

Adaptive diversification of *Escherichia coli* in culture

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Introduction

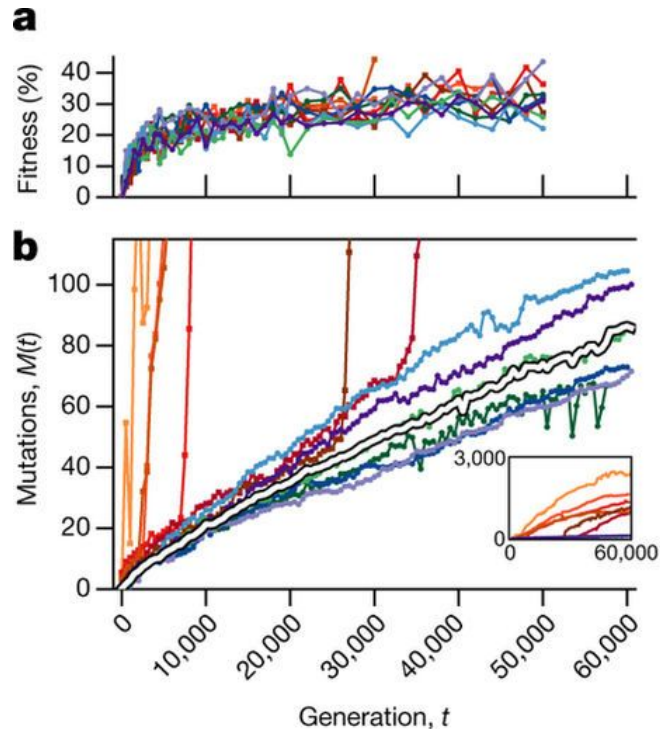
- LTEE
- Population dynamics
- Ara-2
- evoFBA model
- Evo²Sim
- Adaptive diversification
 - Clonal interference
 - Negative frequency-dependent interactions
 - Genetic background
 - Seasonality
- Conclusion

Long term evolution experiment (LTEE)

- Started in 1988 by Richard Lenski
- 12 cultures
 - *E. coli*
- Periodic environment
 - 24 hour cycle (6,6 new generations per day)
 - Batch culture
- 1% is transferred to a new medium after every cycle
- 99% is frozen and stored for future research capabilities
- ~66.000 generations in November 2016

Population dynamics

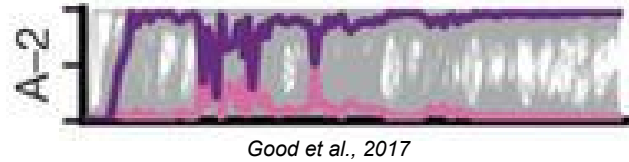
- Fitness increase
 - rate of increase declines
- 6 Non-mutators
 - Mutation speed comparable to bacteria
- 6 Mutators
 - Some become non mutators after evolution



Good et al., 2017

Ara-2

- Mutator culture
- Emergence of 2 lineages L and S
 - ~6500 generations
 - A large glucose-feeding population
 - A small glucose and acetate-feeding population
- Acetate is a byproduct of glucose usage



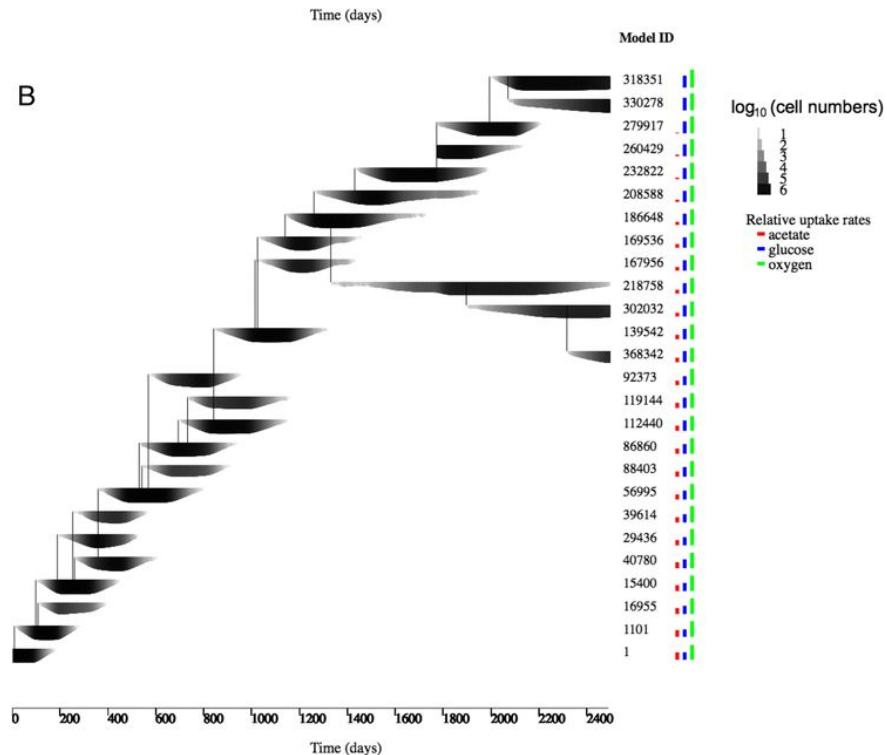
evoFBA, to model adaptive diversification

