From so simple a beginning
Experimental evolution of diversity in *Pseudomonas* populations

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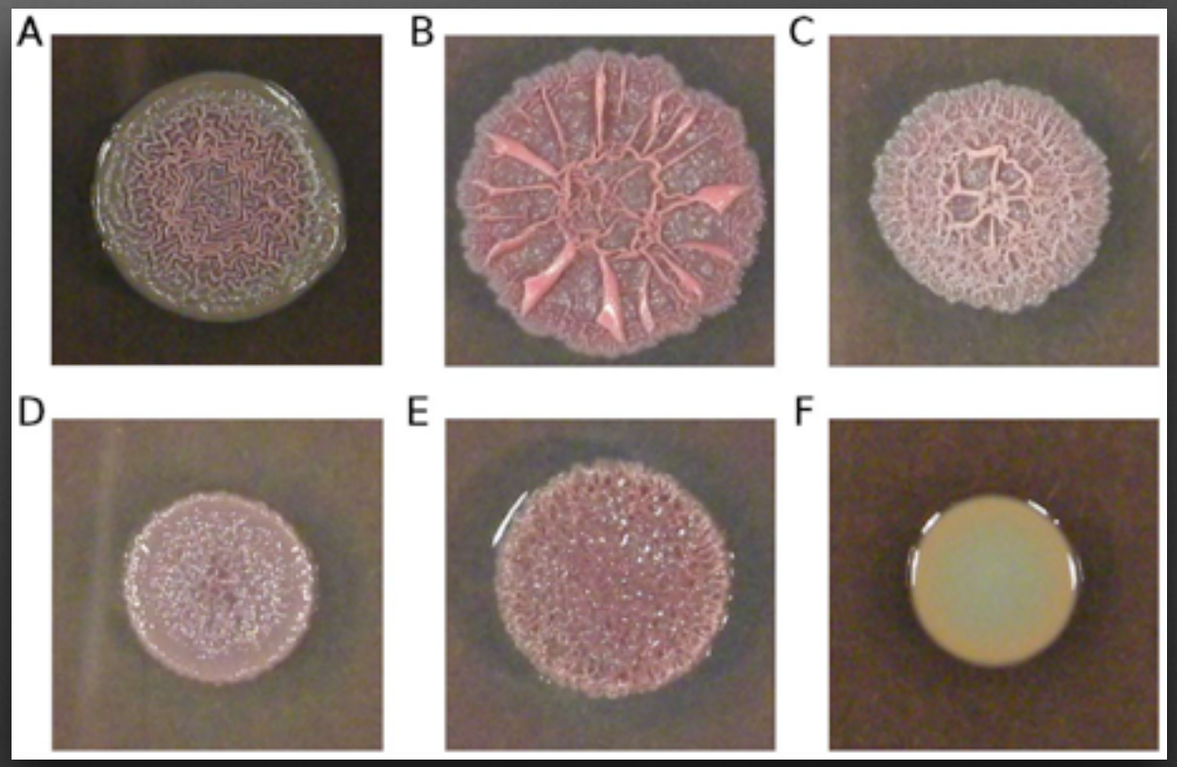
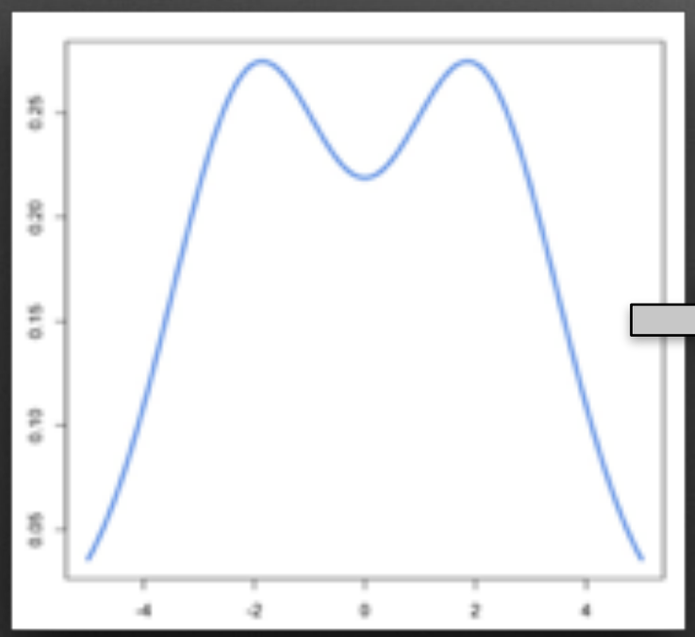
KITP ecoevo17, 23 Aug 2017

