Measuring the Effects of Regulatory Mutations on Bacterial Evolvability

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Labs of Richard Lenski and Charles Ofria

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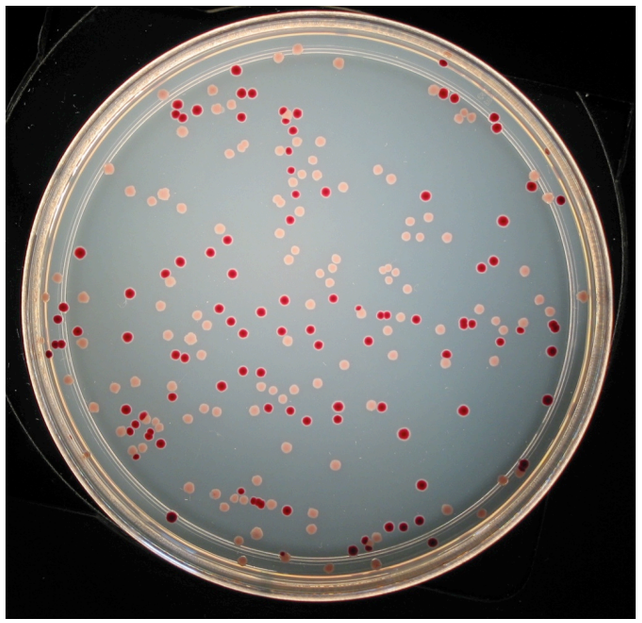
Contending Mutations in Ara-1

- At 500 generations, 4 distinguishable subpopulations:
  (1) *rbs1 topA*, *(2) rbs1* only, (3) *rbs2* only, and (4) no known mutations
- *topA* eventually fixed in this population, but clones carrying it have a lower fitness than their contemporaries?
- *topA* is a global regulator that controls DNA supercoiling.
- Hypothesis: *topA* wins due to greater evolvability

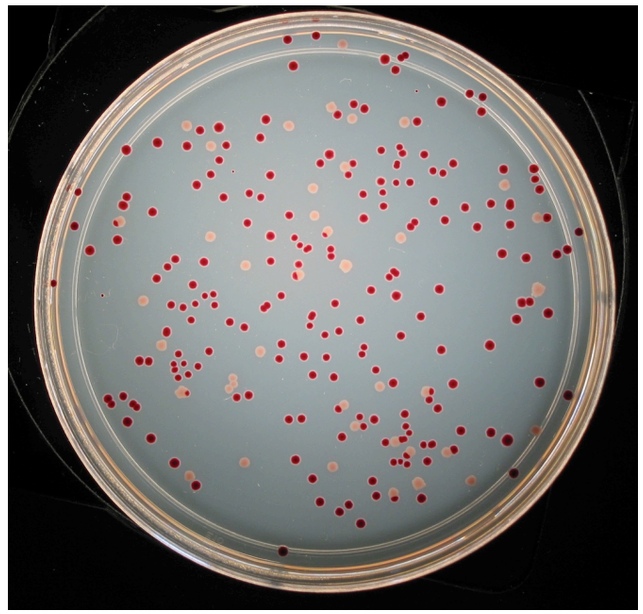
Robert Woods, Ph.D. thesis

Marker Divergence Experiments

Mix neutrally marked variants of a test strain and propagate.
Mutants arise, rise in frequency, and shift marker ratio.



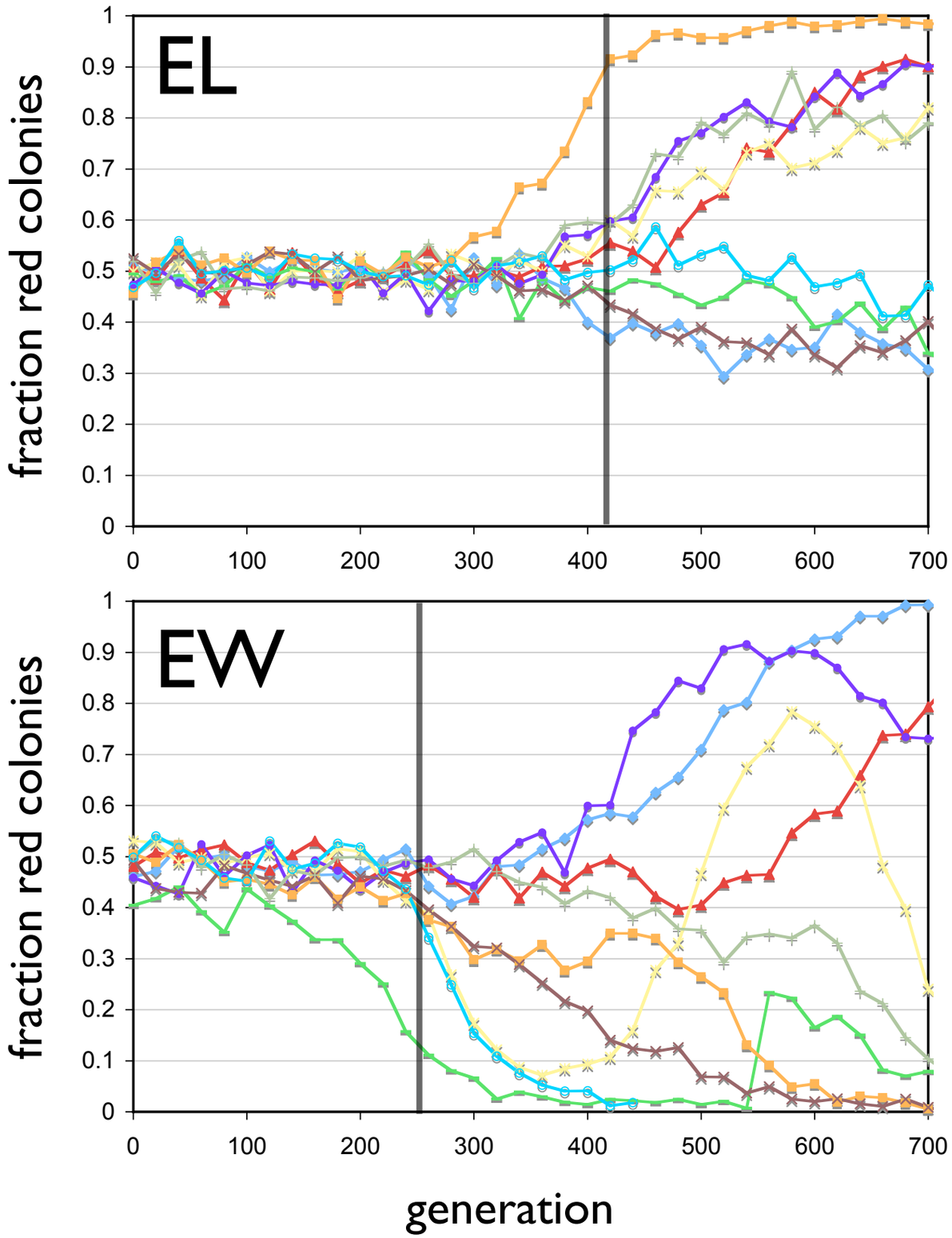
daily 1:100 dilutions
into fresh media



t = 0	
white	110
red	137

t = 300 gen	
white	38
red	203

Marker Divergence Experiments



Eventual Loser (EL)