(Großkopf et al., 2016)

- FBA (as discussed in course)
 - Flow of metabolic pathway is calculated according to specific flux formulas and reactions
- 15 mutable targets
 - Evolution of uptake and reaction
 - Constraint on optimal uptake, forced trade-off
- Mutation rate: 10^{-6} per model cell per generation
- Similar conditions to LTEE

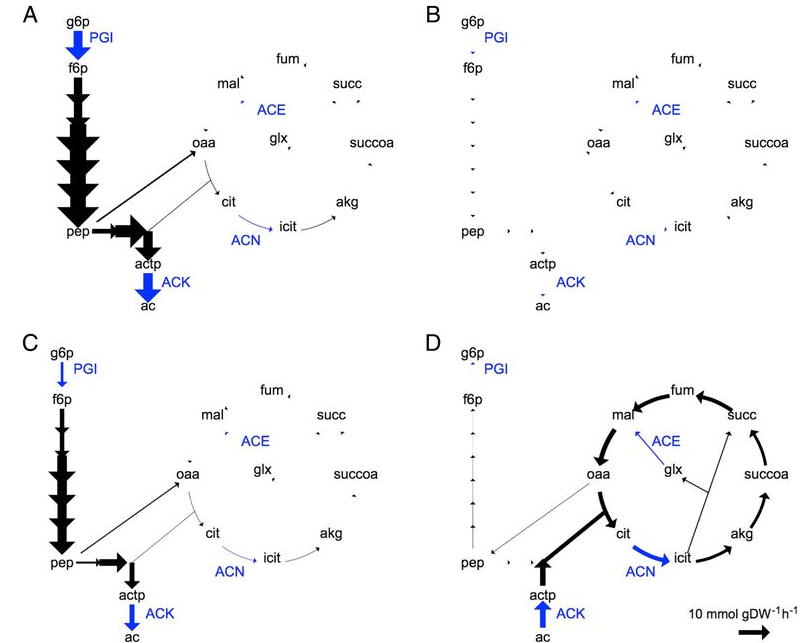
evoFBA, to model adaptive diversification

Divergence of the clones at ~1400 timesteps, which ultimately led to adaptive diversification.



evoFBA, to model adaptive diversification

- Two metabolic clones evolved
- Evolved metabolic pathways
 - A = glucose metabolism in glucose environment
 - B = glucose metabolism in acetate environment
 - C = acetate metabolism in glucose environment
 - D = acetate metabolism in acetate environment
- Clear difference in reaction between the clones in the two circumstances



Großkopf et al., 2016

Adaptive diversification

Diversification is comparable to the *in vitro* ara-2 situation

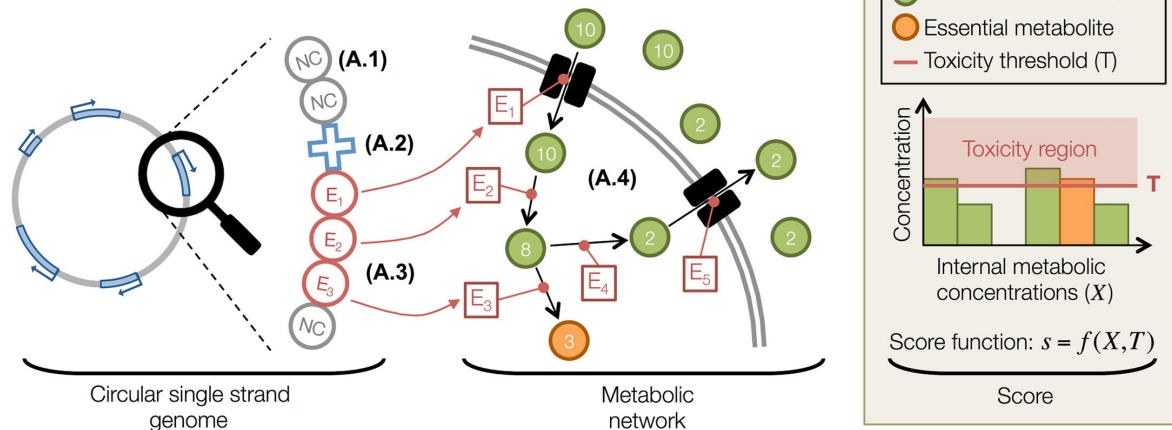
Furthermore, experimental results show that L and S population dynamics are similar to the dynamics of these *in silico* models, when exposed to a changing environment.