Natural communities (of microbes) can be highly diverse

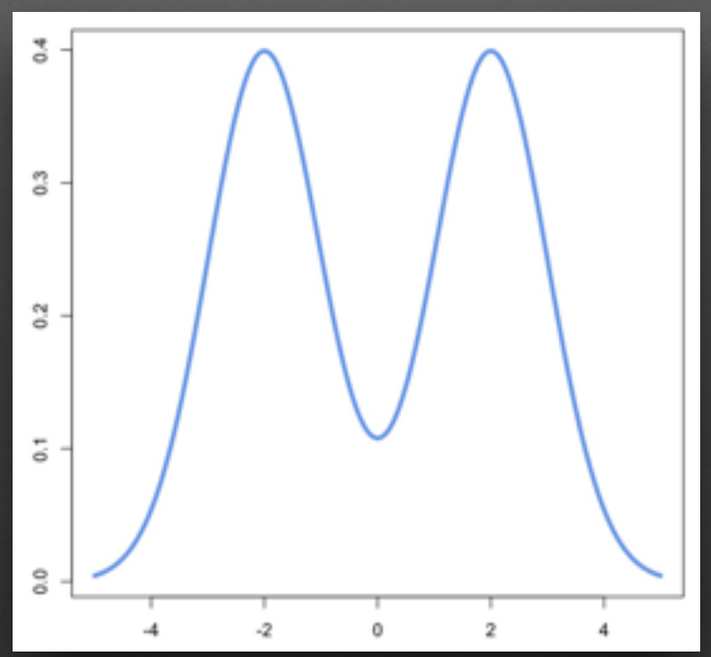


The ecological theory of diversification

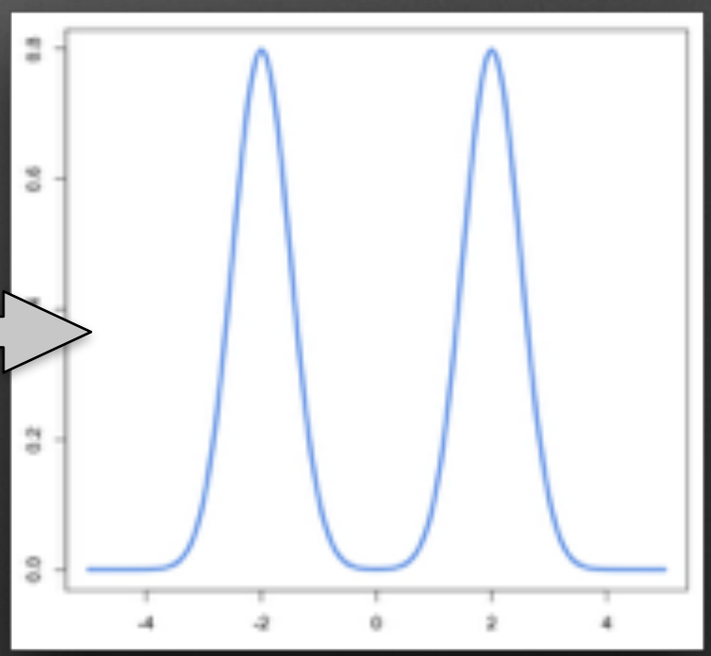
Ecological opportunity



Ecological interactions

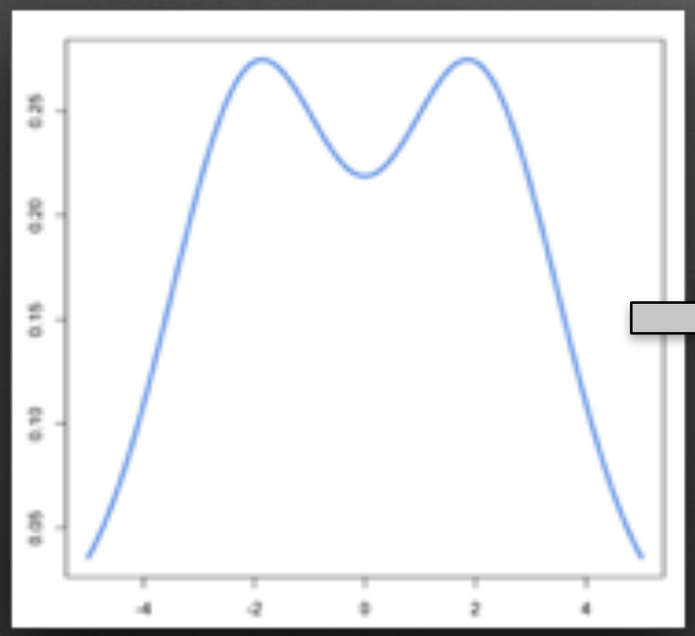


Niche specialization ...speciation

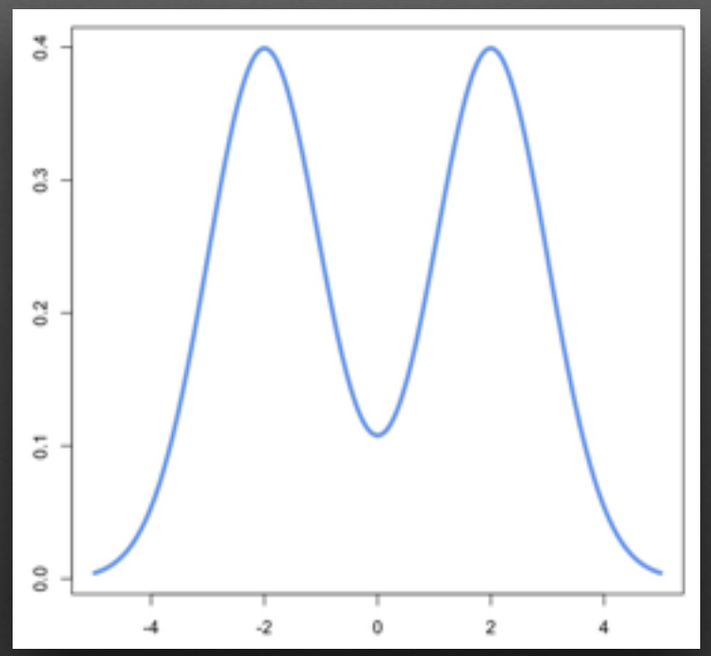


The ecological theory of diversification

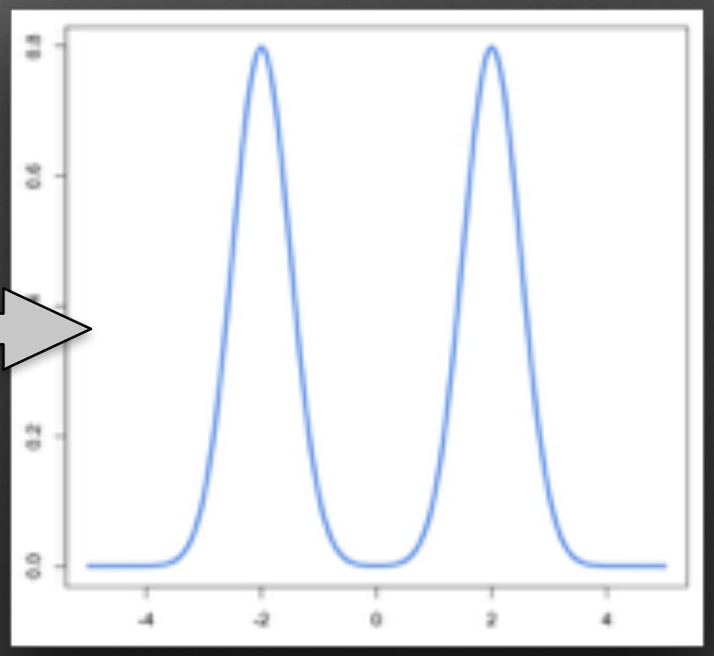
Ecological opportunity



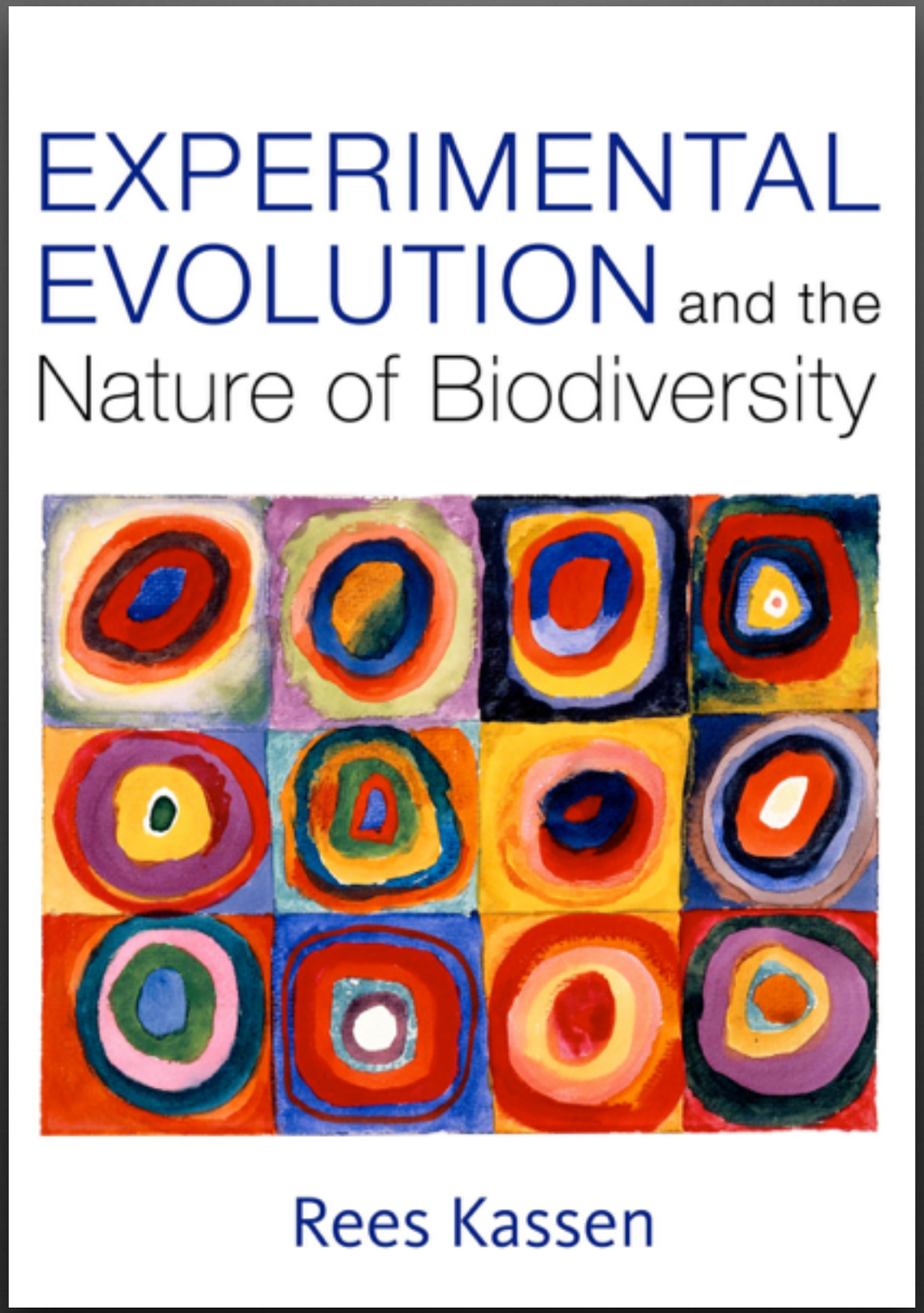
Ecological interactions



Ecological speciation



Whither genetics?