mean generation of significant divergence **409**

genotype: *rbs2* or no known muts

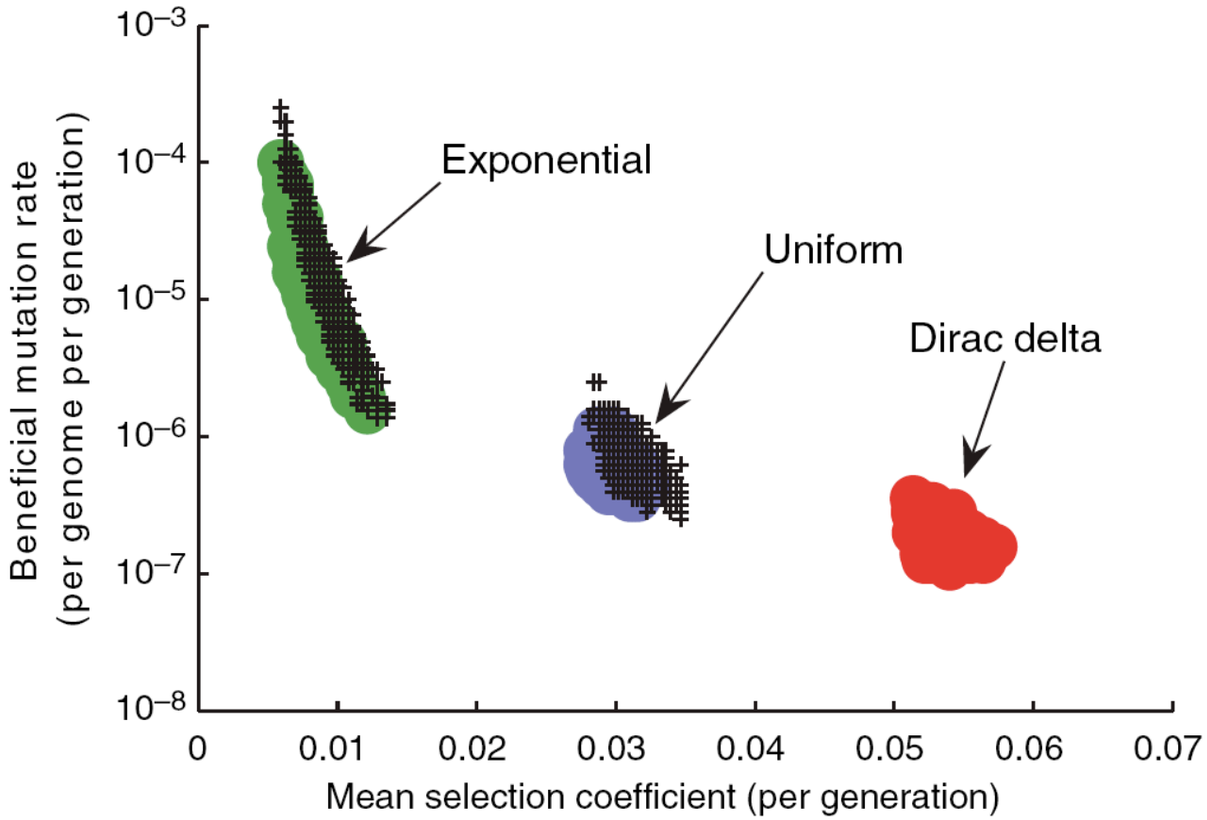
Eventual Winner (EW)

mean generation of significant divergence **248**

genotype: *topA*, *rbs1*

Robert Woods, Ph.D. thesis

Effective Evolutionary Parameters



For any underlying distribution of beneficial mutation effects –

Fixed beneficial mutations approximated well by a single category with one effective selection coefficient and rate.

Hegreness, M., Shoresh, N., Hartl, D., and Kishony, R. (2006) An equivalence principle for the incorporation of favorable mutations in asexual populations. *Science* **311**, 1615-1617.

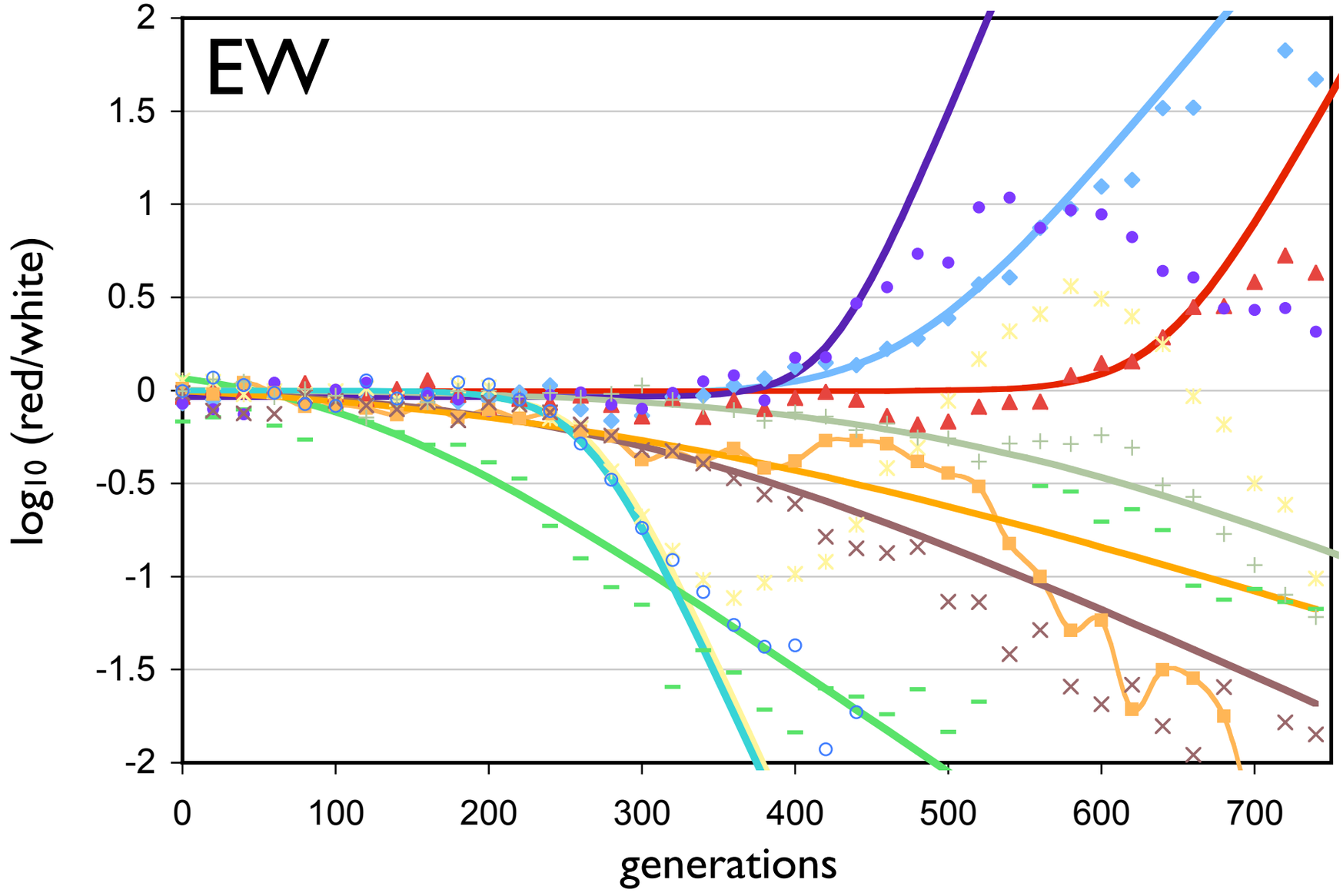
Quantifying Evolvability

1. **Empirical parameters** from marker trajectories.