evoFBA issues

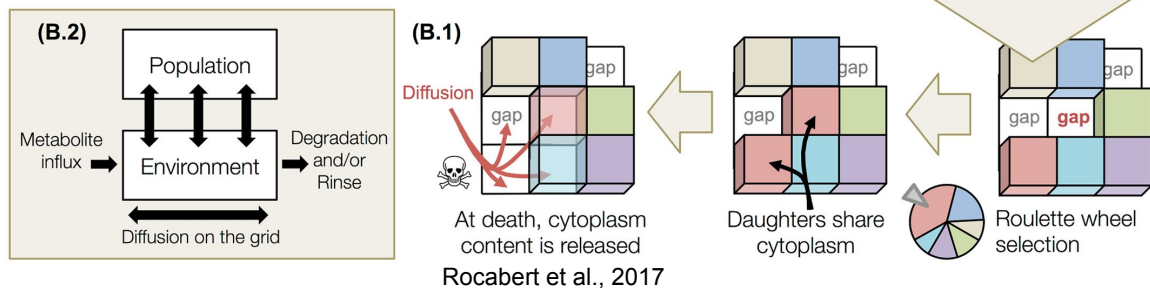
- They define an overall constraint on uptake rates to enforce trade-off
 - Decreases the degrees of freedom
 - Possible different solution is adding toxicity to the metabolic system
- Cross-feeding results are always stable, as opposed to LTEE
 - Explanation given is that stability might still happen in the LTEE
- Not enough evolvability of the model

Evo²Sim

(A) Genotype-to-phenotype mapping

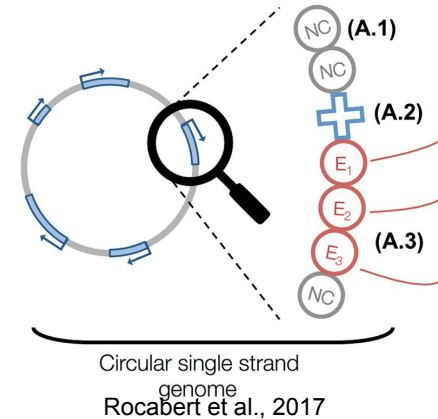


(B) Population-environment level



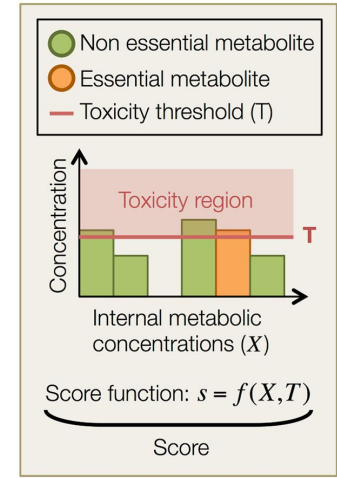
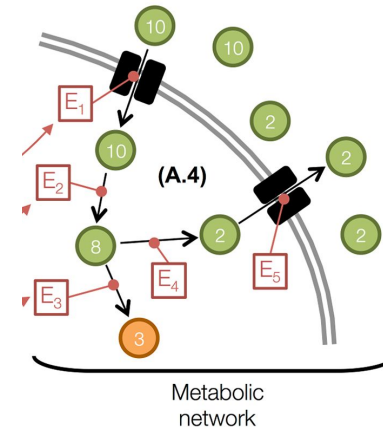
Genome structure

- Coarse-grained genome
 - Protein coding (E), Non-coding (NC), Promoter(+)
- Functional regions
 - Promoter followed by one or multiple E's
- Mutations
 - Enzymatic kinetics
 - Unit type change (10^{-3} per unit per replication)
 - Rearrangements



Metabolic network

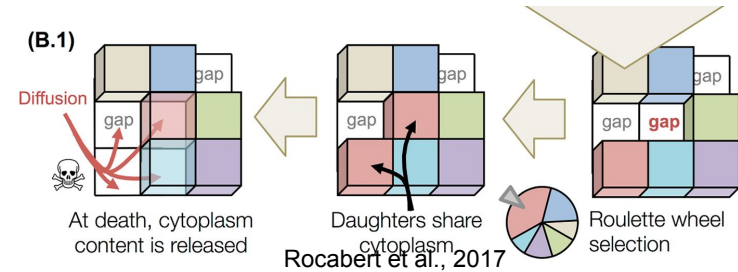
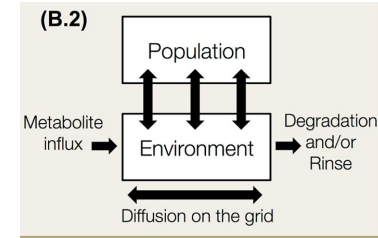
- Pumps and enzymes
- Essential metabolites
 - Define 'score' of cells
- Lethal toxicity threshold