(2014) Roberts and Co., Publishers
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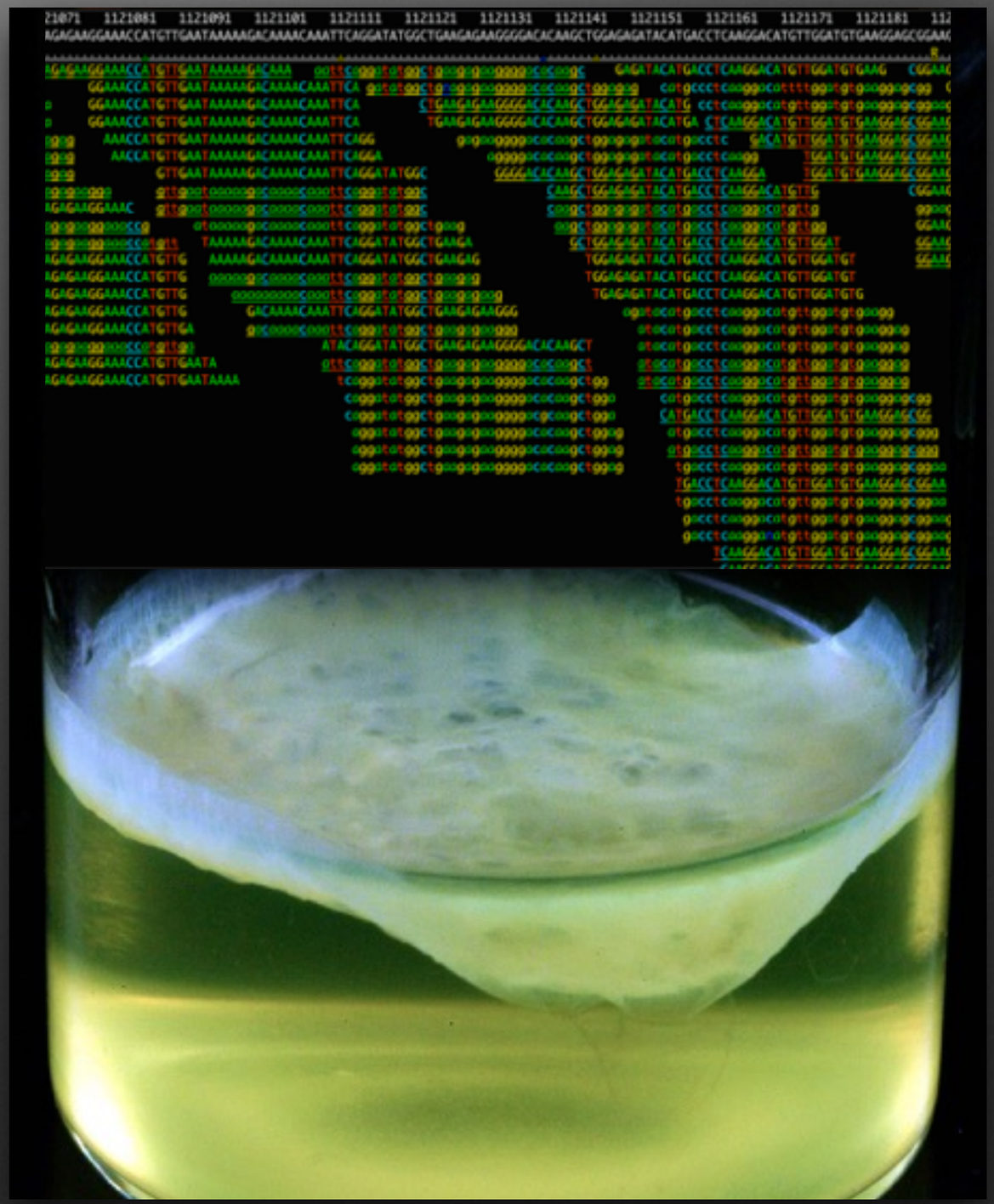
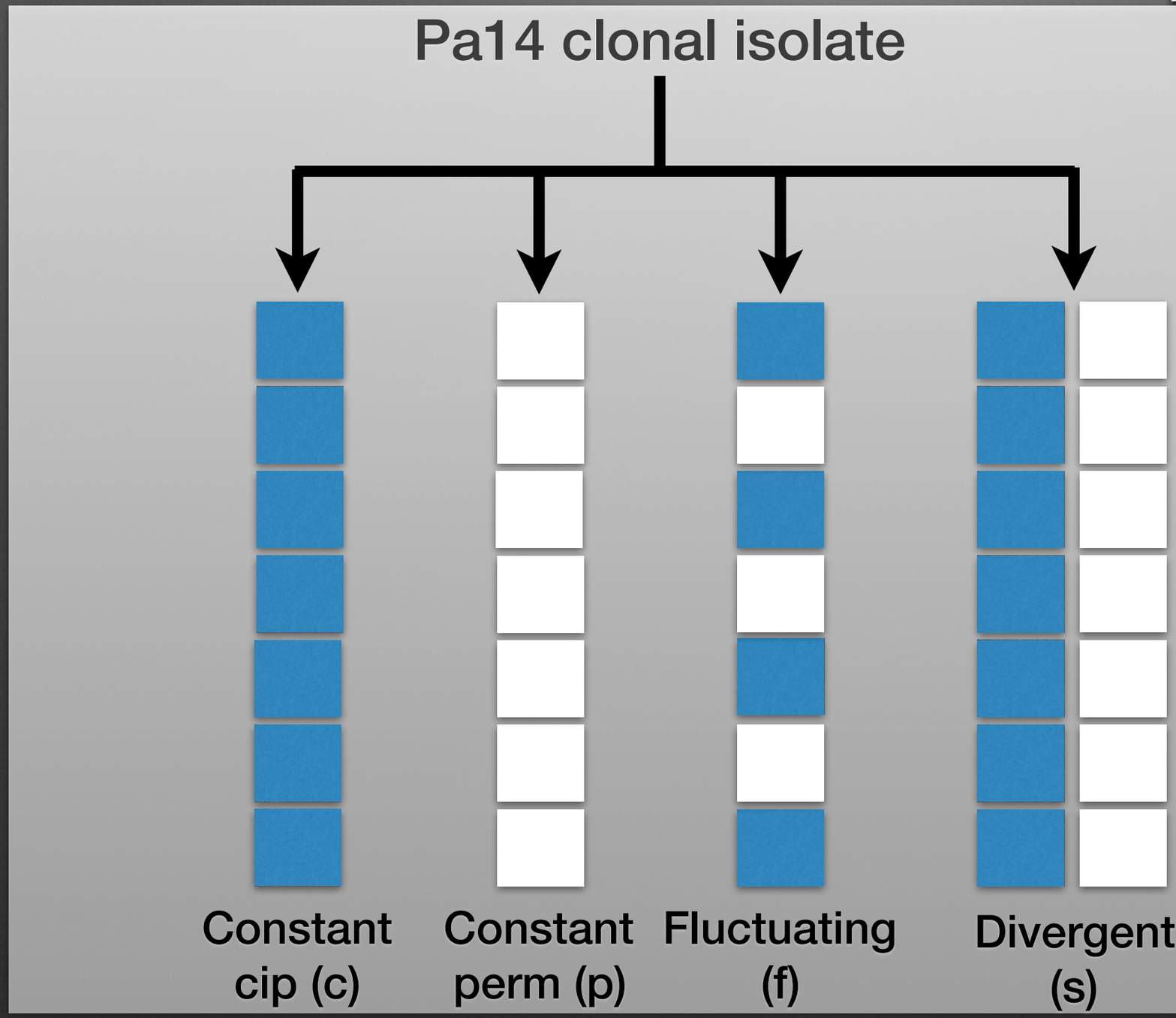
Towards a more general theory of diversification

- The emergence and fate of diversity under divergent selection (with dispersal)
- Ecological drivers of diversification in the CF airway
- The role of resource competition and the genomics of niche specialization

