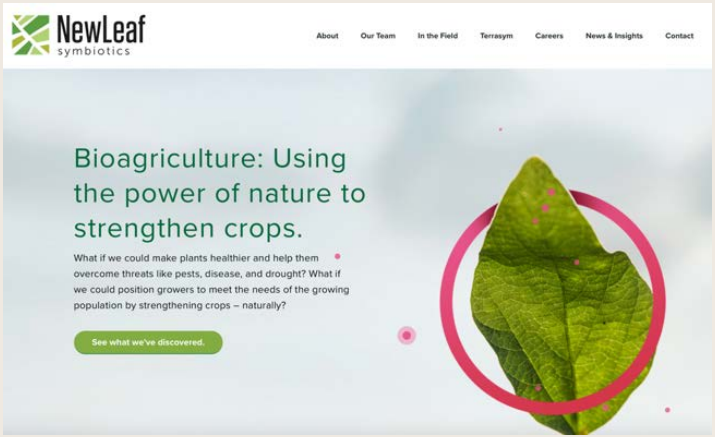


- High flux through reduced C_3, C_4, C_5 compounds
- Can be cultured to >40 g DCW/liter (OD_{600} > 150)



(Ochsner et al., 2014. *Appl Microbiol Biotechnol*)

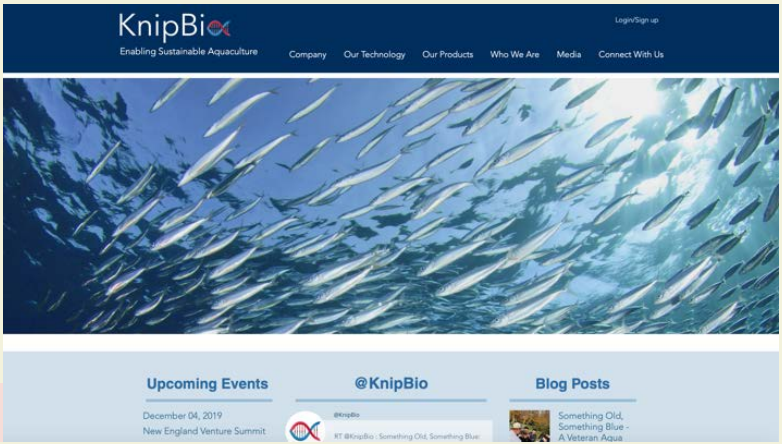
Methylobacterium biotechnology: plant growth & aquafeed ingredient



- Commercialization of plant growth promotion

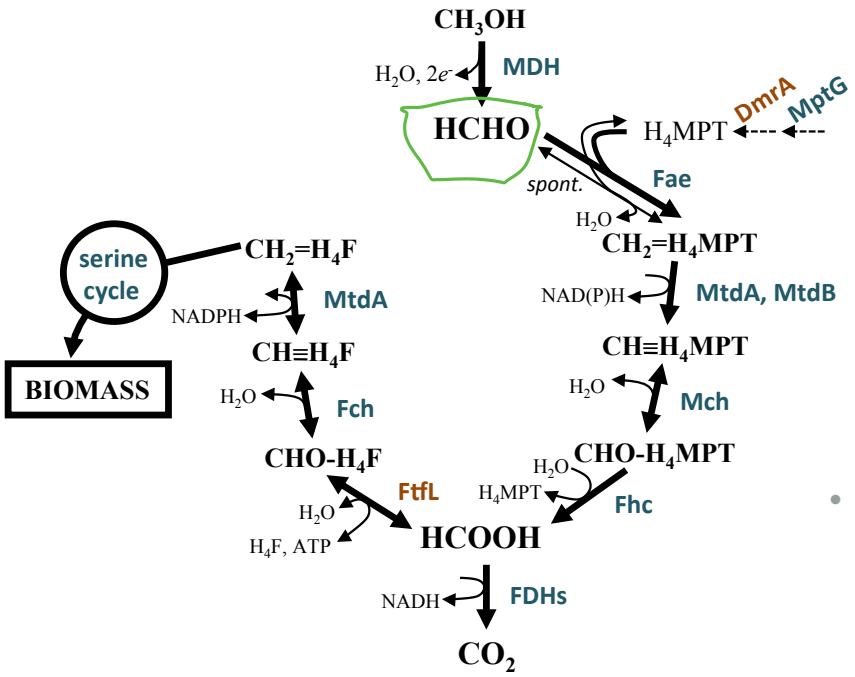
KnipBio
Enabling Sustainable Aquaculture

- Biotechnological platform for aquafeed



- *CJM is co-founder and board member of KnipBio, Inc.*

Formaldehyde as key intermediate

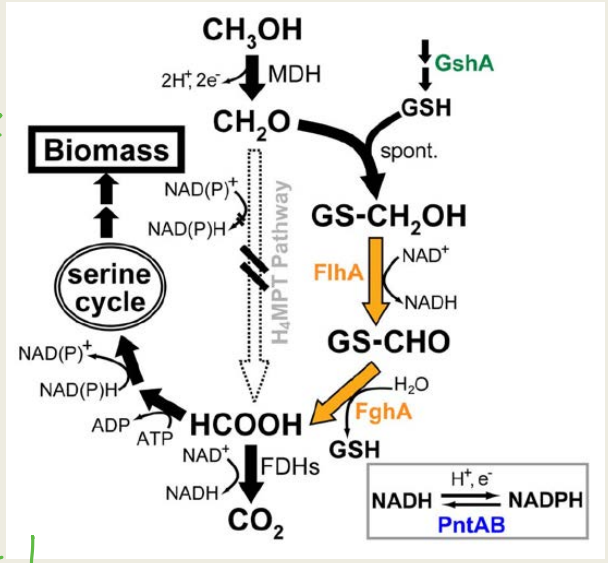


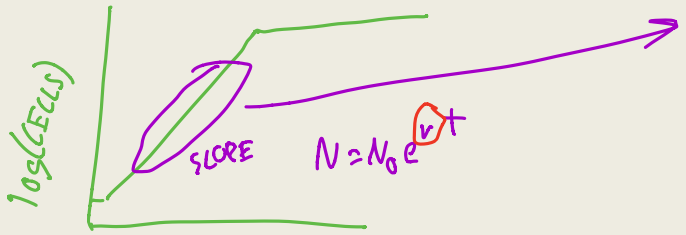
- ***Produced & consumed at 2 mM/s during growth on methanol***

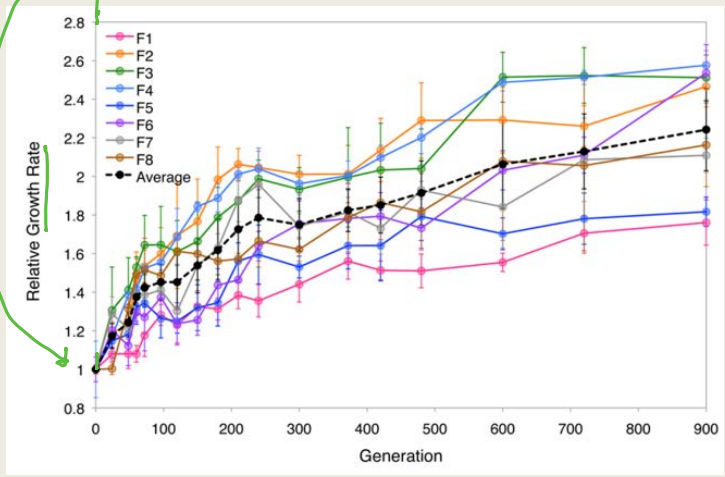
Evolution with new pathway

- GRAD STUDENT → REPLACE THE NATIVE F OXID. PATHWAY w/ FOREIGN PATH.
  - ↳ GROW, BUT MUCH SLOWER

- POSTDOC → STARTED EXP. EVOL. w/ ENGINEERED STRAIN
  - ↳ BATCH TRANSFER w/ $\frac{1}{64}$ DILUTION
    - ↳ 6 GEN/TR.
    - ↳ $N_{FINAL} \approx 1\text{-}2 \cdot 10^9$



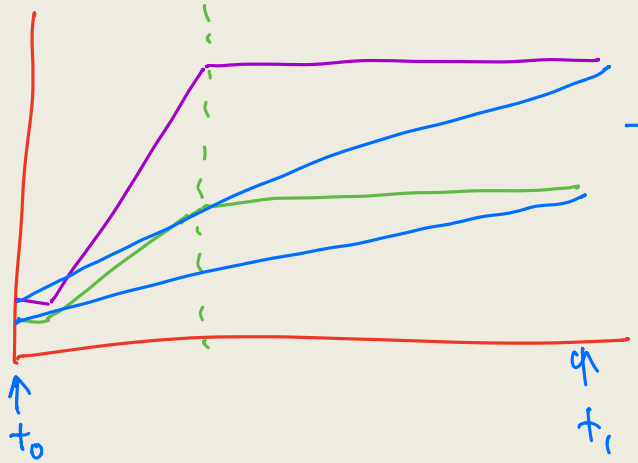




(Chou et al., 2011. *Science;* Lee and Marx, 2013. *Genetics*)

Assay fitness via competition

- PAIRWISE CHANGE IN FREQ.
  - ↳ STRAINS w/ FLUORESCENT PROTEINS
    - ↳ 50K CELLS IN A MINUTE



→ AVERAGE SELECTIVE ADVANTAGE PER GENERATION

Table 2. Fitness of fluorescent strains against wild-type *Methylobacterium*.