Rocabert et al., 2017

Environment

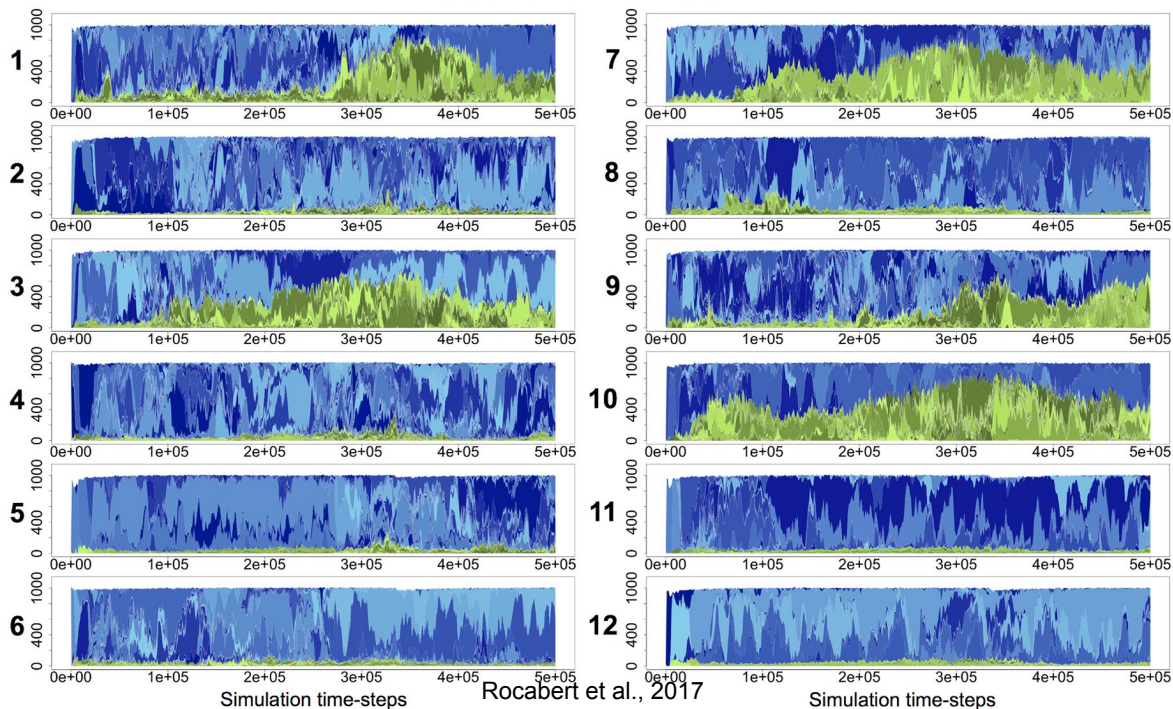
- Batch culture or chemostat
- Diffusing metabolites
 - Degradation rate
- Uptake and release by cells
 - Metabolites are released at death
- Cells can only divide once per timestep



Evo²Sim

In silico evolution of batch culture can lead to stable coexistence of 2 cross-feeding populations

(A) Periodic environment



Adaptive diversification

The evolution of different types of organisms under the effects of evolutionary forces such as selection and mutation

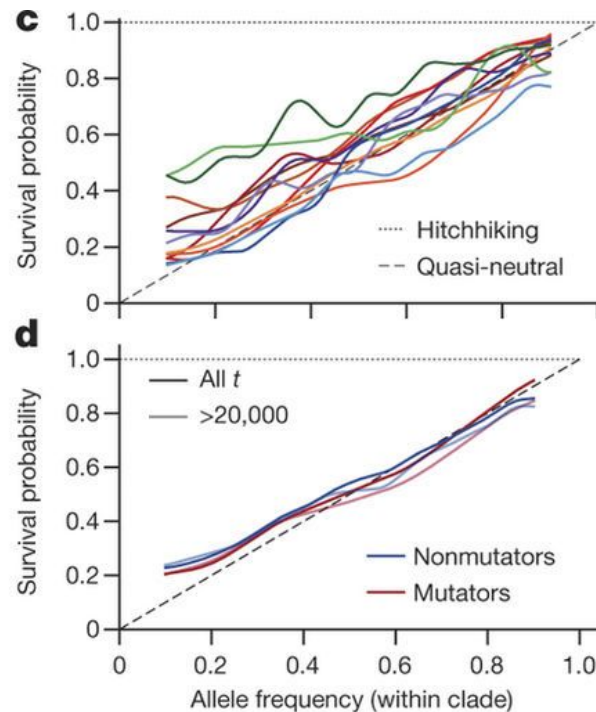
Possible influencing factors:

- Clonal interference
- Negative frequency-dependent interactions
- Genetic background
- Seasonality