2. Population genetics simulations at many combinations of **effective evolutionary parameters** (s and μ).

3. What **evolutionary parameters** produce trajectories with indistinguishable **empirical parameters**?

Fit Empirical Parameters

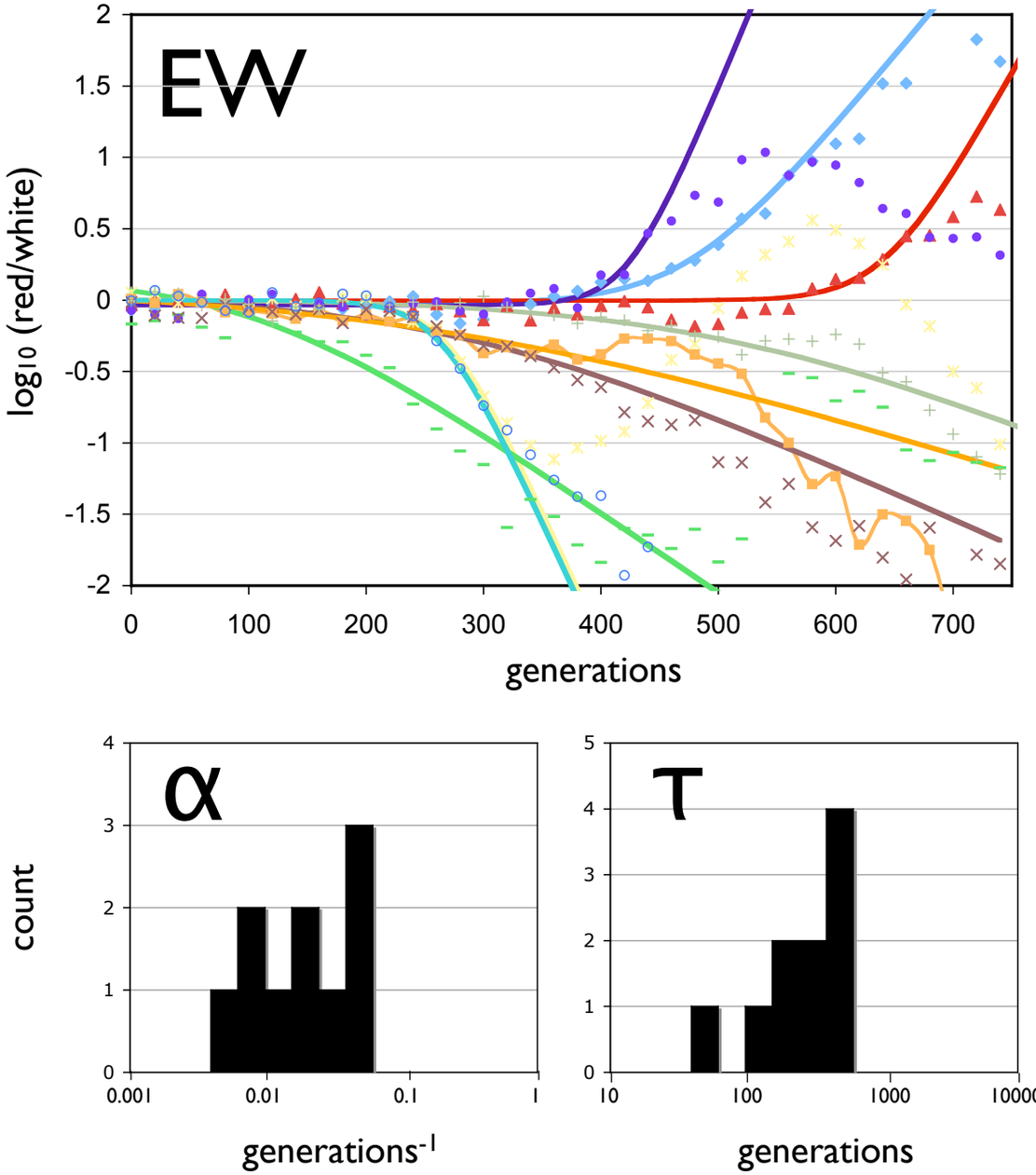


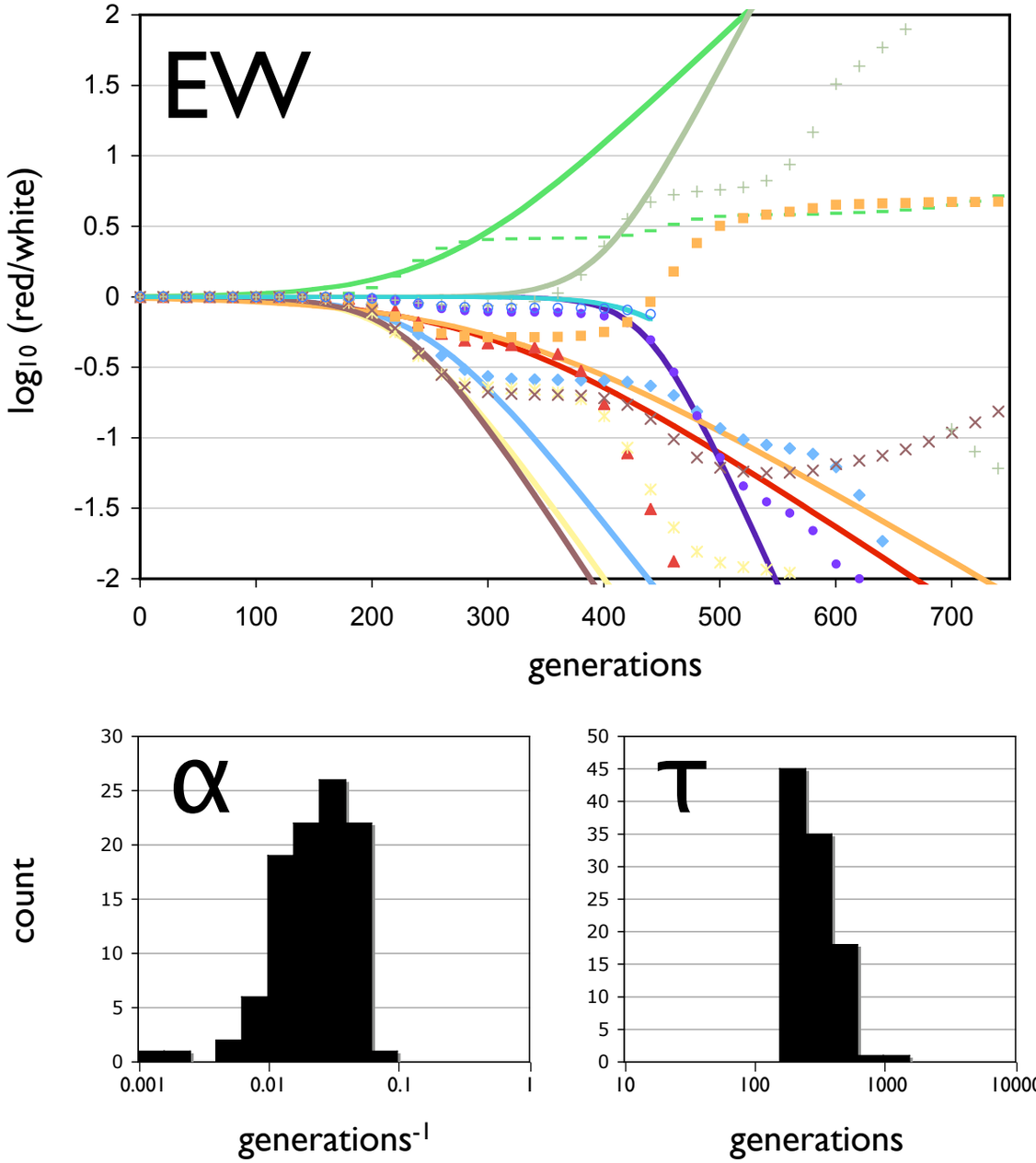
Empirical Equation: $\log_{10}(\text{red/white}) = c_w \log_{10}(1+\tfrac{1}{2} e^{\alpha(t-\tau)})$