Strains	Methanol		Succinate	
	Plate count	Flow cytometer	Plate count	Flow cytometer
CM1176	0.9948±0.0036	0.9955±0.0036	0.9908±0.0131	0.9968±0.0012
CM1178	0.9798±0.0179	0.9799±0.0016	0.9790±0.0056	0.9869±0.0009
CM1180	1.0130±0.0078	1.0021±0.0030	0.9942±0.0093	0.9996±0.0003

$$W = \frac{\log\left(\frac{R_1 \cdot 64}{R_0}\right)}{\log\left(\frac{(1 - R_1) \cdot 64}{1 - R_0}\right)}$$

(Lee et al., 2009. *Evolution*)

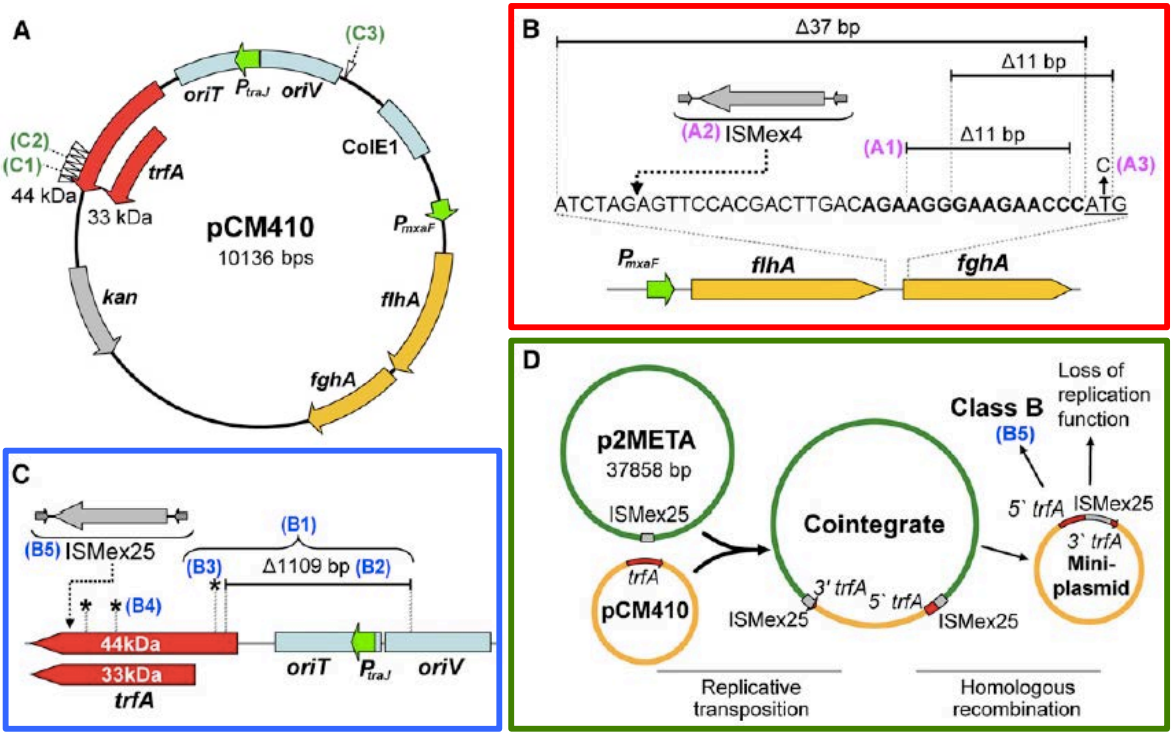
What types of mutations to test in combination?

1. COMBINED PAIRS OF ALLELES FROM DIFFERENT POPULATIONS, BUT AFFECTING THE SAME, INTRODUCED PATHWAY

2. COMBINED ALLELES FROM A SINGLE ADAPTIVE TRAJECTORY

Beneficial from different lineages affecting same pathway

btw/ 2 GENES PATHWAY



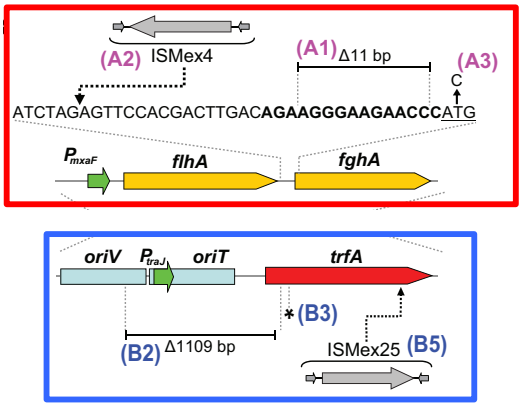
PLASMID REPLICATION

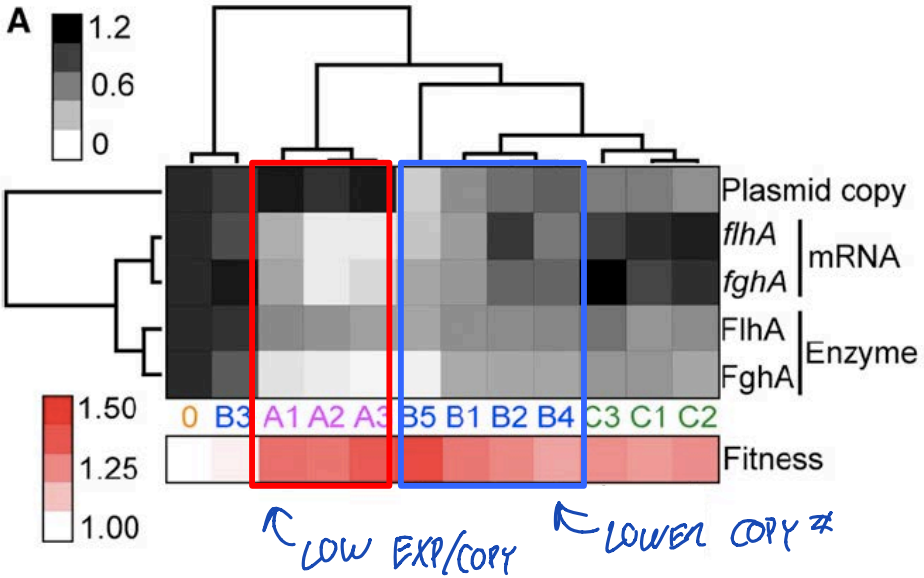
INTEGRATE PLASMID INTO HOST GENOME

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

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

Expression per copy vs. copy#

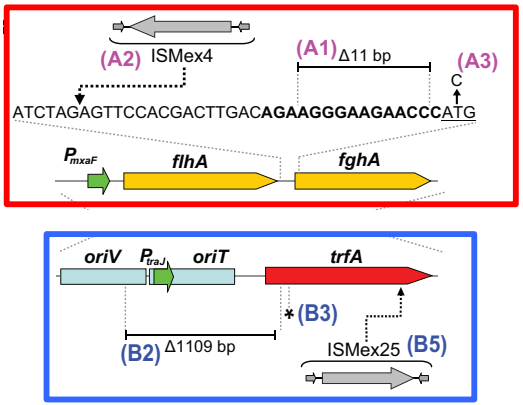


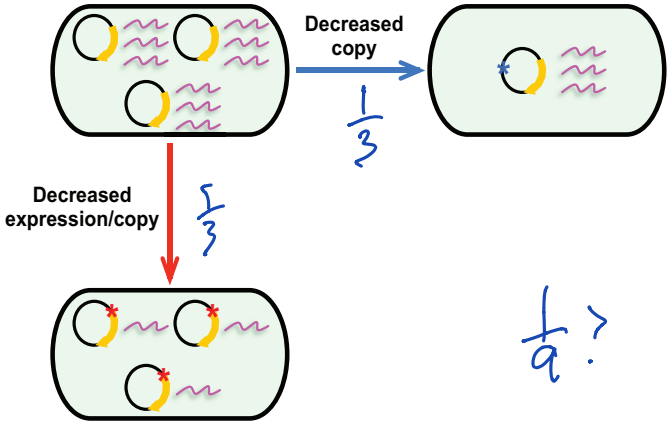


- Distinct, independent mechanisms to **reduce** expression of the GSH pathway enzymes
- But no promoter mutations?...