Clonal interference

Beneficial mutation outcompeted by other, more beneficial mutations

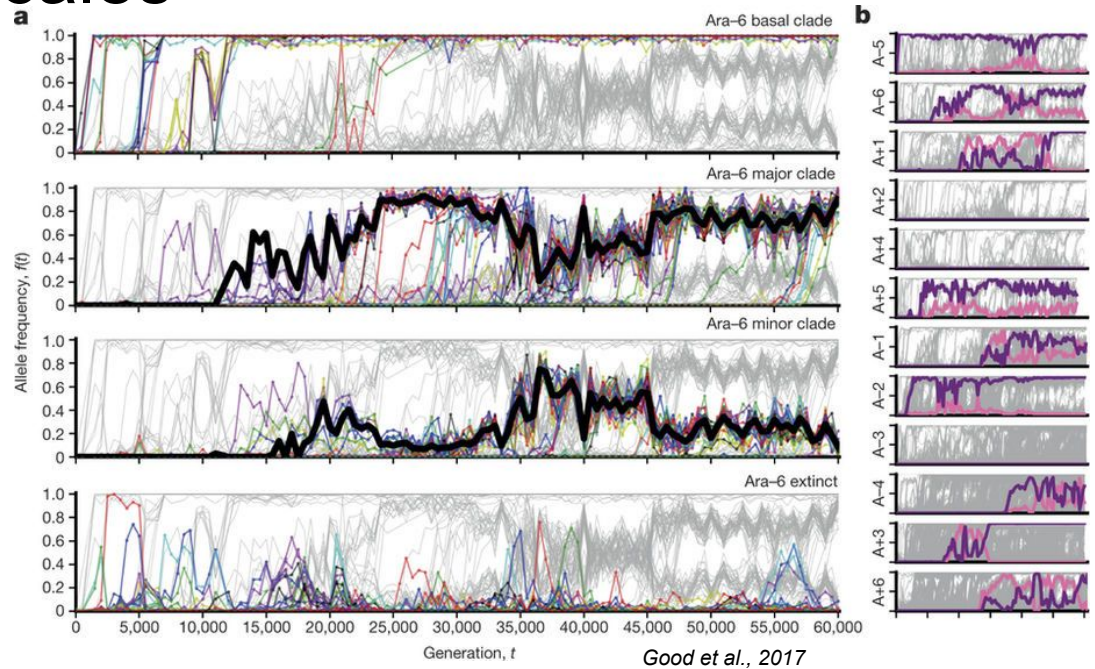
- Many mutants persist at intermediate frequencies
 - Often reversals in frequency or extinctions
- Fixation probability $\ll 100\%$
 - Consistent with strong clonal interference
- Quasi-neutrality
 - Implies that adaptation in LTEE is not mutation limited



Good et al., 2017

Separation of timescales

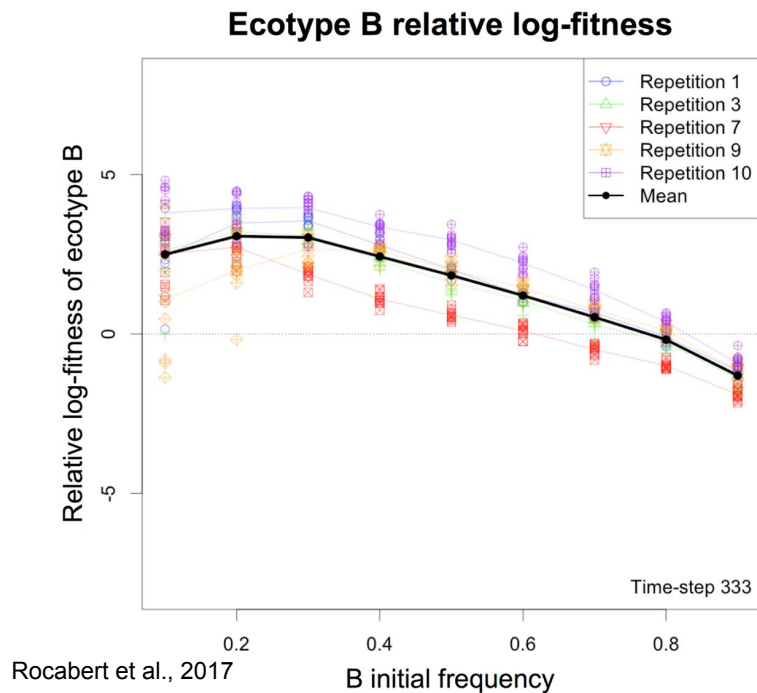
- Inter-clade fixations
- Intra-clade fixations



- Can not fully be explained with clonal interference
 - Eventually one cohort of mutations should fixate in the whole population