c_w winning color (-1,+1) α divergence rate τ waiting time

Experimental Data

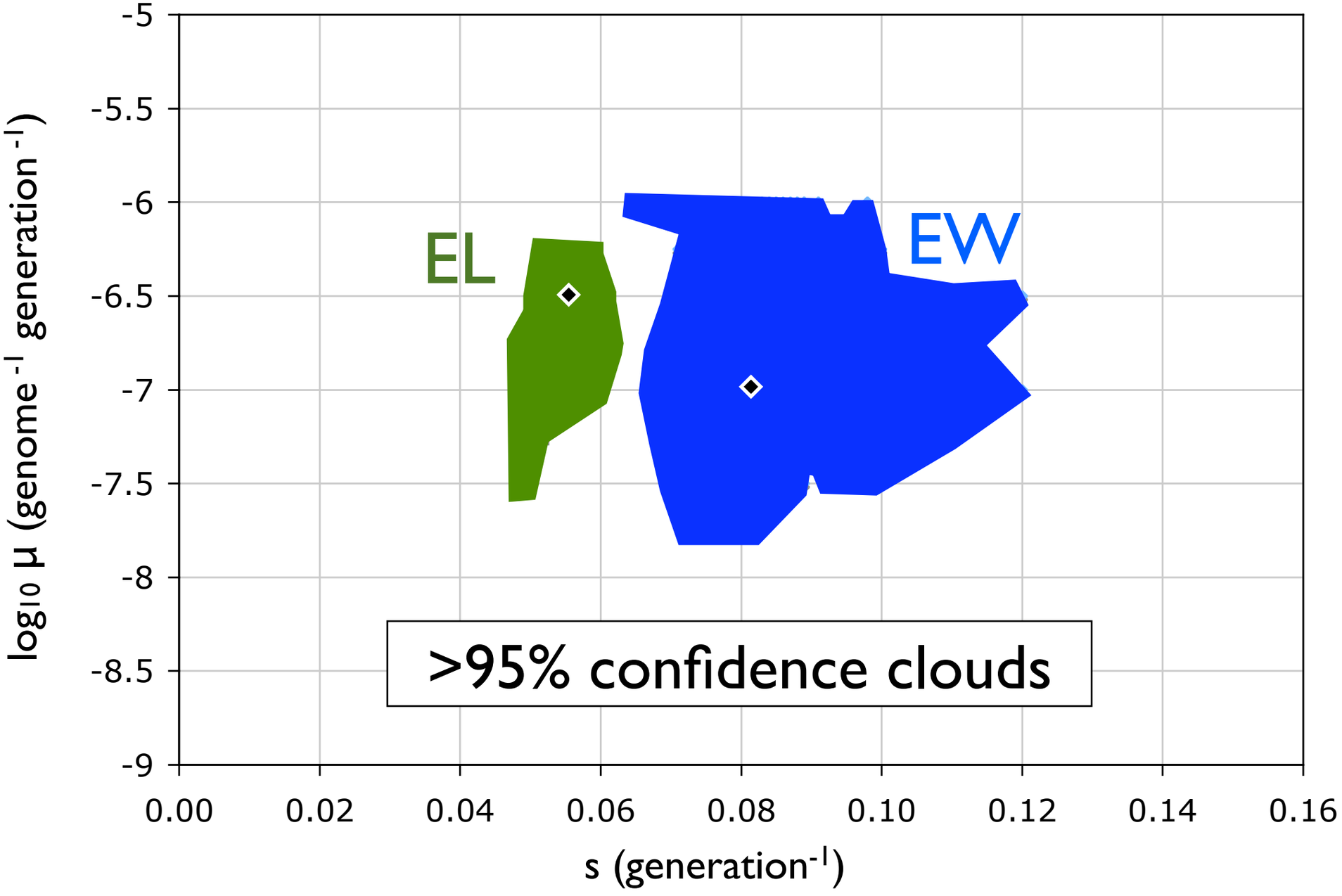
Population Genetics Simulations





ML Effective Parameters: $s = 0.081$ gen^{-1}, $\mu = 10^{-7}$ genome^{-1} gen^{-1}
average fitness effect and rate of beneficial mutations

A Difference in Evolvability



EW clones also achieve a higher final fitness than EL after 883 generations, despite initial fitness disadvantage.

Why a Difference in Evolvability?

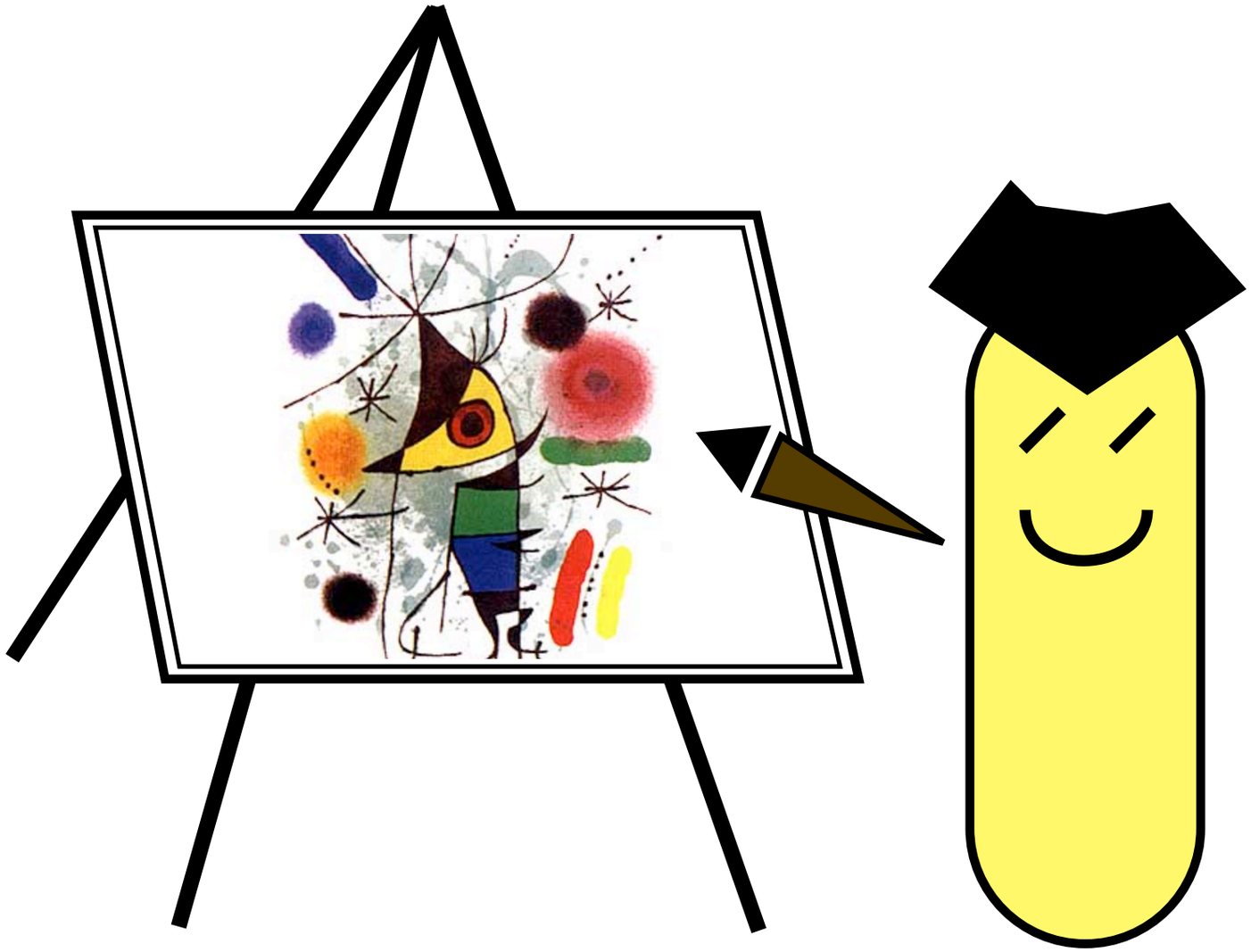
1. The beneficial mutation in *topA* restricts further adaptation less than alternatives.

2. In the genetic background of the *topA* regulatory mutation, other mutations are more beneficial.

- Next mutation is in *spoT* (another global regulator) in long-term experiment. Is this also the case in marker divergence experiments?

- Gene and genome sequencing ongoing...

Is it possible to encourage more creative bacteria?



<u>Stereotypes applied to bacteria</u>–

- Drugs that remove barriers to creativity?
- Sick mutants that would be rare in nature?