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

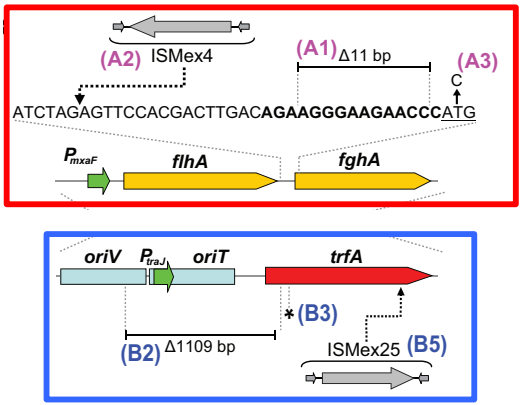
Independent effects upon expression

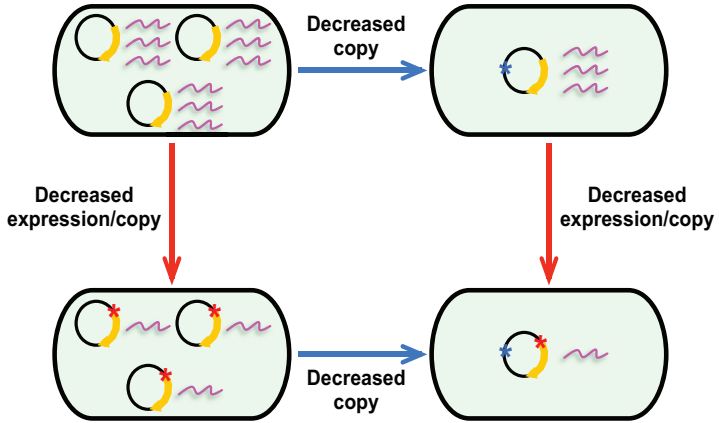




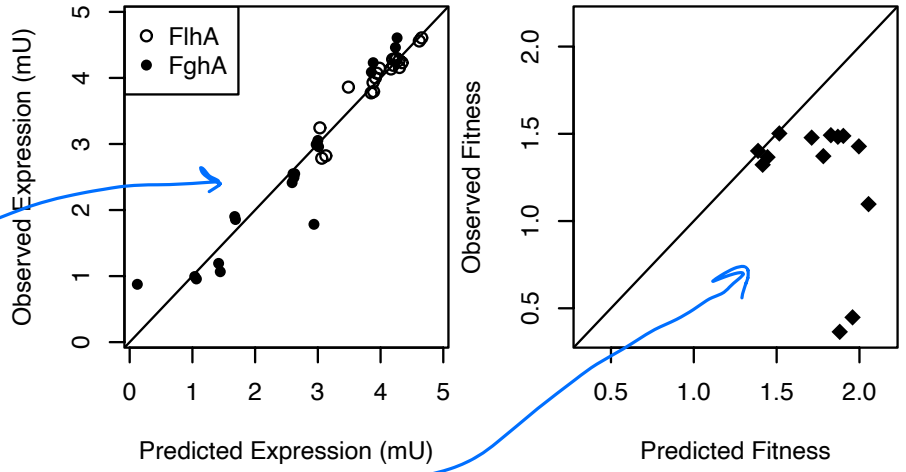
(Chou et al., *PLOS Genetics*, 2014)

Independent effects upon expression



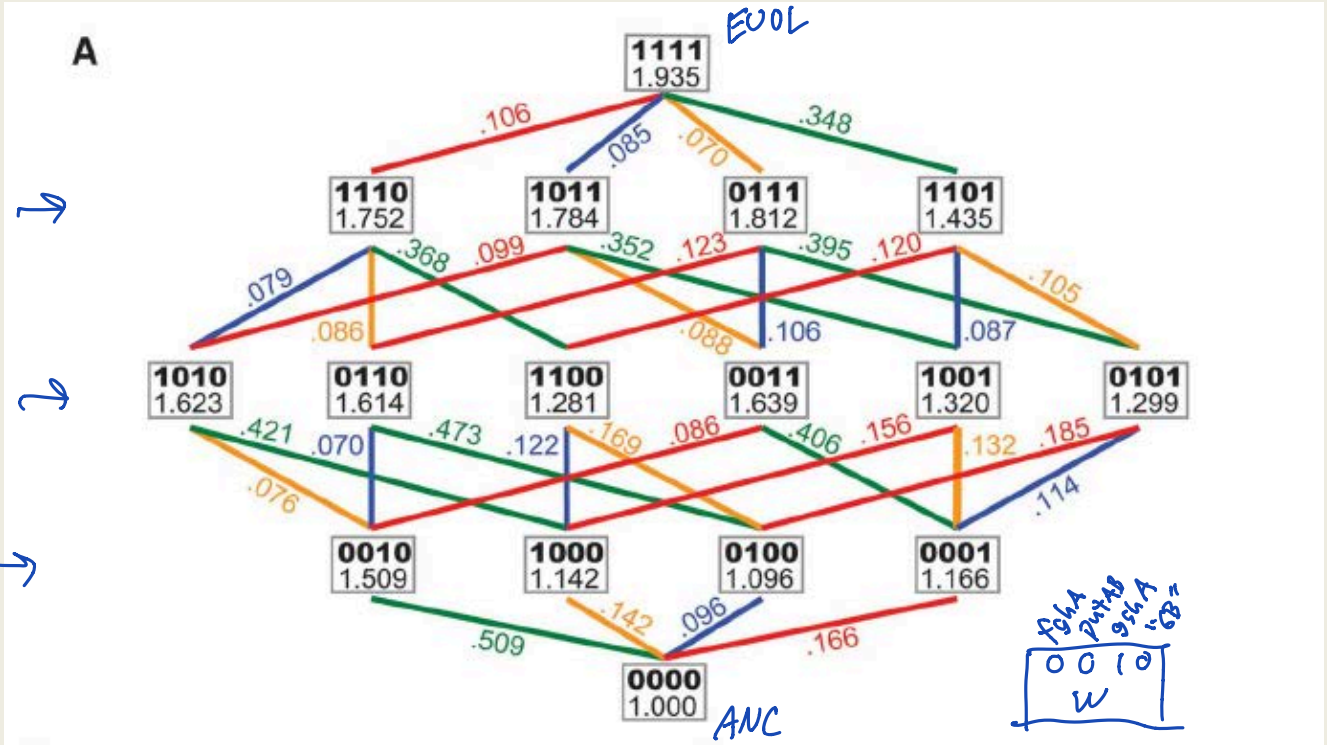


- Two classes should interact independently:
  - $E_{AB} = E_A \times E_B$
  - Yes
- Indep. upon fitness?
  - **No.**