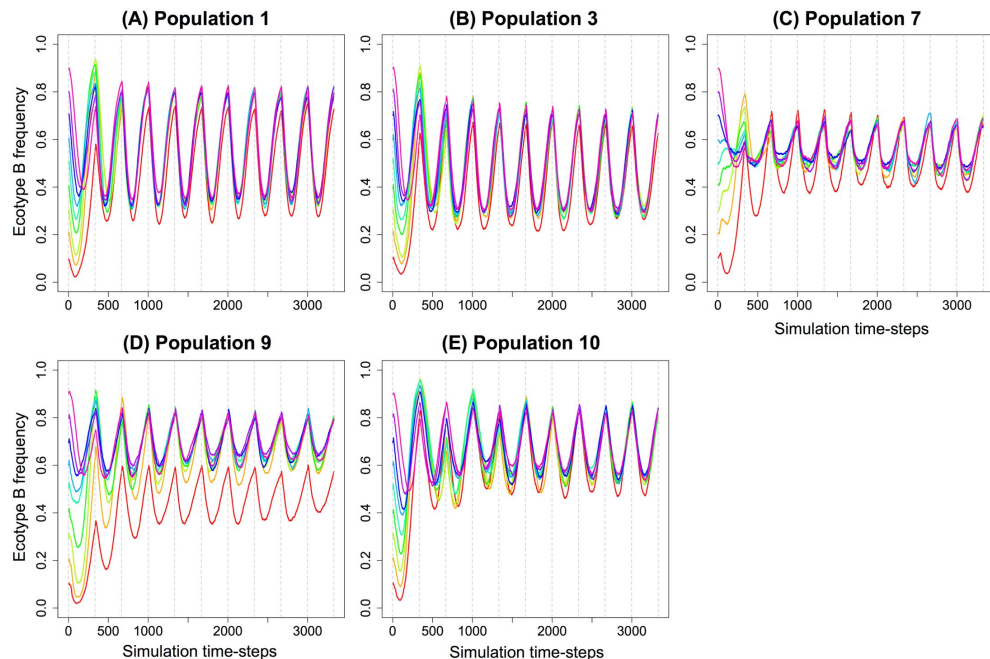
Negative frequency dependent interaction

- Short term competition experiment
- Acetate ecotype B favored when frequency low and penalized when abundant



Negative frequency dependent interaction

- Relative frequencies of A and B should stabilize over time
- Observed in the LTEE dynamics
- Influence of cross-feeding



Genetic background

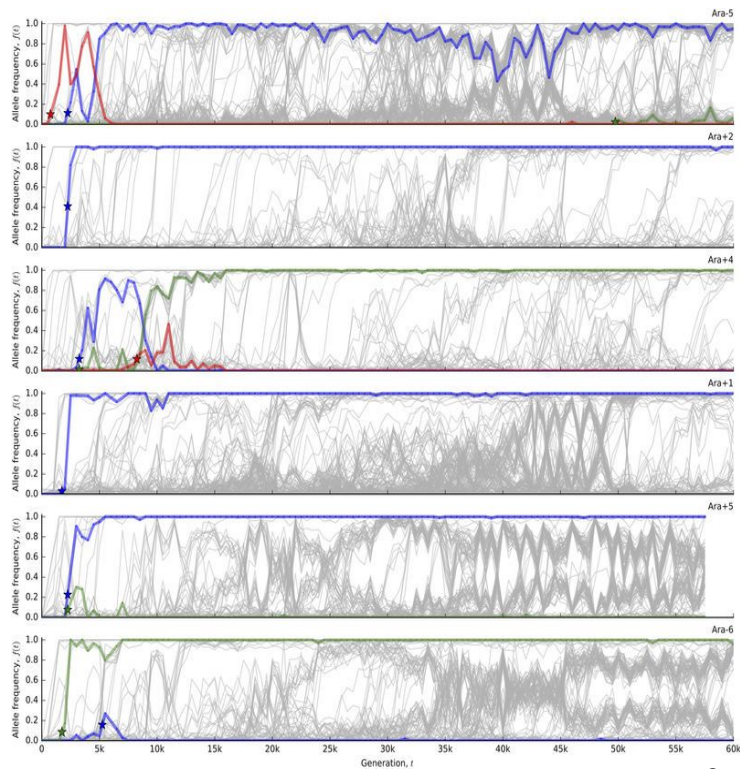
Steps in emergence of the S population:

1. Mutation in *spoT* increases overall fitness
2. Mutation in *acs* promotor and *arcA*
3. Increase in transcription of *acs*, *acnB* and *aceB*
 - a. Genes needed for acetate consumption
4. Mutation in *gntR* contributes to the ability of the S lineage to invade the L population via a negative frequency-dependent interaction