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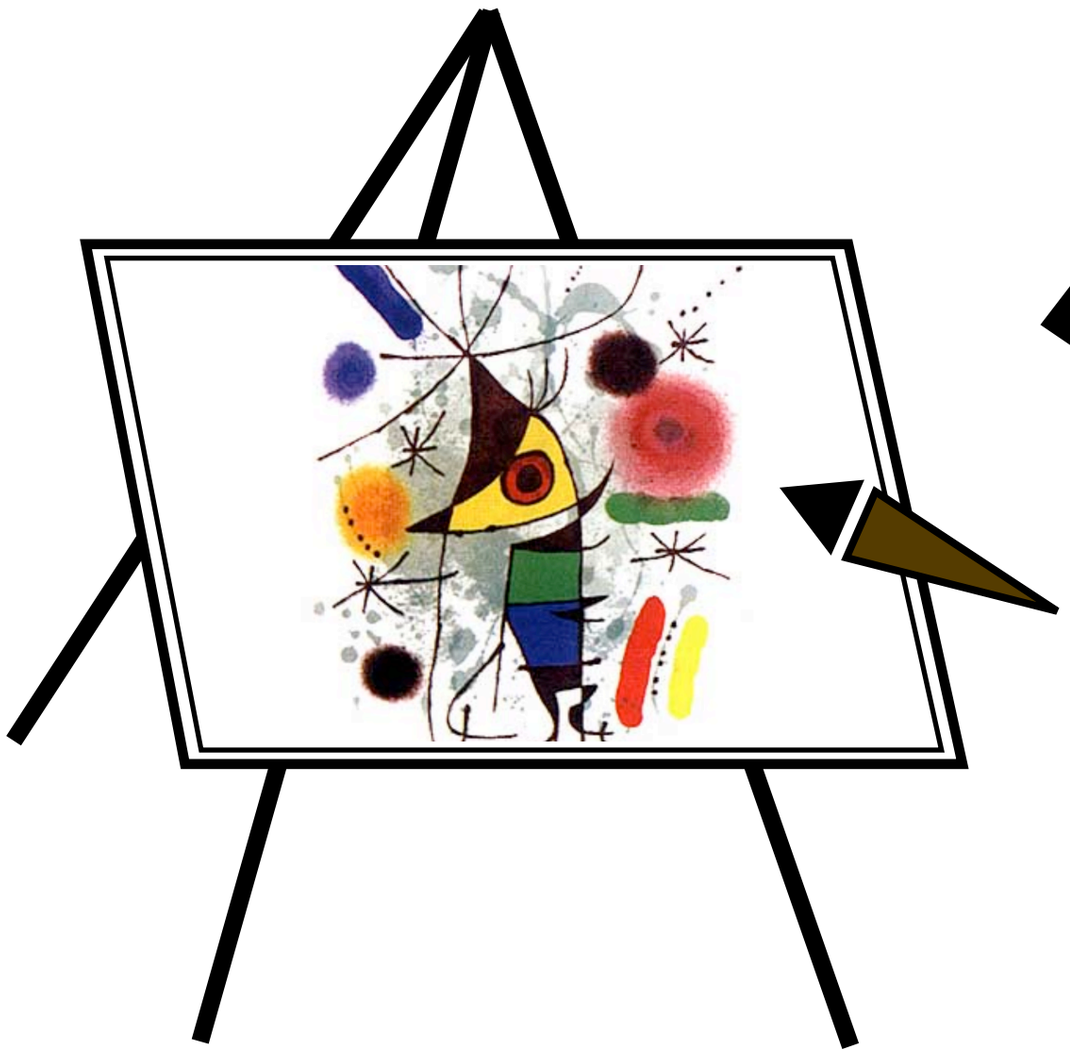