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

Fitness values of mutational combinations



AFTER 600 GEN: 9 MUTATIONS

3 IN C_1 METAB:

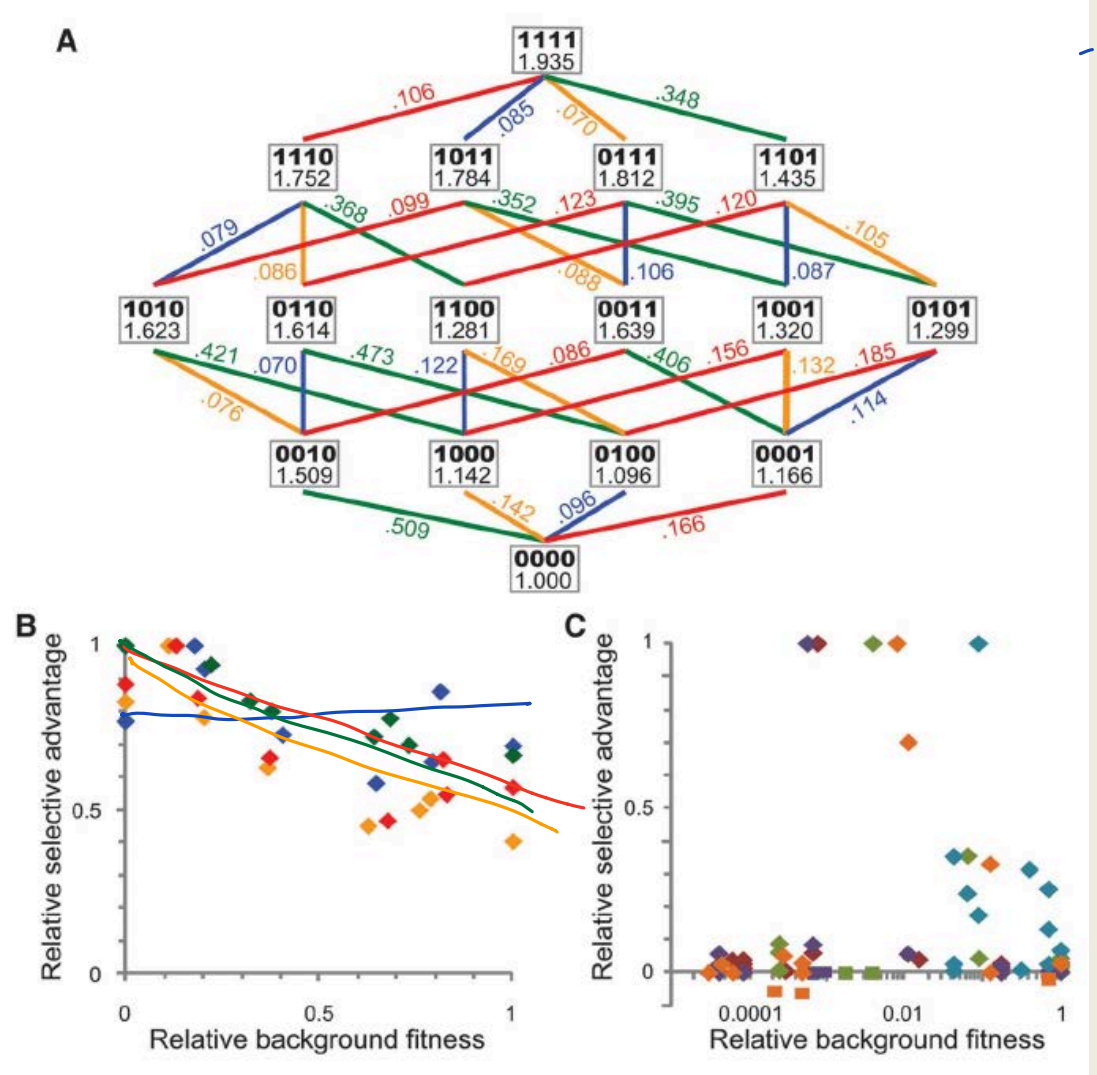
6 OTHERS → LUMPED AS ONE "ALLELE" (RED)

* NO SIGN EPISTASIS: ALL ALLELES BEN. ON ALL BACKGROUNDS
  ↳ EVERY TRAJECTORY POSSIBLE

* MAGNITUDE: 3/4 ALLELES DECLINE IN MAGNITUDE W/ W

(Chou et al., 2011. *Science*)

Epistasis across genes vs. within protein



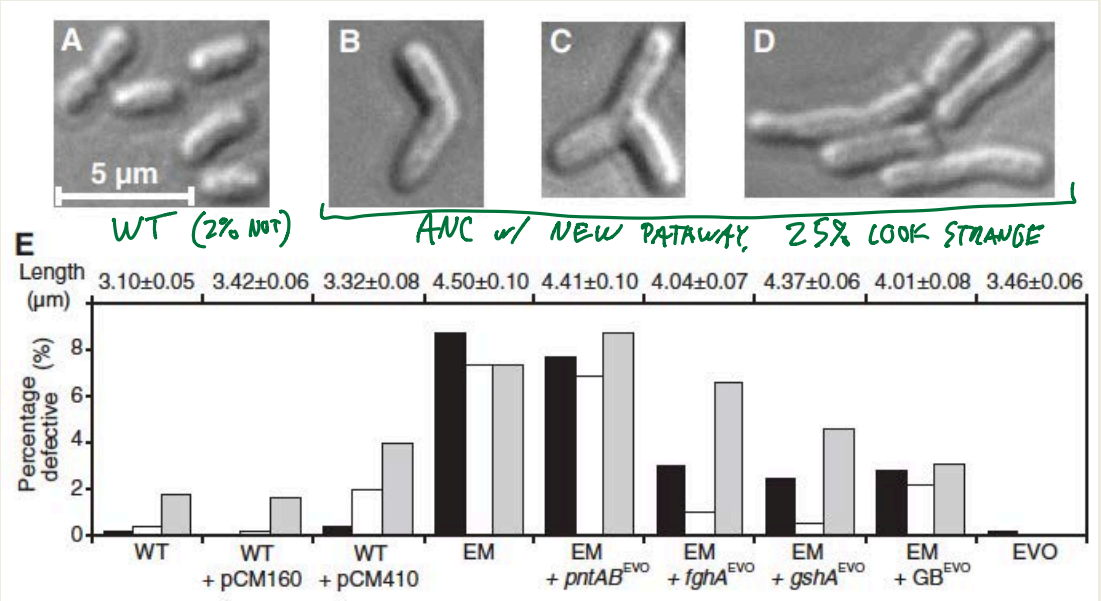
- IN (B), PLOTTED RELATIVE s (DIVIDED BY MAX s) AGAINST RELATIVE FITNESS (SCALED FROM ZERO TO ONE)

ACROSS GENOME
- SEE "DIMINISHING RETURNS"
  - ↳ ANTAGONISTIC
- $\varepsilon < \bar{s}$
- SCALES GENERICALLY w/ w (FOR 3/4)

*CONTRIBUTES TO ADAPTATION SLOWING DOWN

(Chou et al., 2011. *Science*)

What might cause diminishing returns?

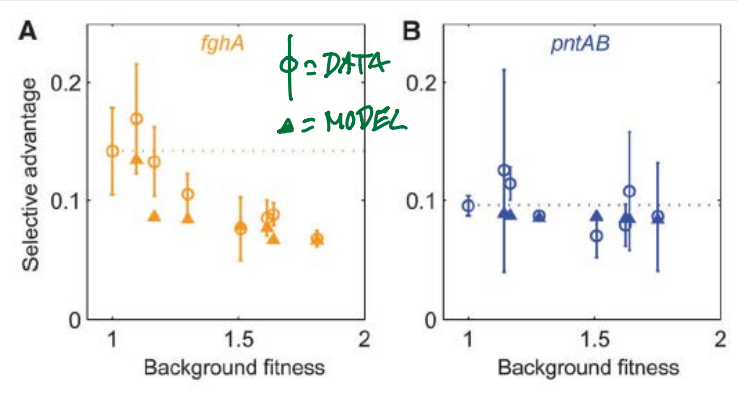


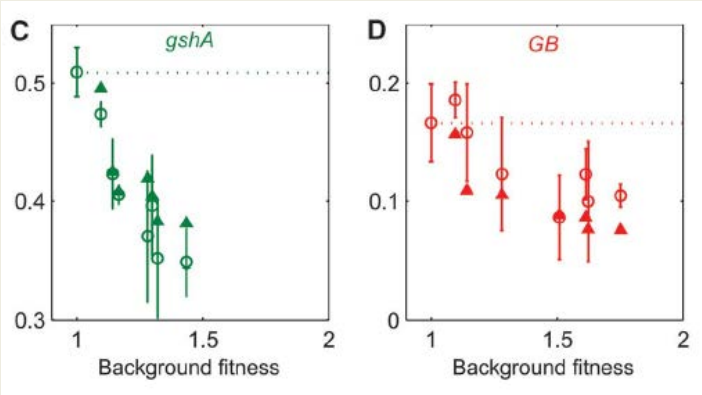
- EXPRESSION OF FOREIGN PATHWAY CAUSES THE CELLULAR ABNORMAL.
  - ↳ COST, LOOKED LIKE PROT. EXP
- EVOLVES AWAY

	LOWER # DEFECTIVE	DIMINISHING RETURNS FOR W
pntAB	NO	NO
OTHER THREE	YES	YES

(Chou et al., 2011. *Science*)

What might cause diminishing returns?