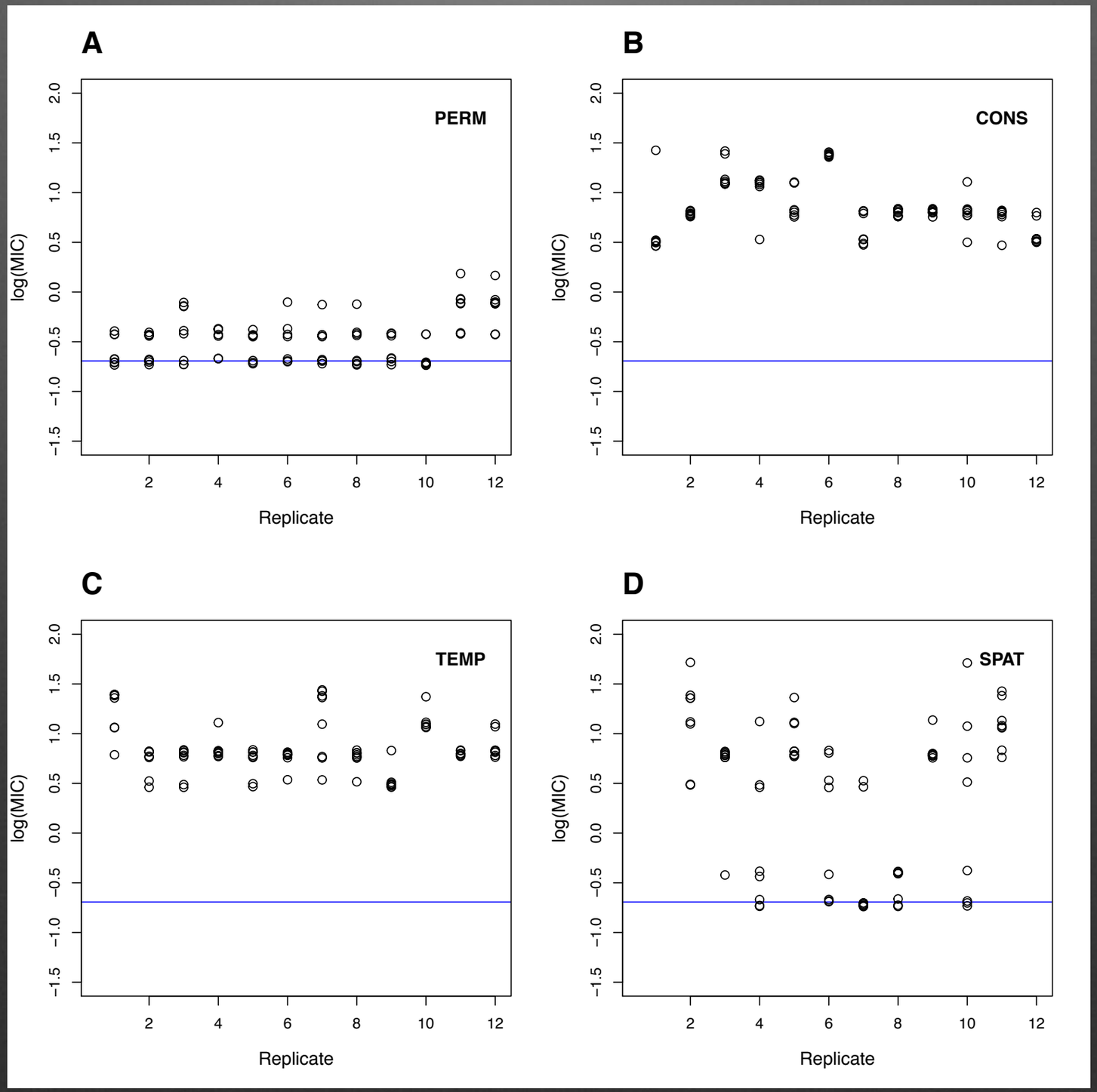


Diversification under divergent selection with dispersal

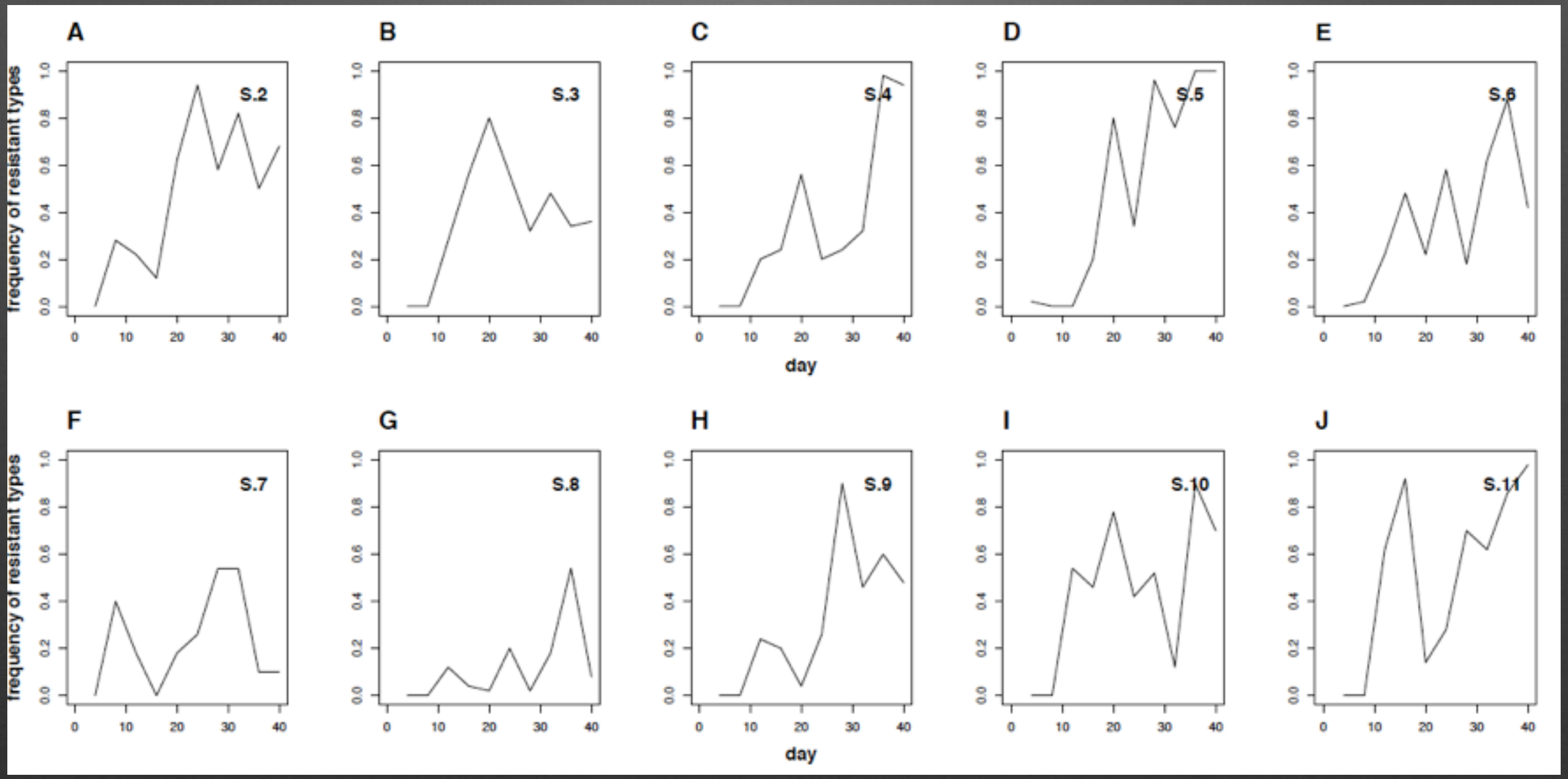


- *Pseudomonas aeruginosa*
- ~250 generations in LB + 0.3 ug/ml ciprofloxacin (sub-inhibitory)
- 12 replicates
- MIC, fitness of at least 8 isolates from each population