Importance of genetic background

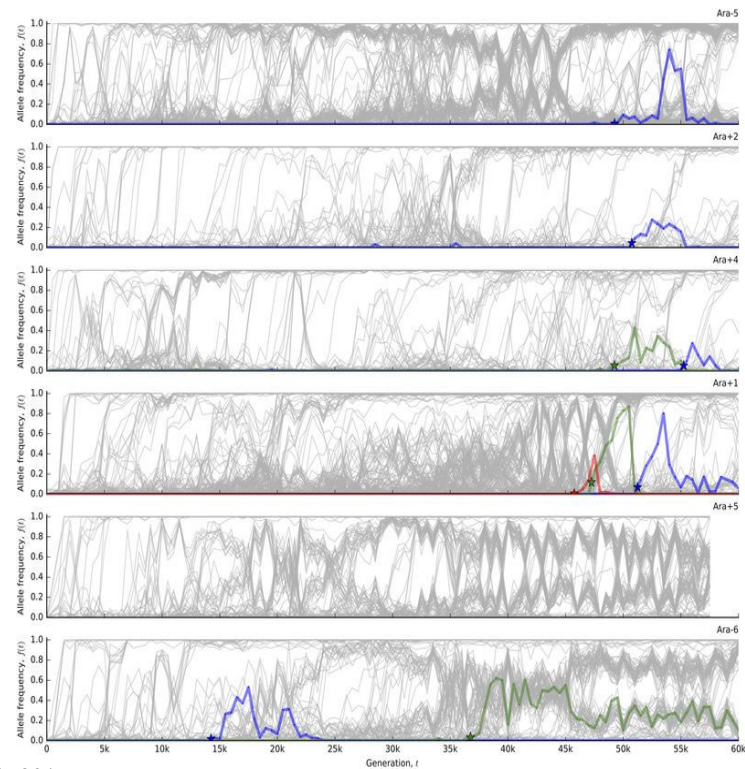
- The effect of introducing *arcA*^S was different in cells with a different genetic background
- The evolved *arcA*^S allele conferred S-specific traits only in the S-evolved background
 - Importance of epistatic interactions

- Occurrence of early and late evolving genes
 - Opening of new evolutionary paths by earlier mutations



Early occurring genes

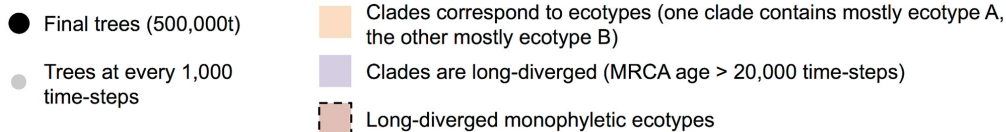
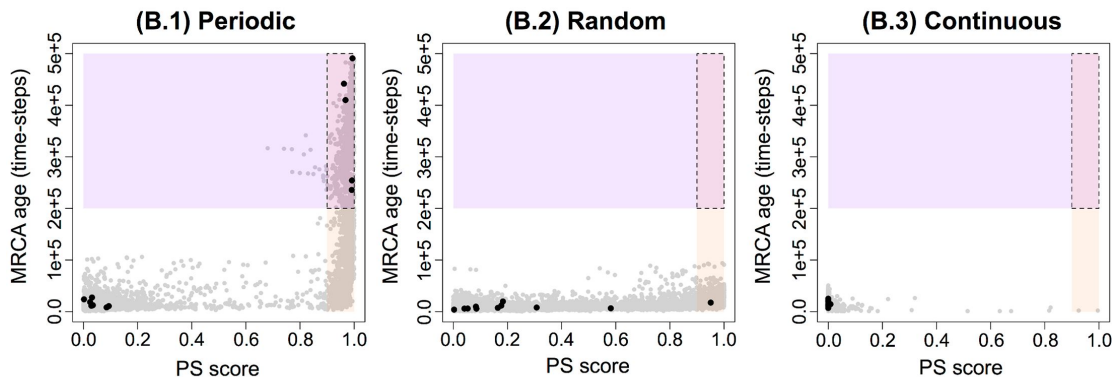
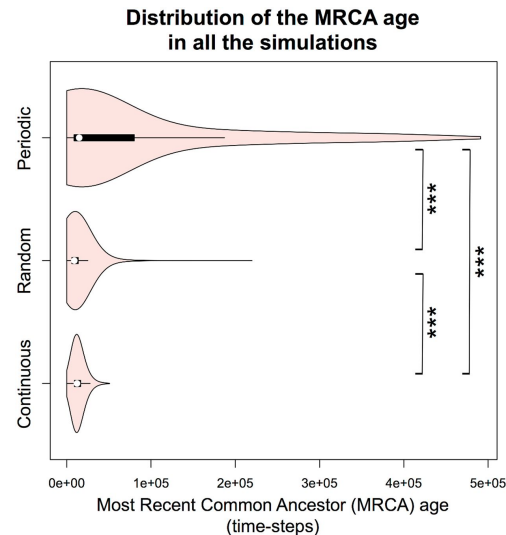
Good et al., 2017