FITNESS = (GROWTH w/o COSTS) − COSTS

b_0 c_0

$W_{ANC} = 1.14 - 0.14 = 1$

$W_i = \lambda_i \cdot b_0 - \theta_i \cdot c_0$

PROPOSED: NULL $W_{ij} = \lambda_i \lambda_j b_0 - \theta_i \theta_j c_0$ ← FIT PARAM. FROM WT & SINGLES

↳ PREDICT DOUBLE → QUAD STRAINS

$R^2 = 0.97$ AS TWO PHENOTYPE

$R^2 = 0.64$ $(W_{AB} = W_A \cdot W_B)$

· EACH BEN. ALLELE COULD AFFECT ONE OR BOTH TRAITS (λ_i, θ_i)

↳ PROT. REP. IN STRANGE CELLS

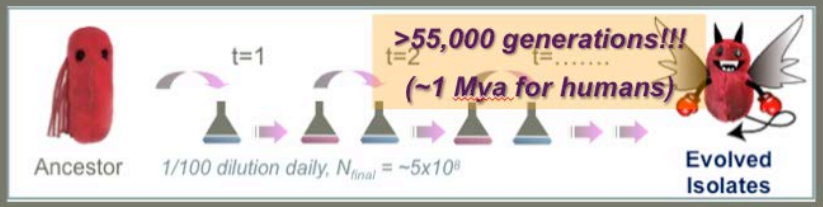
(Chou et al., 2011. *Science*)

Classic system: Lenski long-term evolution

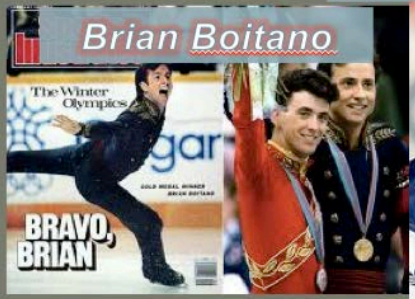
Experimental evolution: the Lenski populations

1.) Approach and model system | 2.) Major findings | 3.) Tradeoffs | 4.) Conclusions

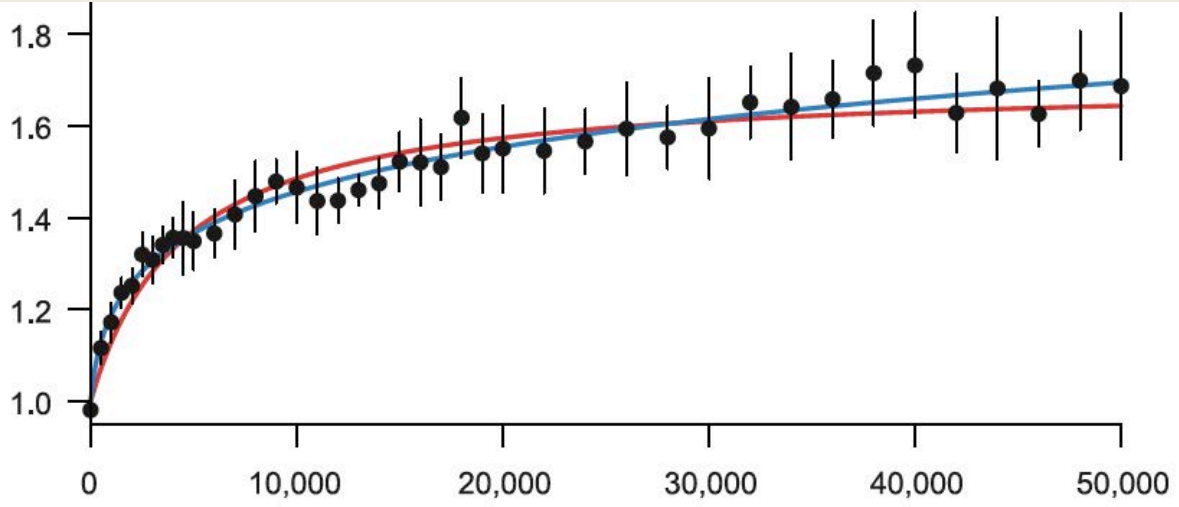
- On February 24th, 1988, Rich Lenski started 12 populations of *E. coli* B in minimal glucose medium



- On February 24th, 1988: amidst the Calgary Winter Olympics:

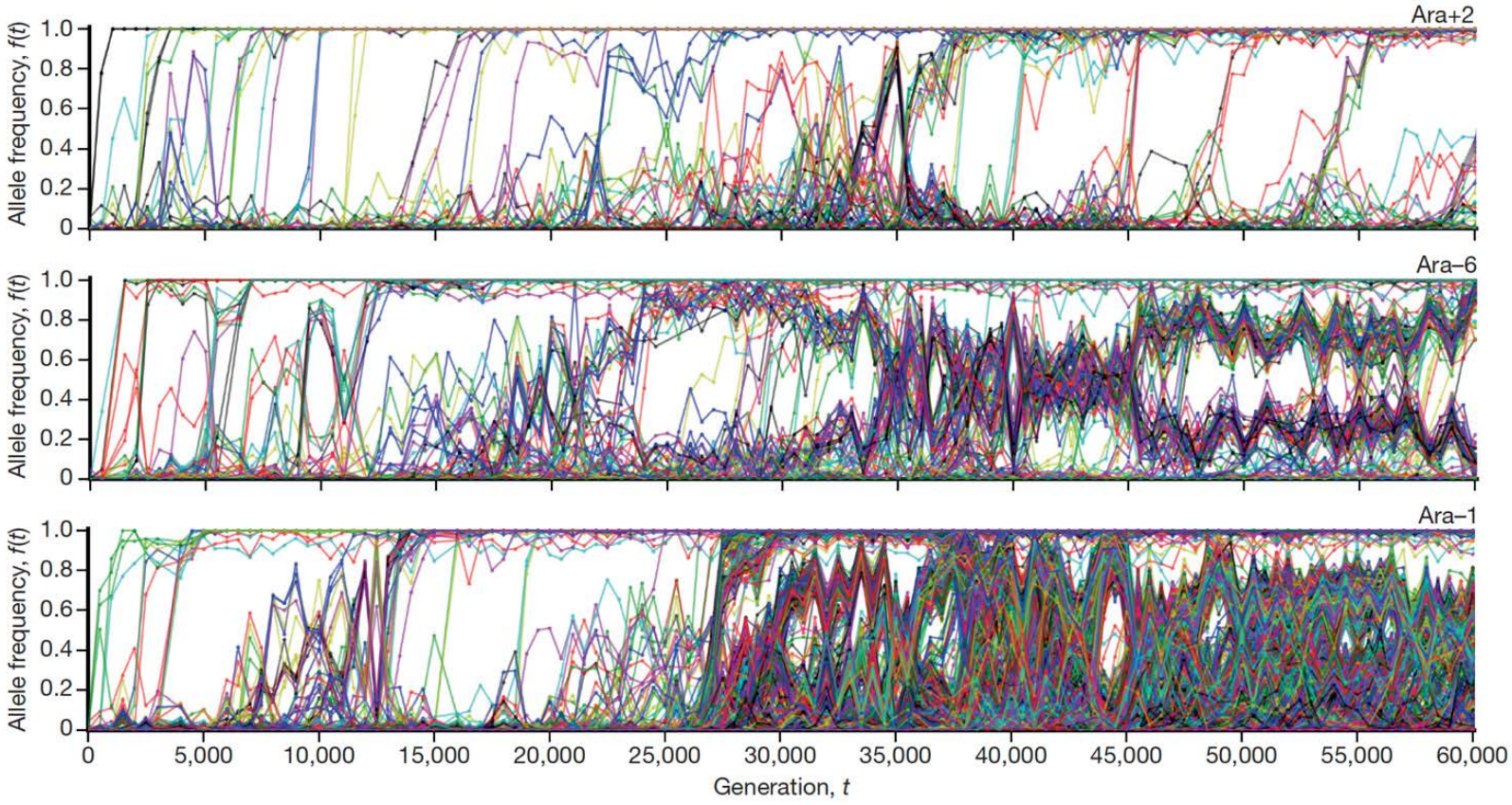


Continued improvement for 25+ years



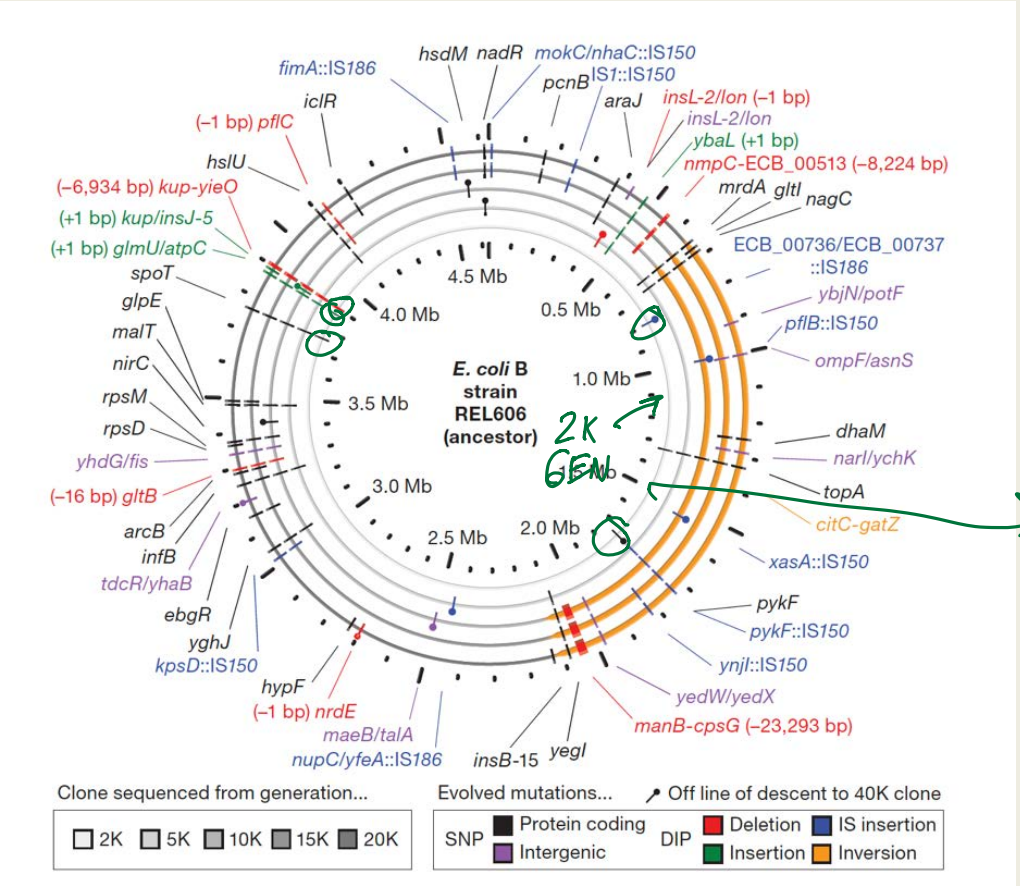
(Wiser et al., 2014. *Science*)

Picture of genomic change during adaptation



(Wiser et al., 2014. *Science*)

Picture of genomic change during adaptation

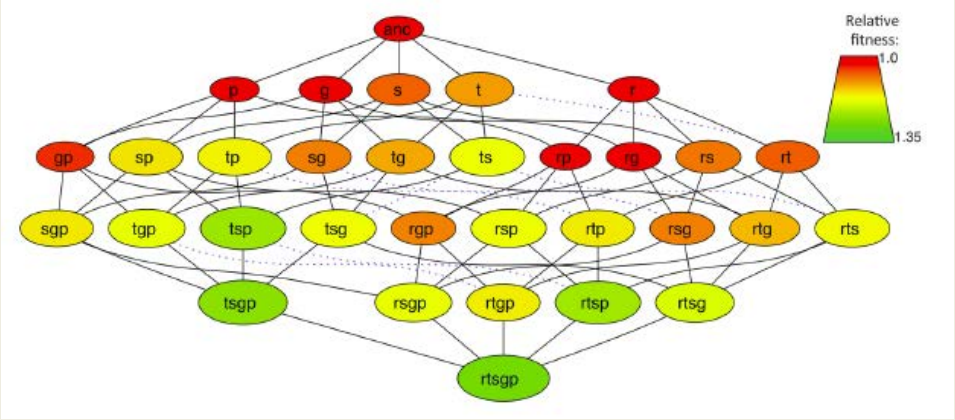


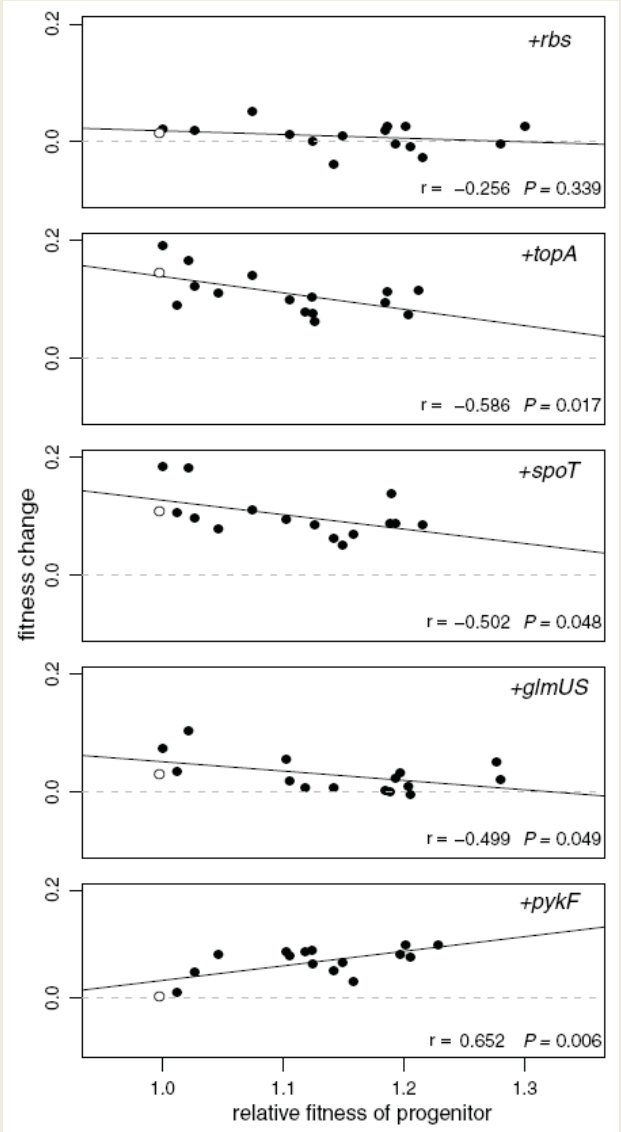
ALL COMBOS OF 5 MUT. FROM E.coli EVOL. ON GLUCOSE

(Barrick et al., 2009. *Nature*; Good et al., 2017. *Nature*)

Diminishing returns in *E. coli*

- $\varepsilon < \bar{s}$
- NO SIGNIFICANT SIGN EPISTASIS
- 4/5 ALLELES w/ DIMINISHING RETURNS

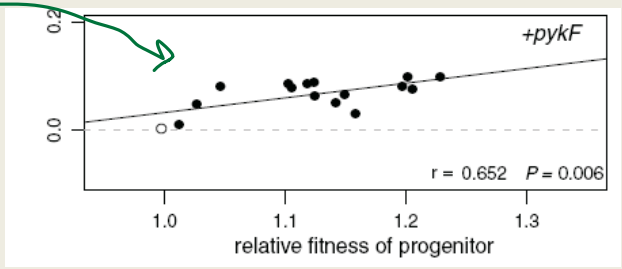




(Khan et al., 2011. *Science*)

Some loci have synergistic effects

- 1/5 FROM E.coli (PTS SYSTEM FOR GLUCOSE TRANSPORT)

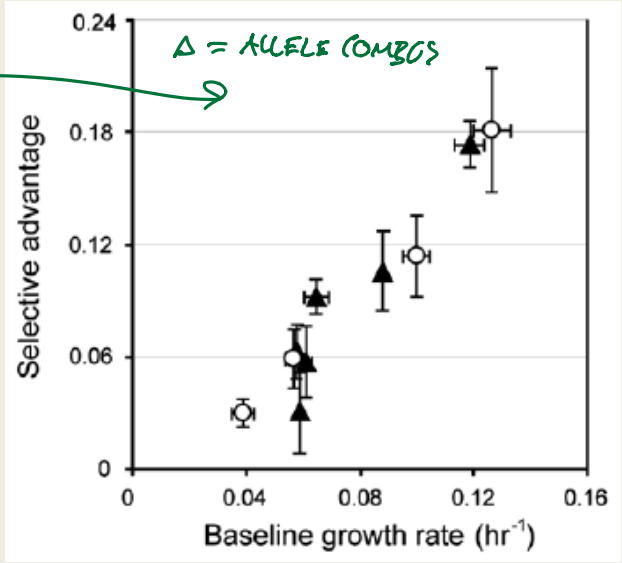


- ONE ALLELE (Co TRANSPONTER)

- EPISTASIS = G × G

- GENOTYPEXENVIRON = G × E "BY"