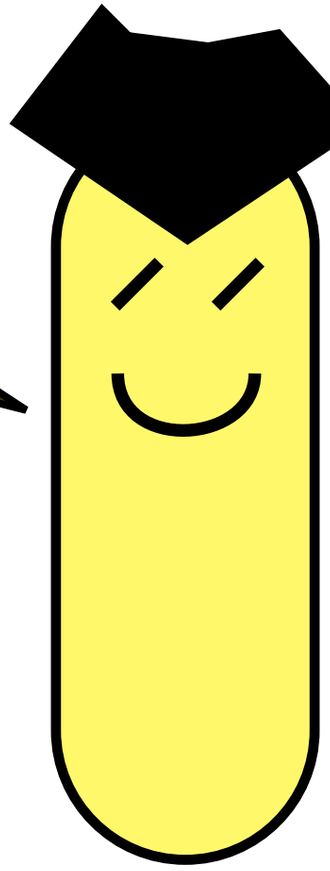

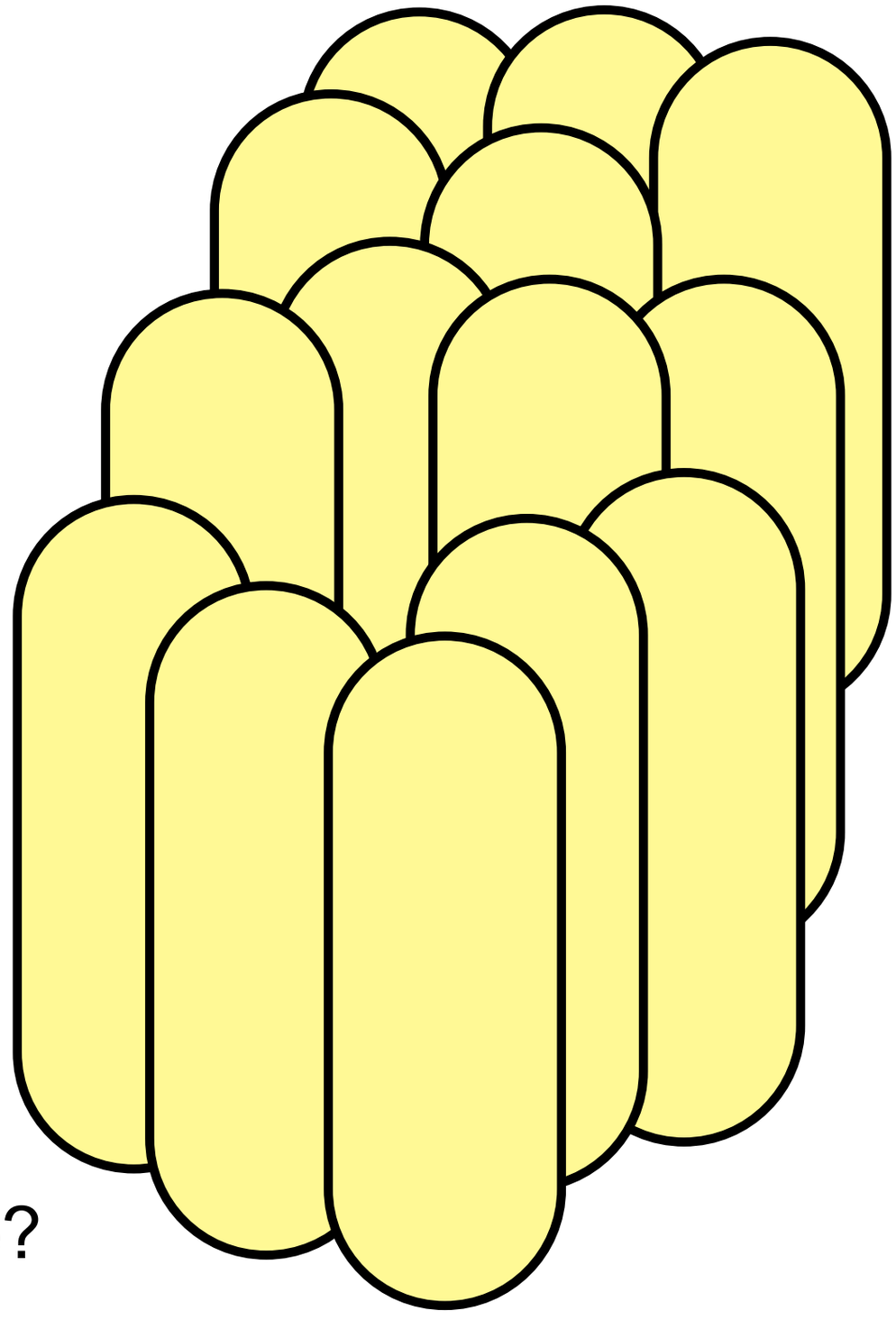

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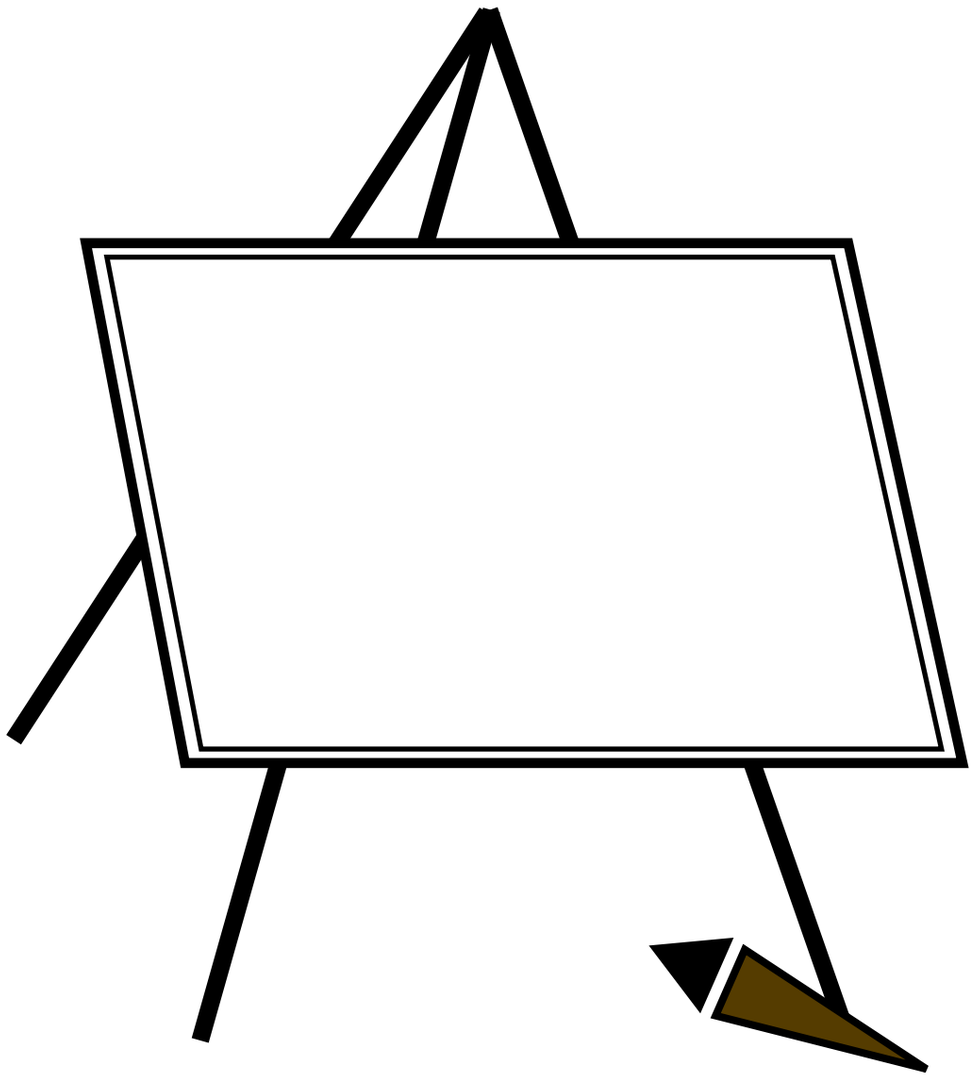