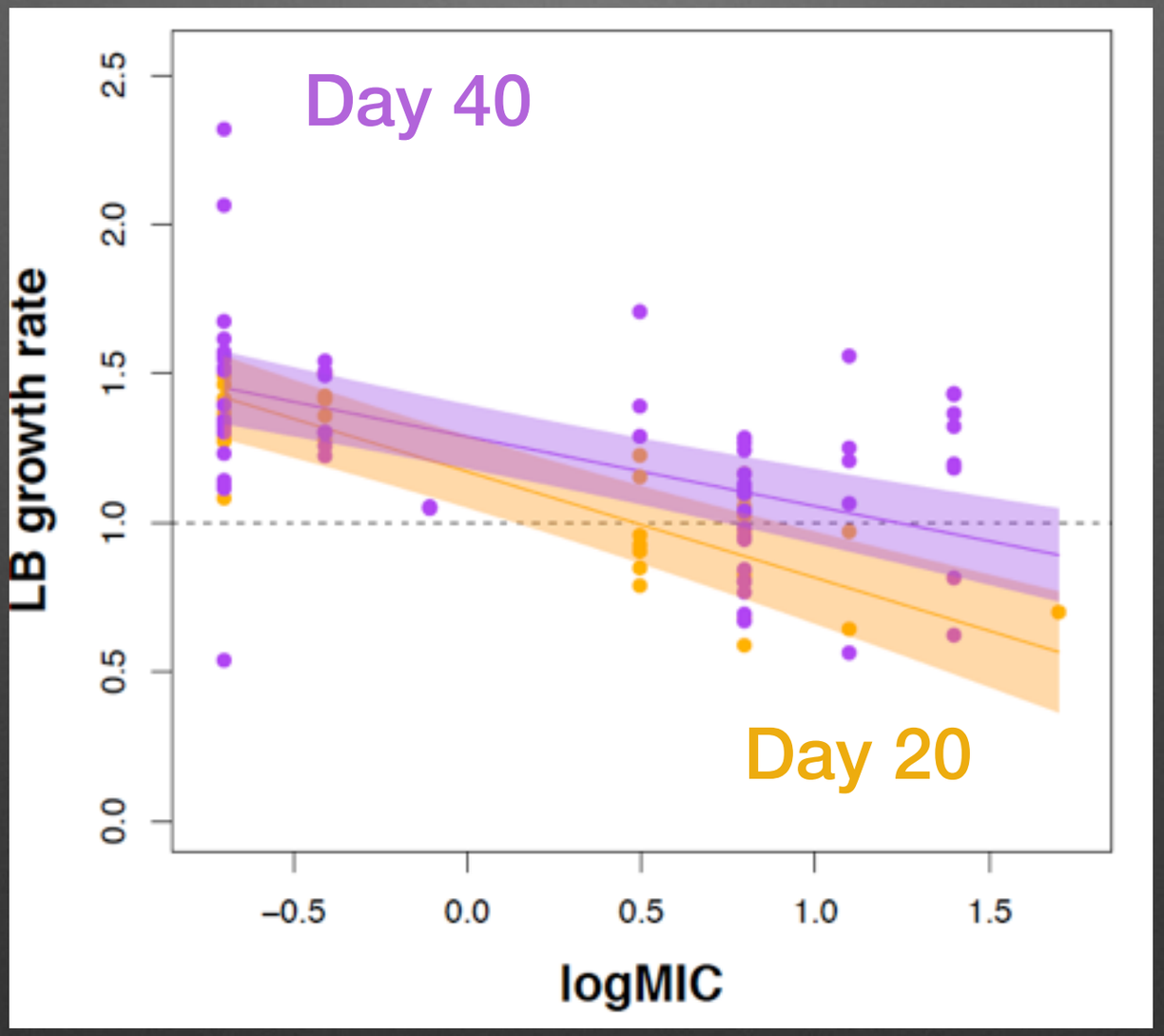
After 20 days (~130 generations)



Resistant and sensitive types coexist through time…

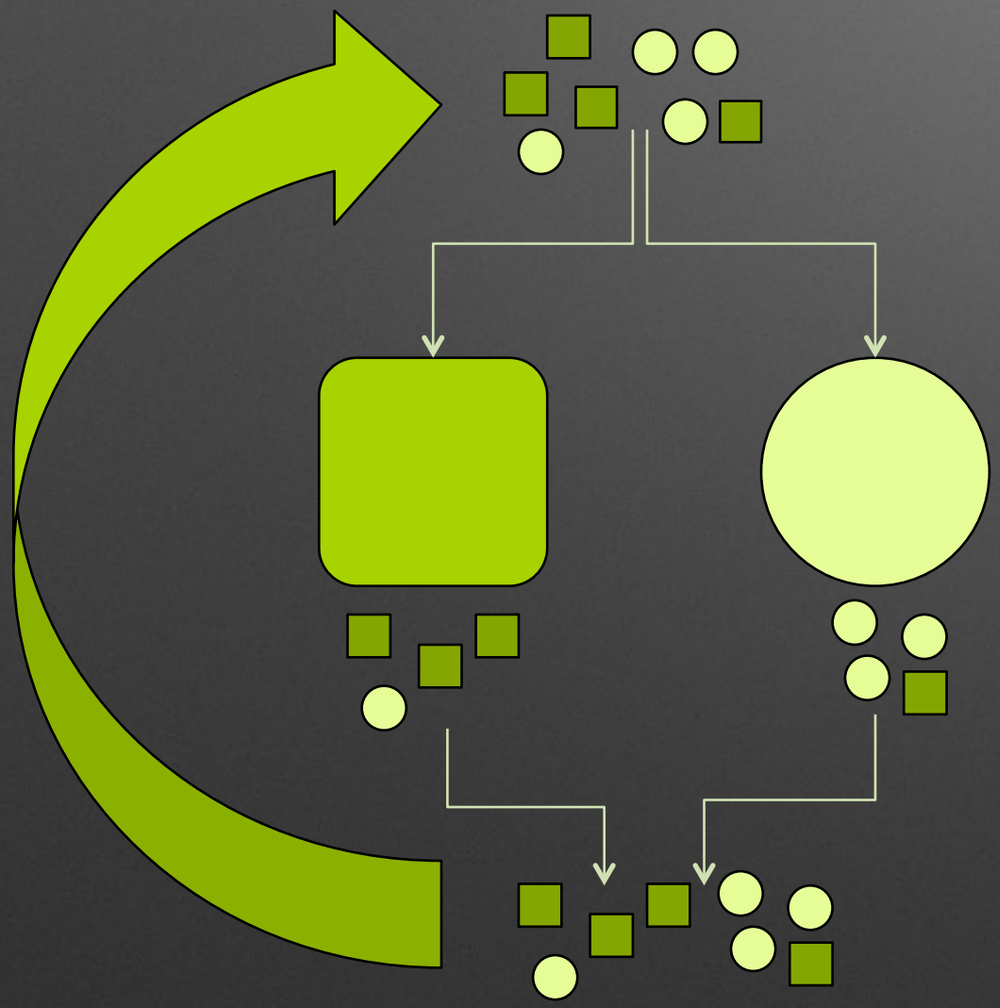


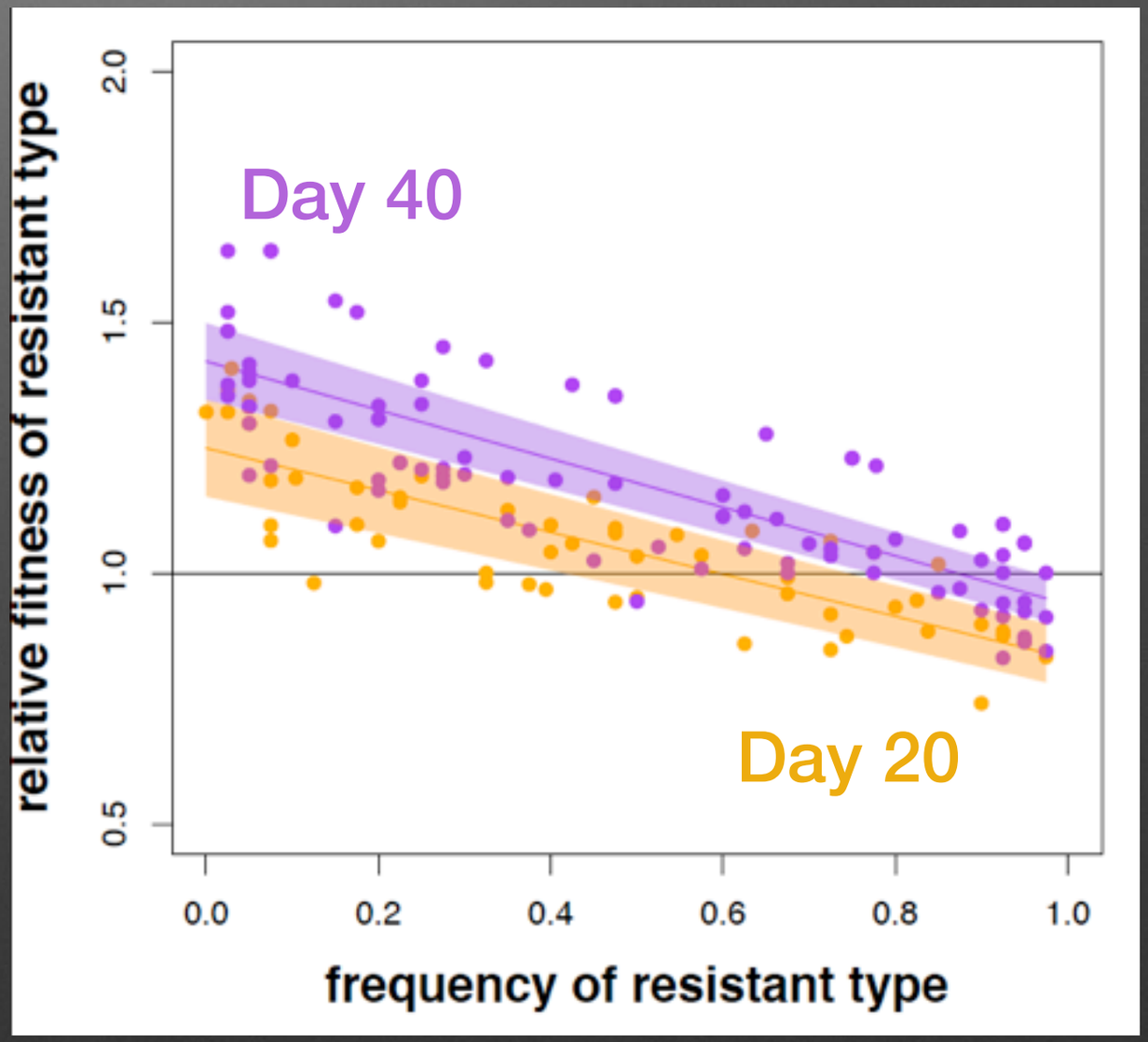
Costs of resistance underpin coexistence



But these costs evolve!