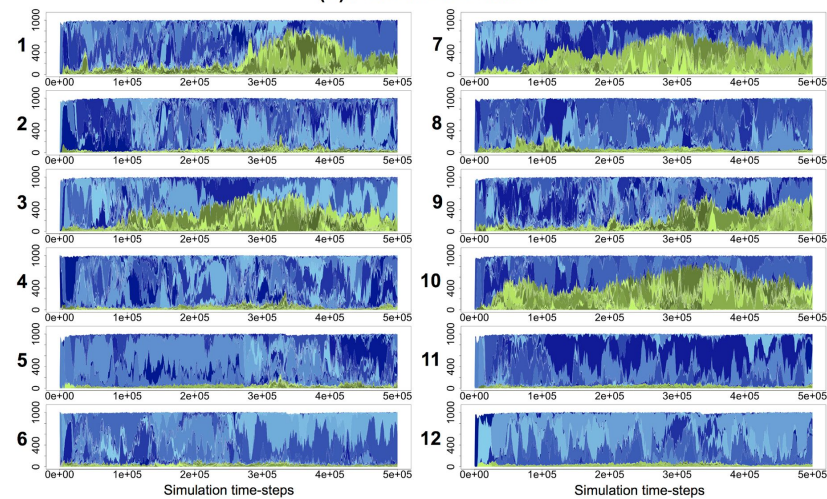
Late occurring genes

Seasonality

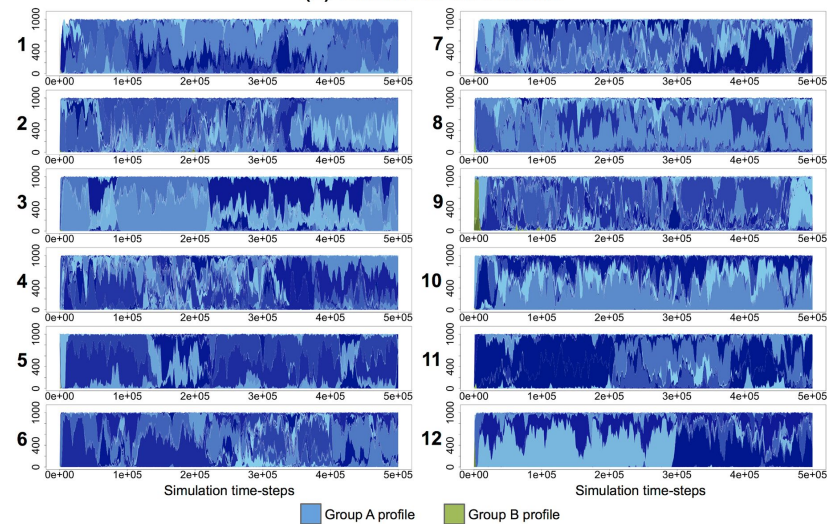
- Evo²Sim
- MRCA age reflects the stability of a polymorphism
- Deepest trees in periodic environment
- Long lived polymorphism in periodic environment



(A) Periodic environment

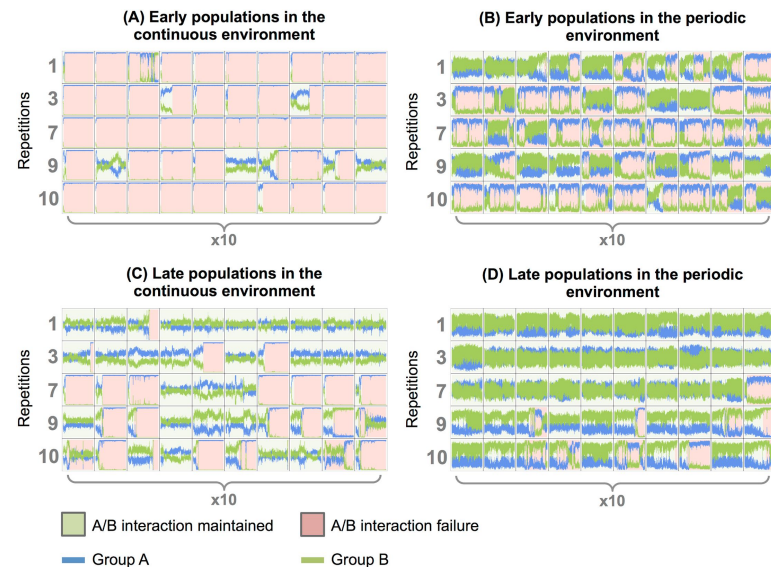


(B) Continuous environment



Seasonality

- Stability of interaction when placed in different environment
- Early populations not robust; 6% persists
- Later populations more robust; 50% persists



Conclusion

Negative frequency-dependent interactions, seasonality and genetic background are essential for adaptive diversification of *E. coli* in culture, leading to the emergence of stable coexistence

Clonal interference does not appear to be important for adaptive diversification. It could, however, be an explanation for the appearance of non-stable coexistence as observed in the LTEE

References

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