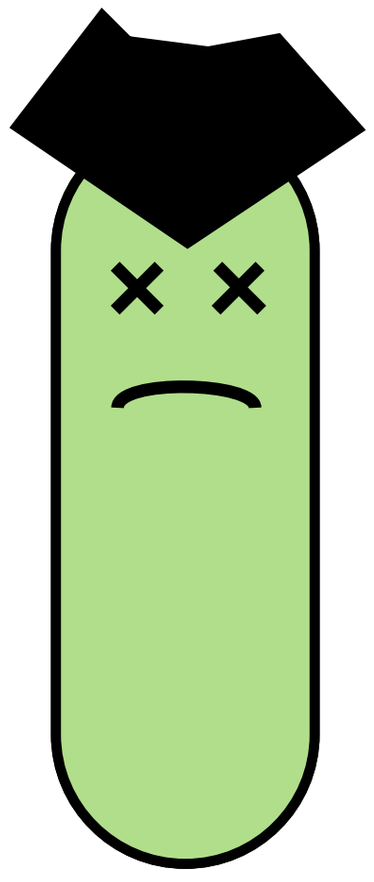

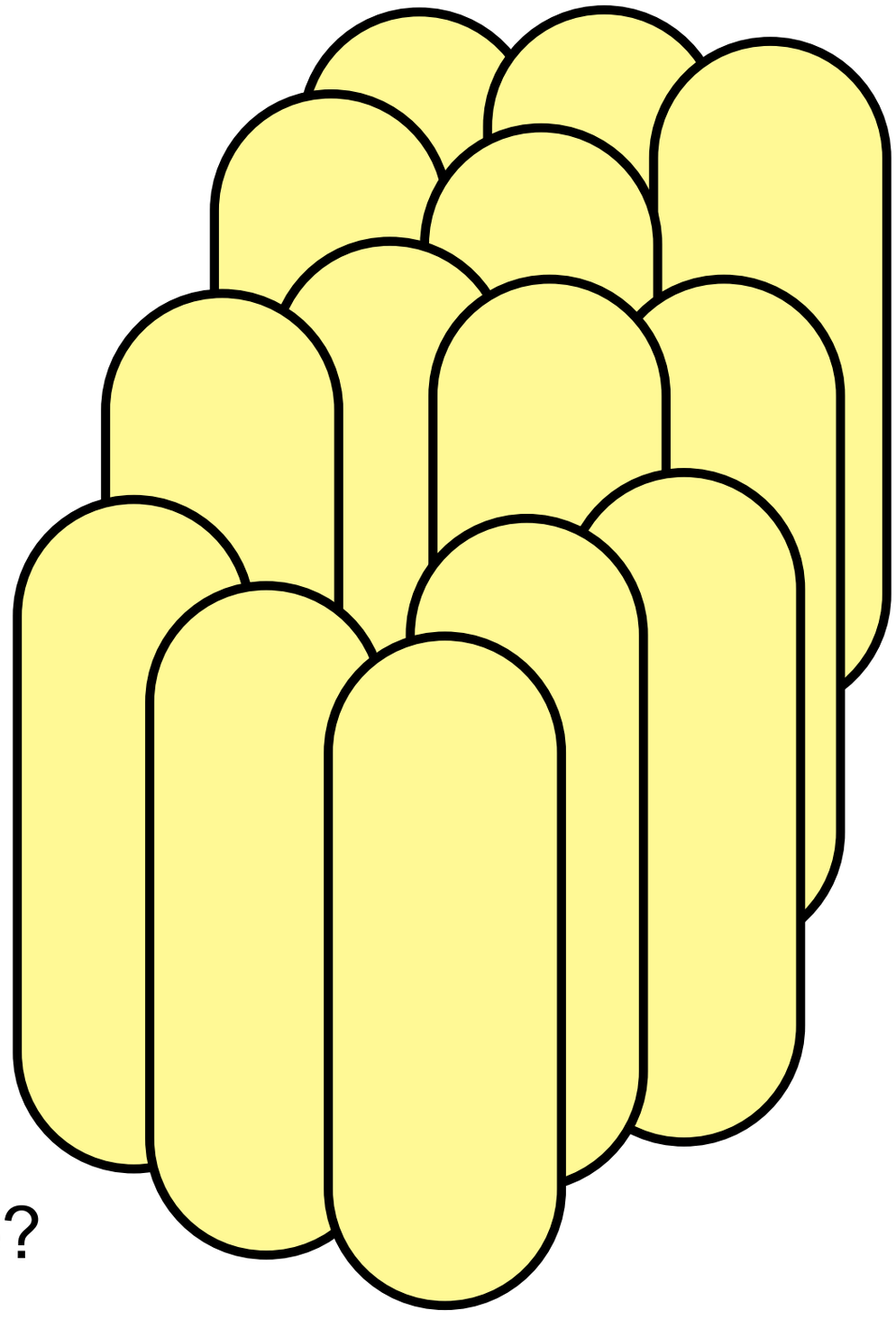

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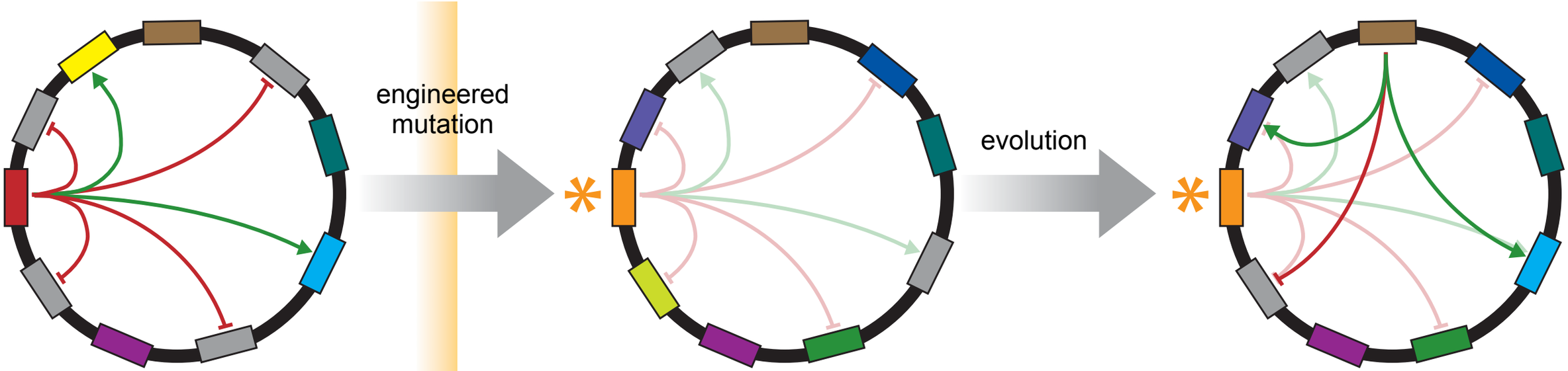
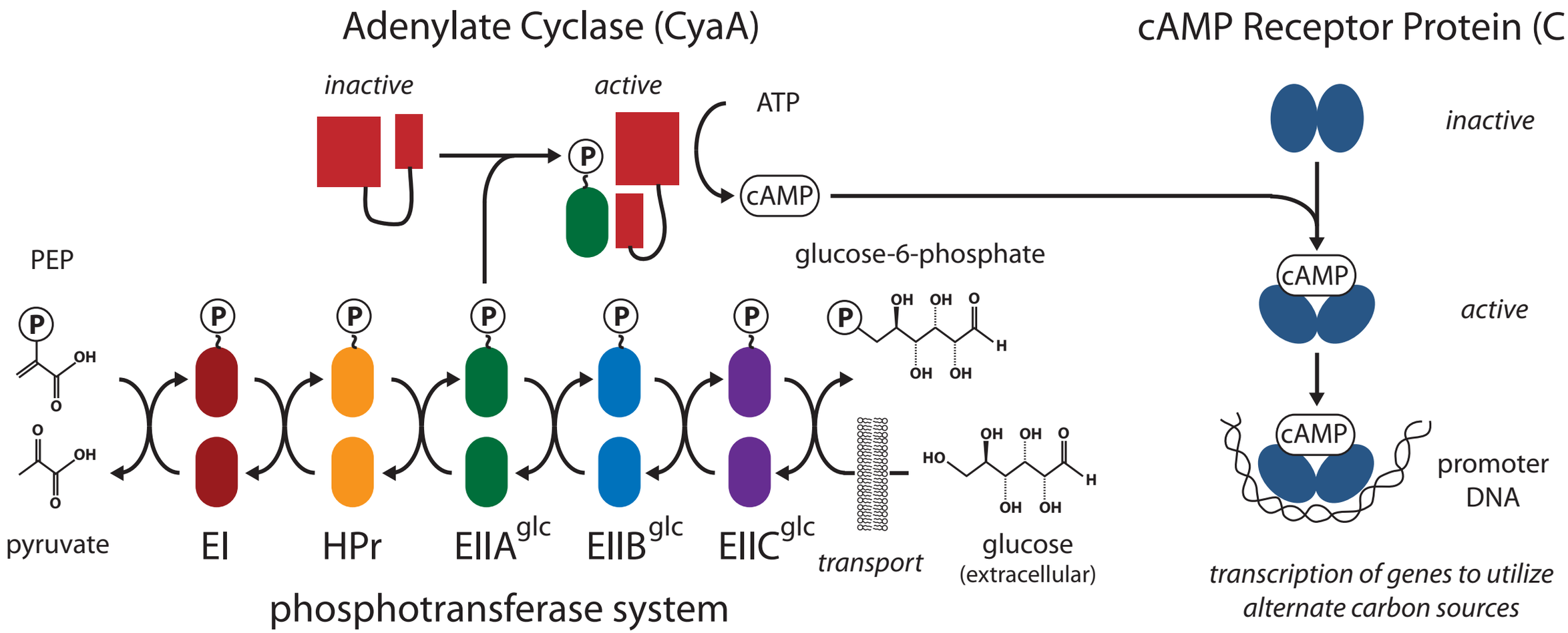
Regulation, Evolvability, Modularity



- Decanalize existing predictive patterns.
- Better to **rebuild** than to **rewire**?
- Remove hubs or specific connections?
- Changing or constant environment?

Carbon Catabolite Repression

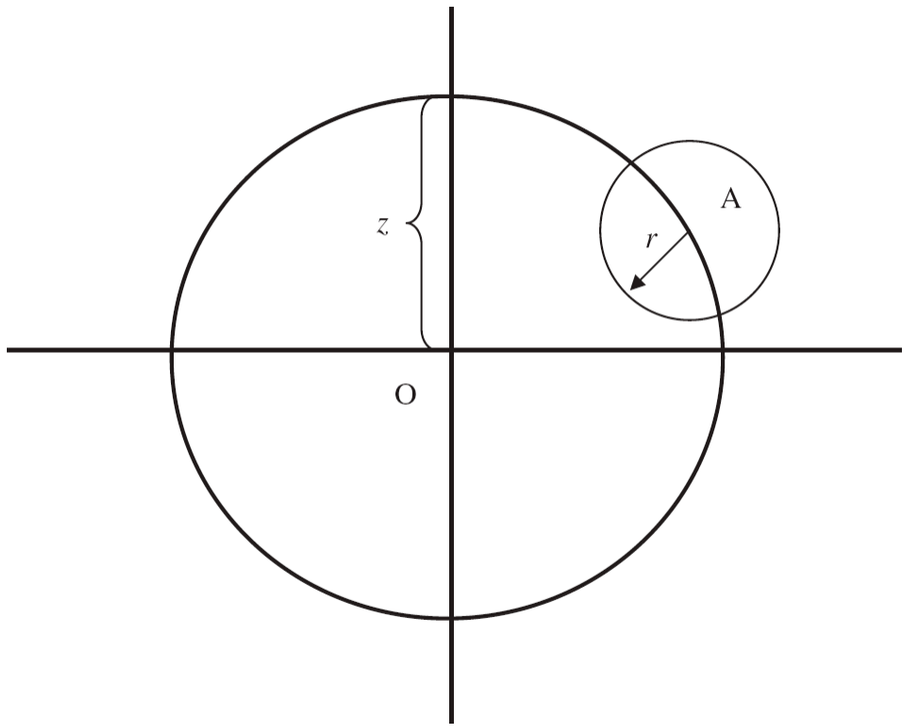


When glucose is present, **do not activate** genes for utilizing other carbon compounds.

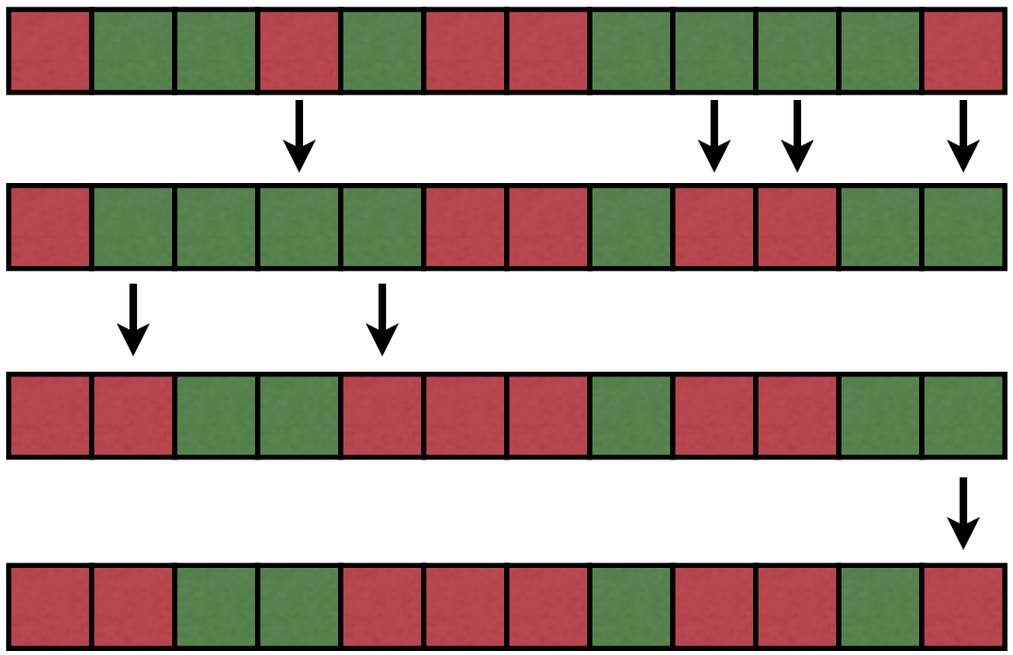
What is the null model?