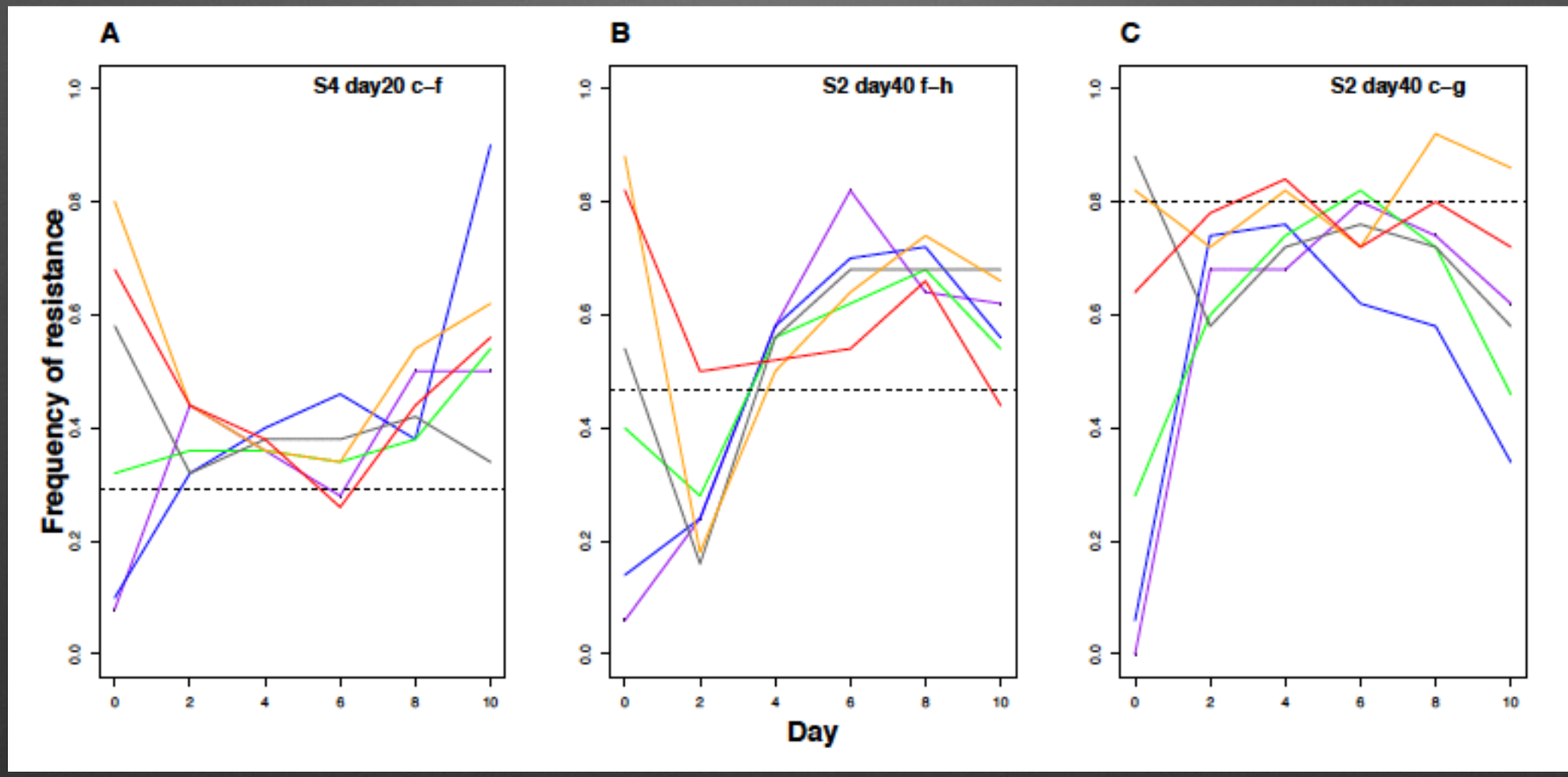
The Levene model* predicts stable coexistence through negative frequency dependent selection