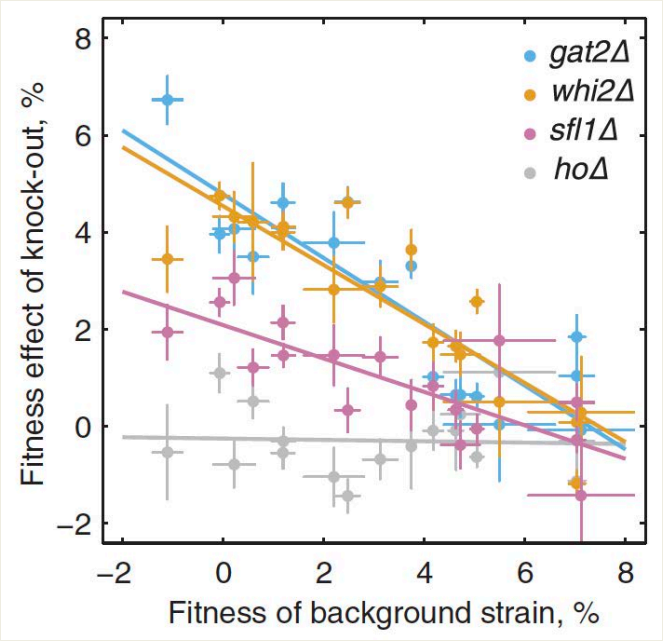
↳ GREW FAST WT STRAIN TO CHANGE GROWTH RATE



(Khan et al., 2011. *Science*; Chou et al., 2009. *PLOS Genetics*)

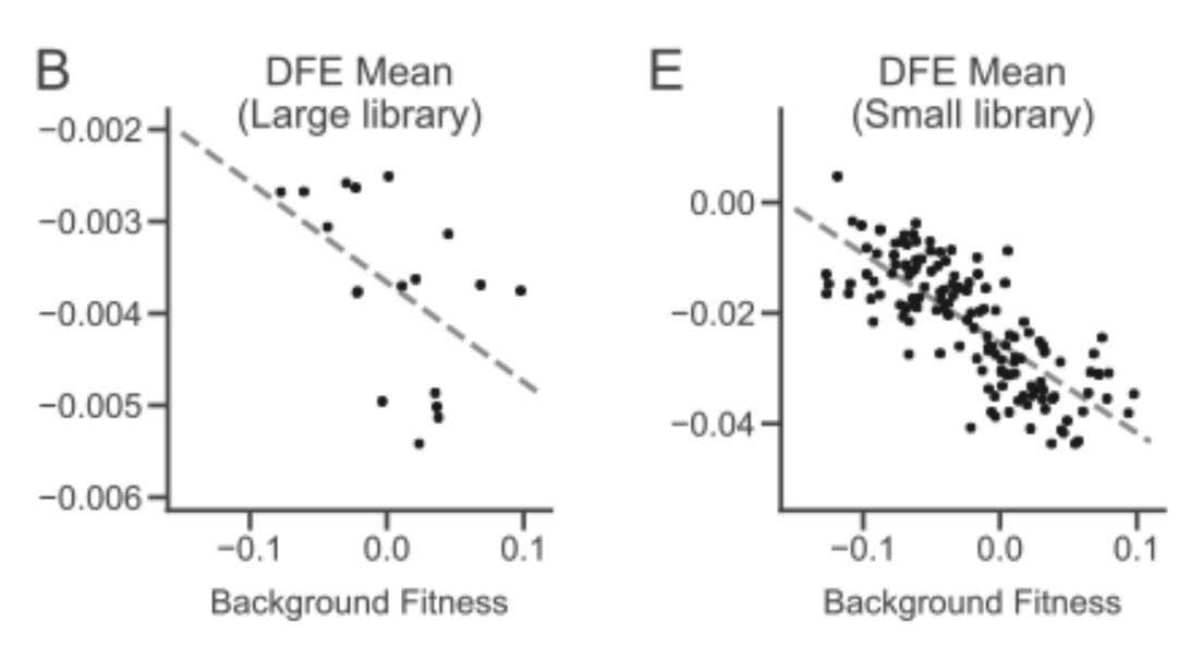
Continued observation of diminishing returns

- YEAST EVOLUTION IN RICH MEDIUM IN DESAI LAB
  - 3/4 TESTED MUT. W/ DIMINISHING.



(Kryazhimskiy et al., 2014. *Science*)

Epistasis with beneficial mutations vs. deleterious

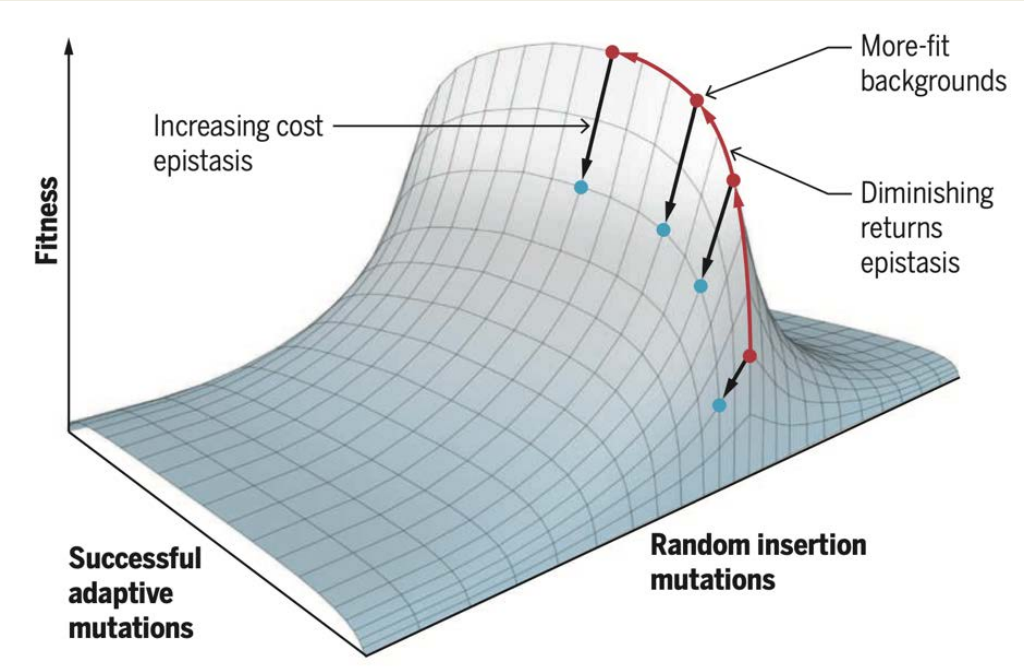


- NEW STUDY INTRODUCED MANY DELETERIOUS MUTATIONS INTO MANY FIT STRAINS

- GENERIC TREND w/ FITNESS, MUTATIONS HAD BIGGEST EFFECT ON HIGH FITNESS BACKGROUNDS

(Johnson et al., 2019. *Science*)

Epistasis with beneficial mutations vs. deleterious



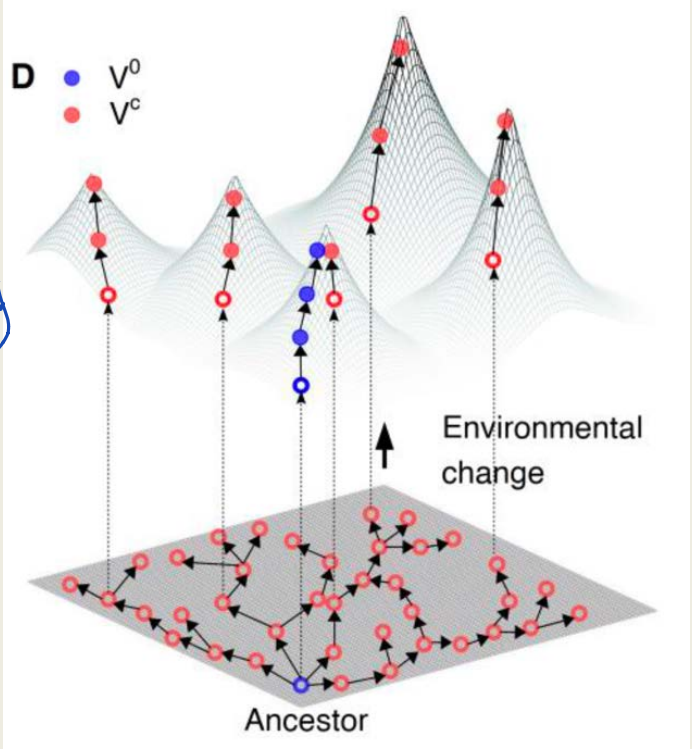
- DIFFERENT TRENDS FOR DELETERIOUS VS BEN. ALLELES

WHY??