Complication: Fitness effects of engineered mutations.

Fisher's Geometric Model



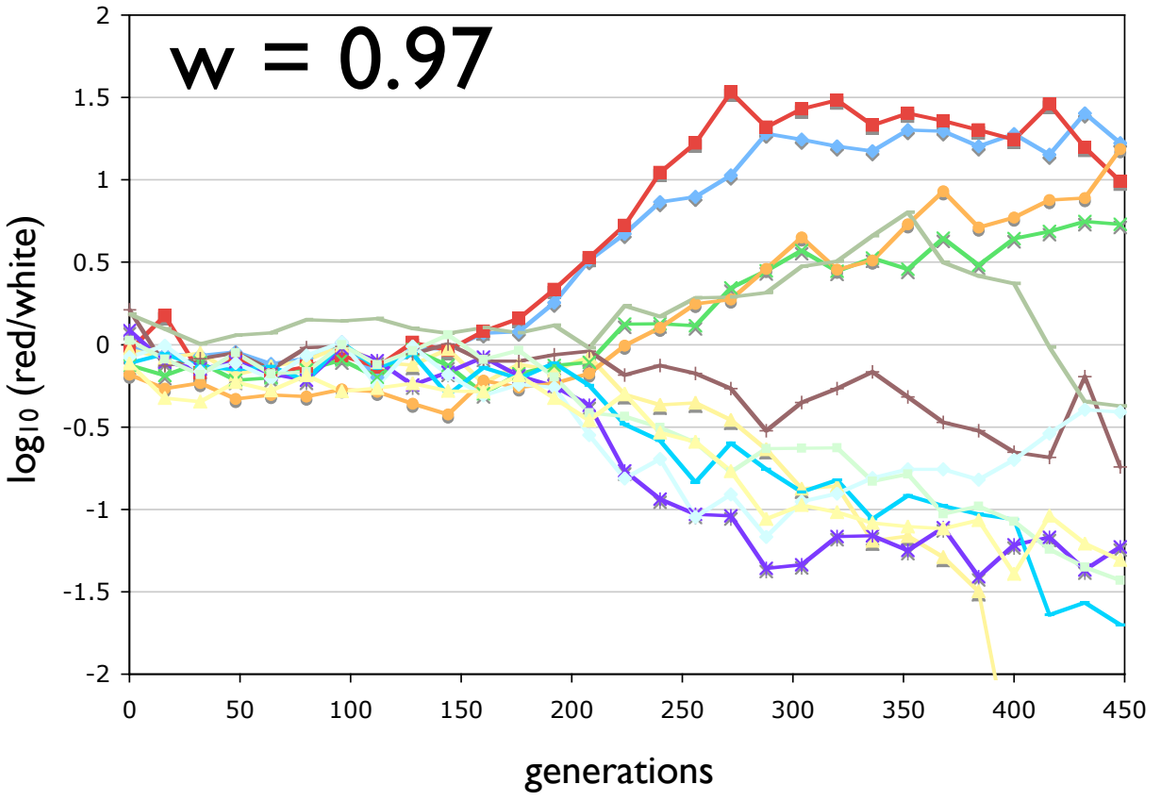
Orr, H.A. (2006) The distribution of fitness effects among beneficial mutations in Fisher's geometric model of adaptation. *J Theor Biol* **238**, 279-285.

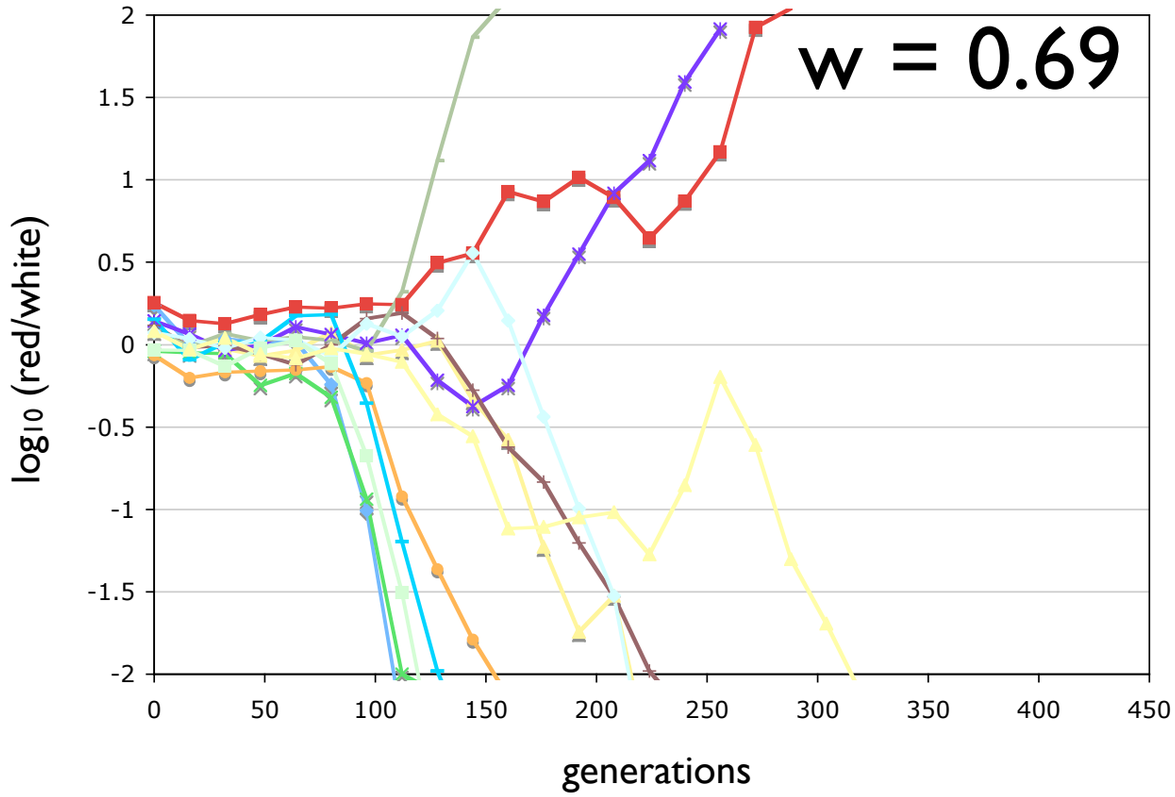


Are there better models, for (re)optimization of gene regulatory networks?

Rif^{R} Marker Divergence Series

Rifampicin resistance: Mutations in RNA polymerase that perturb global gene expression.





- Series of 10 initial strains with known mutations and fitnesses from 0.68 to 0.99 (relative to Rif^{S} ancestor).
- General expectation for change in effective evolutionary parameters for a given initial fitness defect?

Mark Kauth

Future Directions

- Assumption of rsT << 1 violated in population genetics simulations. Use stochastic simulations?
- Extract other information from MD curves?
- Reconstruct the order and tempo of mutations in long-term *E. coli* lines to search for other mutations that may have increased evolvability.
- Screen sequence-tagged libraries for mutations that increase evolvability in many environments. (Highly parallel marker divergence experiments.)