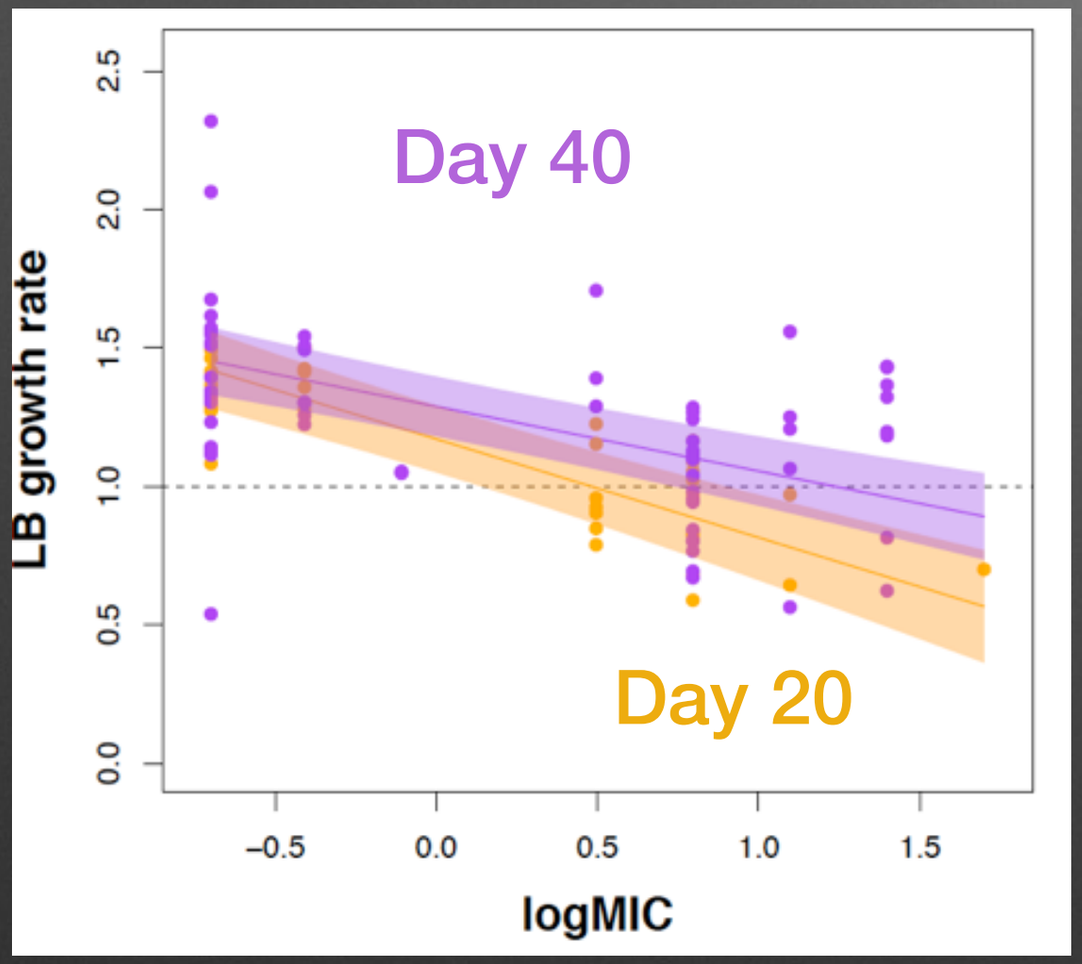


*Levene (1953) *Am Nat* 87: 331-333

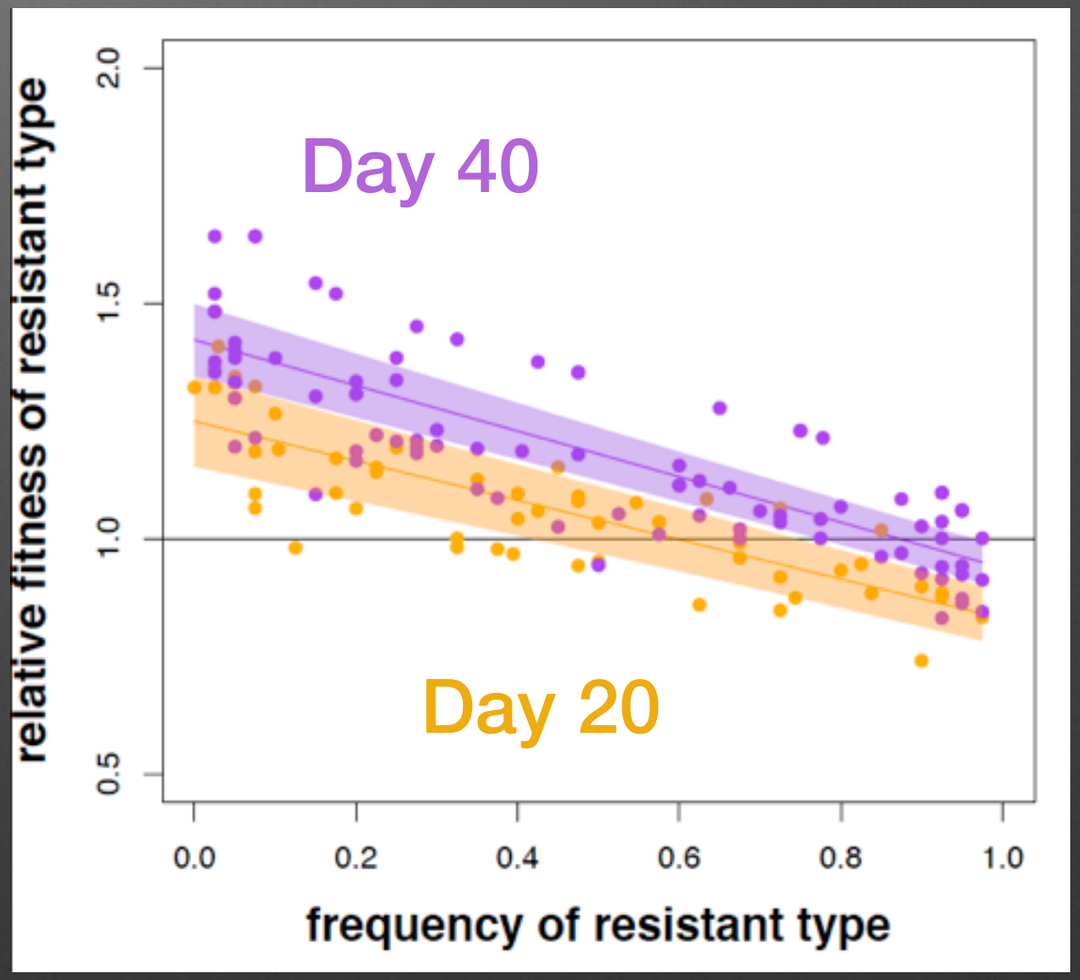
Variable dynamics are due to clonal interference, not stochastic effects



Resistance eventually predominates because of compensatory evolution



Trade-offs evolve…



which shifts the equilibrium

Divergent selection

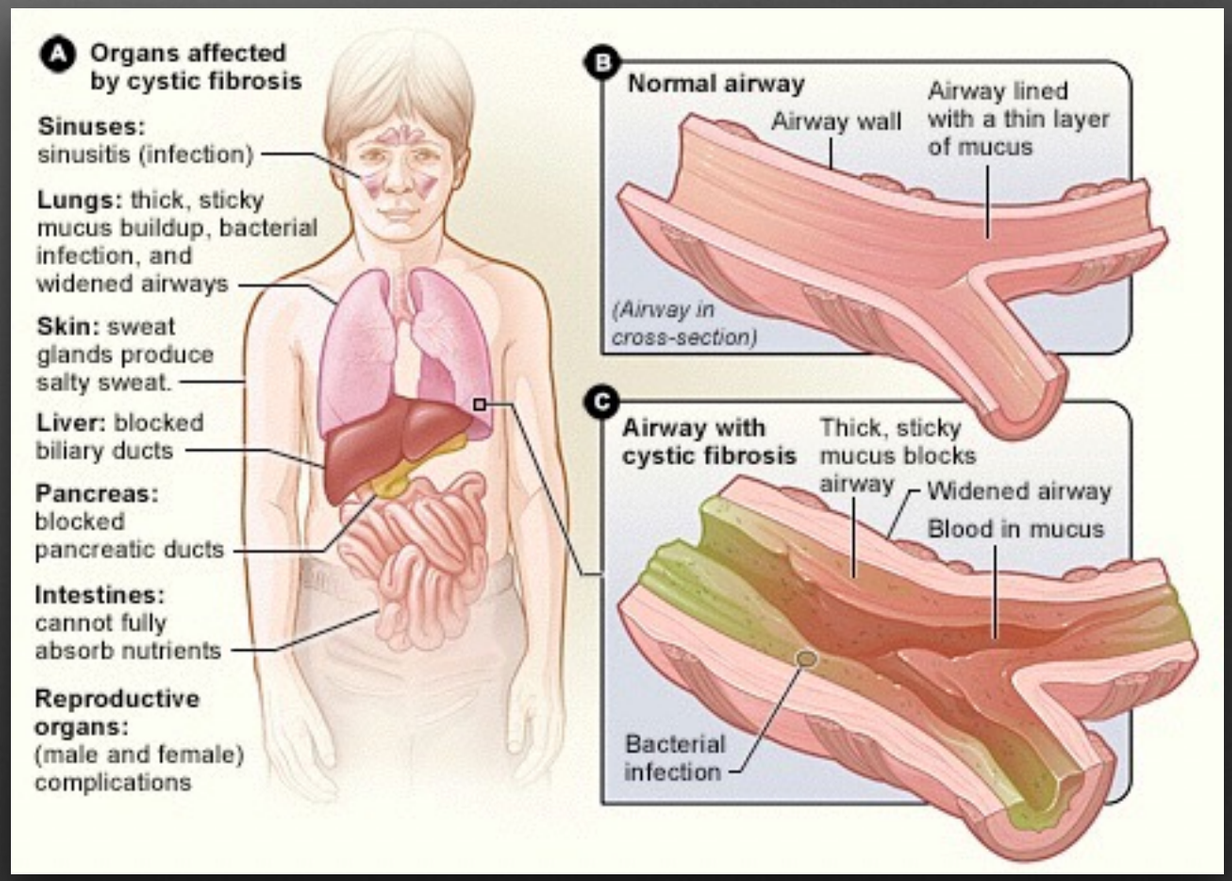
- Leads to the emergence of quasi-stable coexistence between resistant and sensitive types

- Coexistence is underlain by a trade-off between resistance and growth rate in the absence of drug

- Diversity is eventually lost because resistant types evolve to become generalists due to the fixation of compensatory mutations

Cystic fibrosis

- Genetic disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene
- Impacts chloride ion transport which, among other effects, results in reduced ability to clear fluid from lower respiratory tract
- As treatments have improved, median survival has increased from 8 years (1974) to ~40 years now



60-70% of Canadian adult CF patients are chronically infected with *Pa*

- Ubiquitous, opportunistic Gram negative pathogen
- Can also cause acute pneumonia and other infections (wounds, urinary tract, etc…)
- Increased morbidity and mortality, independent of lung function