(Miller, 2019. *Science*)

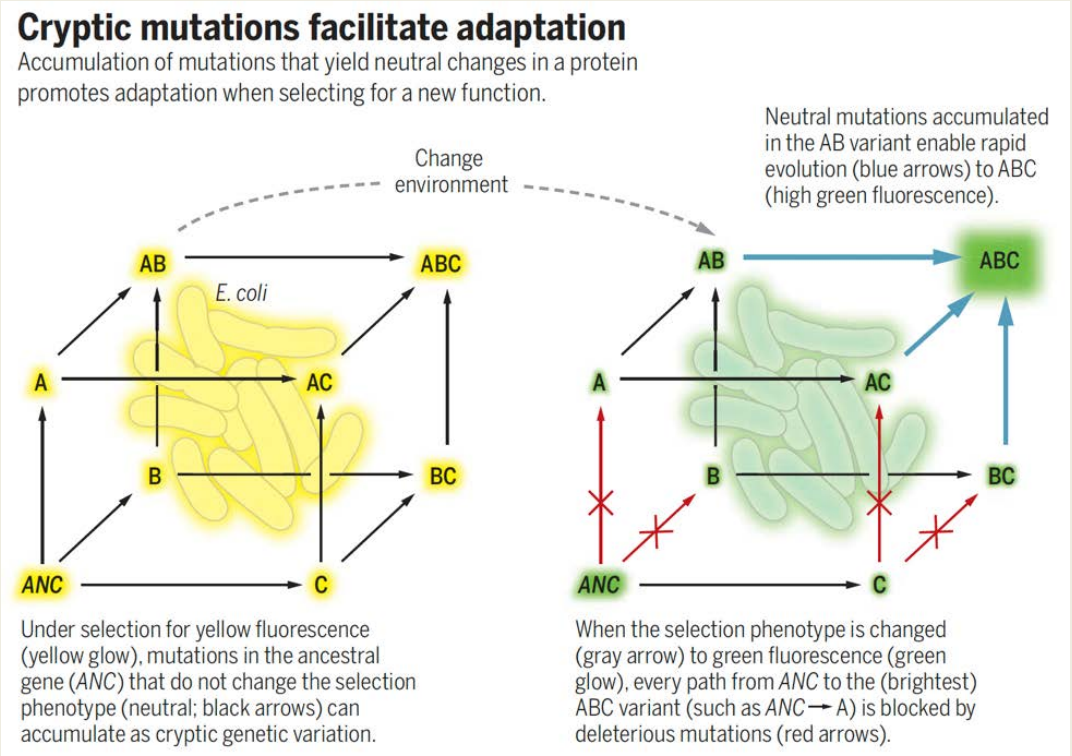
Changing environments allows cryptic variation to escape local fitness peaks

- CAN STARTING FROM MULTIPLE PLACES GENETICALLY PREVENT GETTING TRAPPED ON LOCAL PEAK?
  - GENERATE NEUTRAL VARIATION UNDER PURIFYING SELECTION (YFP & YELLOW FLUOR.)
    - ↳ "CRYPTIC VARIATION"
  - NOW SELECT FOR NOVEL TRAIT (YFP FOR GREEN FLUORESCENCE)



(Zheng et al., 2019. *Science*)

Changing environments allows cryptic variation to escape local fitness peaks



- ONE ANSWER TO HOW TO CROSS A FITNESS VALLEY

(Lee and Marx, 2019. *Science*)

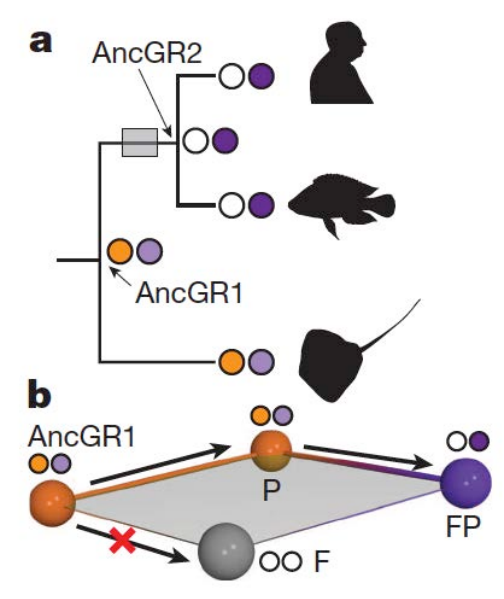
Specific epistasis: basis of historical contingency

LETTER

doi:10.1038/nature13410

Historical contingency and its biophysical basis in glucocorticoid receptor evolution

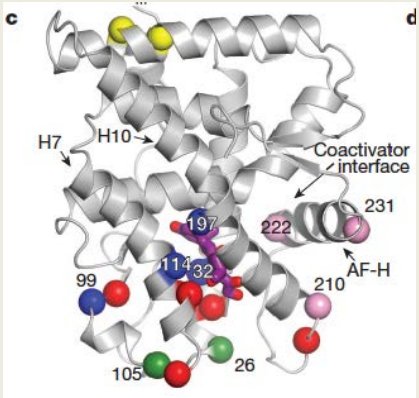
Michael J. Harms[1,2] & Joseph W. Thornton[2,3]



- EPISTASIS HAS AFFECTED PROTEIN EVOL. IN THE PAST

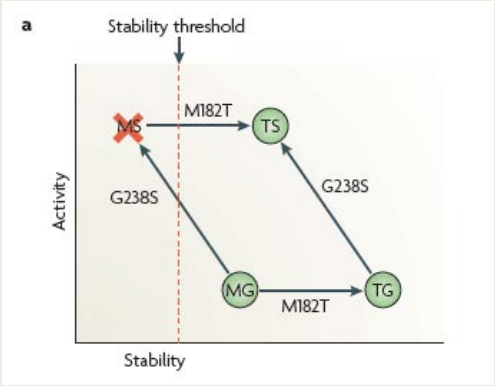
- THORNTON LAB DID PHYLOGENETIC RECONSTRUCTION OF EXTINCT NODES & TEST PHENOTYPES FOR HORMONE RECEPTOR FAMILY
  - FOUND MUTATIONS THAT CHANGED SPECIFICITY
  - NEUTRAL MUT. FOR SPECIFICTY THAT ARE REQ. FOR FXNL CHANGES

SPECIFIC AAE MUTATIONS →

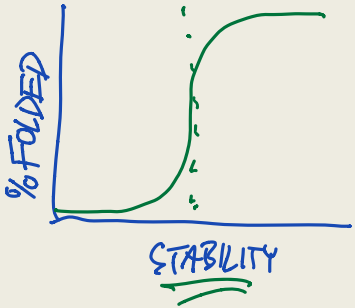


(Harms and Thornton, 2014. *Nature*)

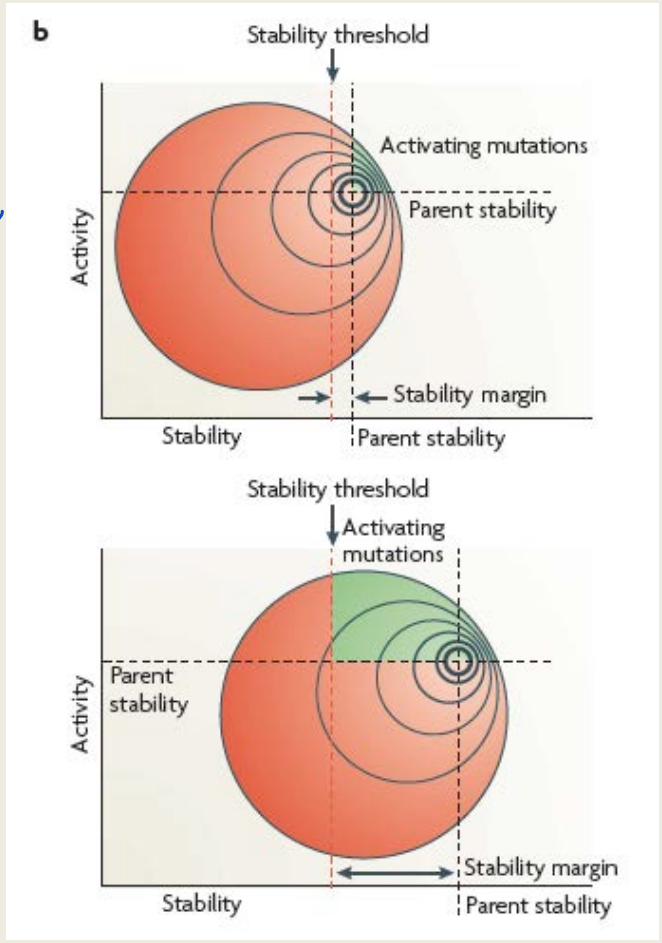
Generic epistasis: stability



- GENERIC TREND FOR PROTEINS RELATES TO FOLDING STABILITY



- STABILITY & ACTIVITY ARE BOTH RARE TRAITS



(Romero and Arnold, 2009. *Nat. Rev. Mol. Cell. Biol.*)