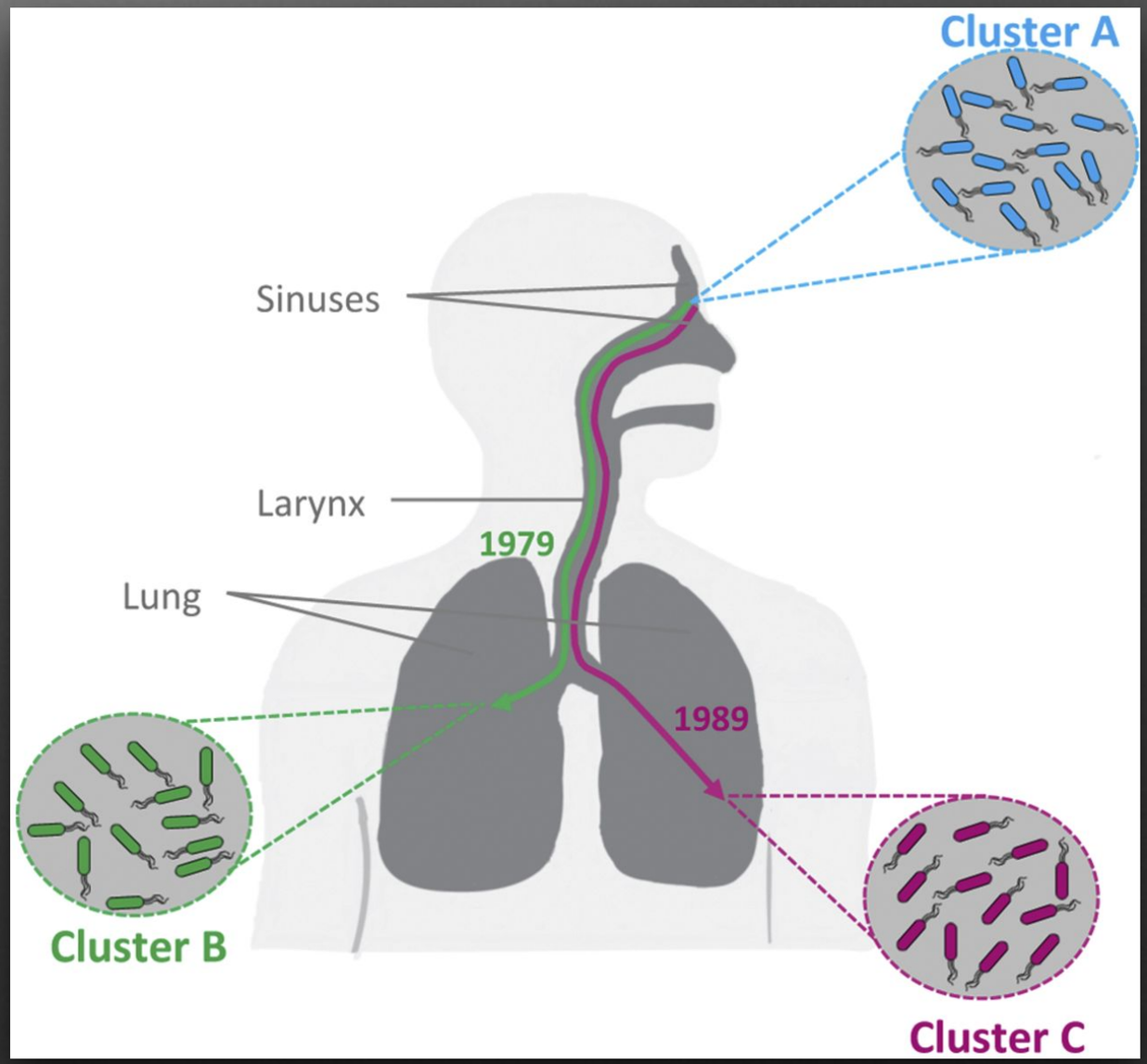
Artist: Eva Markvoort Photo: Cyrus McEachern
Courtesy of 65RedRose.com #4Eva

Patho-adaptation by *Pa* to the CF lung

- Loss of virulence factors (an adaptation to evade immune system detection)
- Antibiotic resistance
- Loss of motility- tendency to form biofilms and unattached microcolonies
- Rapid *in situ* phenotypic and genetic diversification

What drives diversification?

- Spatial structure associated with airway compartments that differ in environmental conditions
- Many different resources (ecological opportunity)
- Thick mucous reduces dispersal



Markussen et al (2014) *mBio* 5: e01592-14

An experimental test

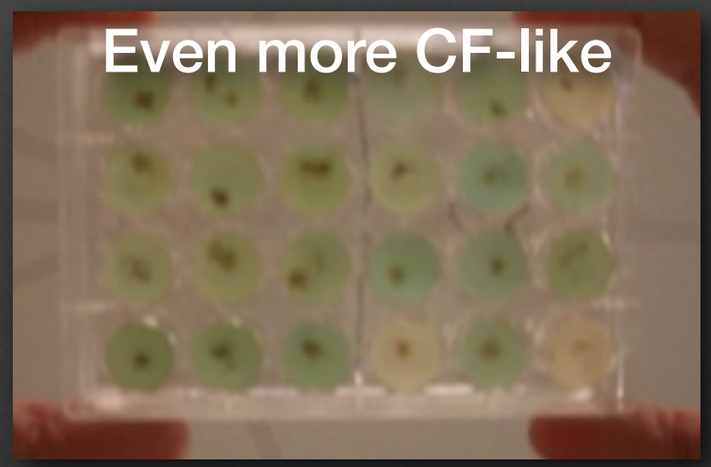
Pa14 clonal isolate

~ 220 generations
30 replicates

MIN	SCFM*
MIN + MUCIN	SCFM + MUCIN

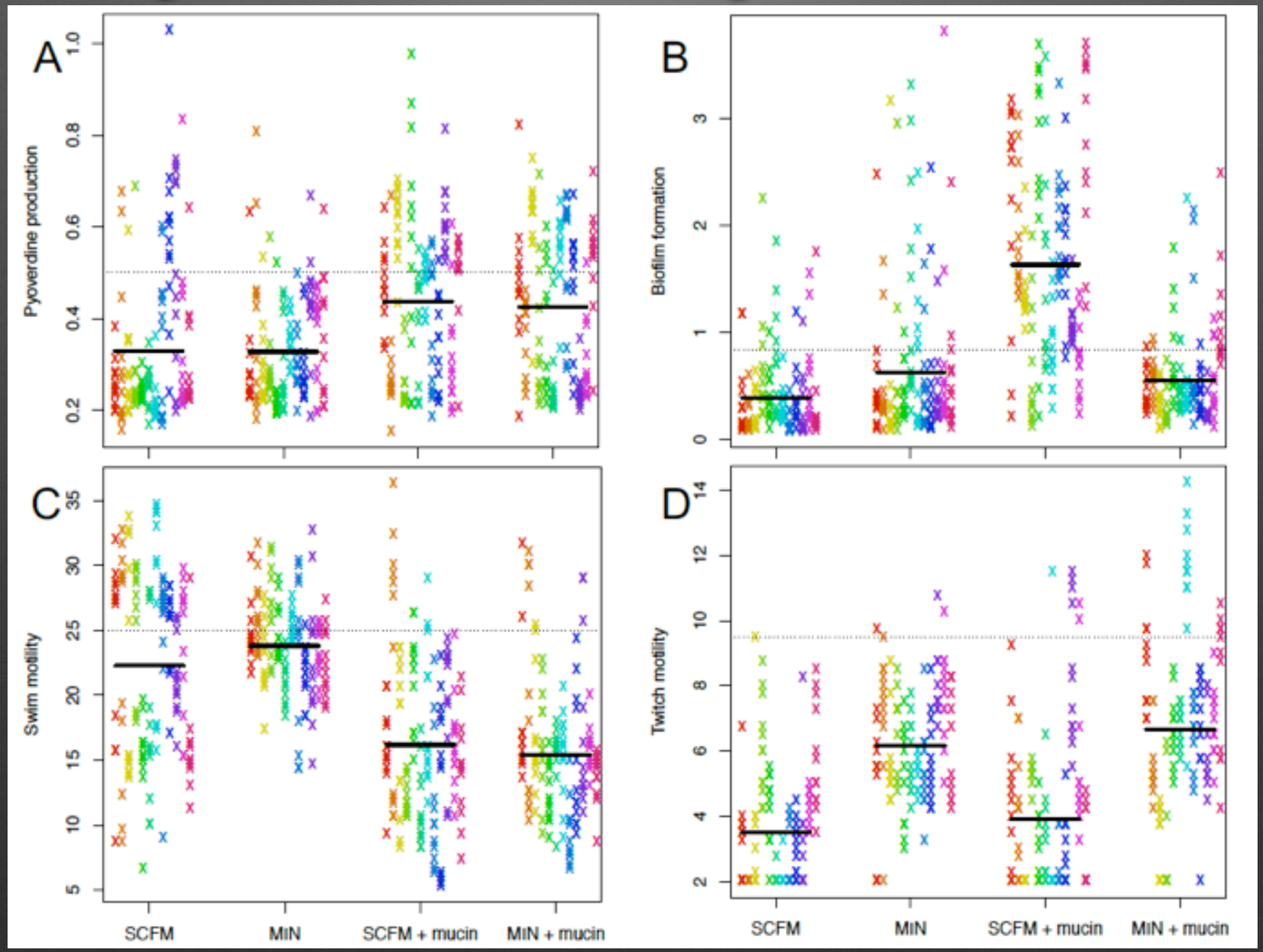
Spatial structure

Nutritional complexity



*Synthetic CF Medium after Palmer et al. (2007) *J Bacteriol* 189: 8079-8087

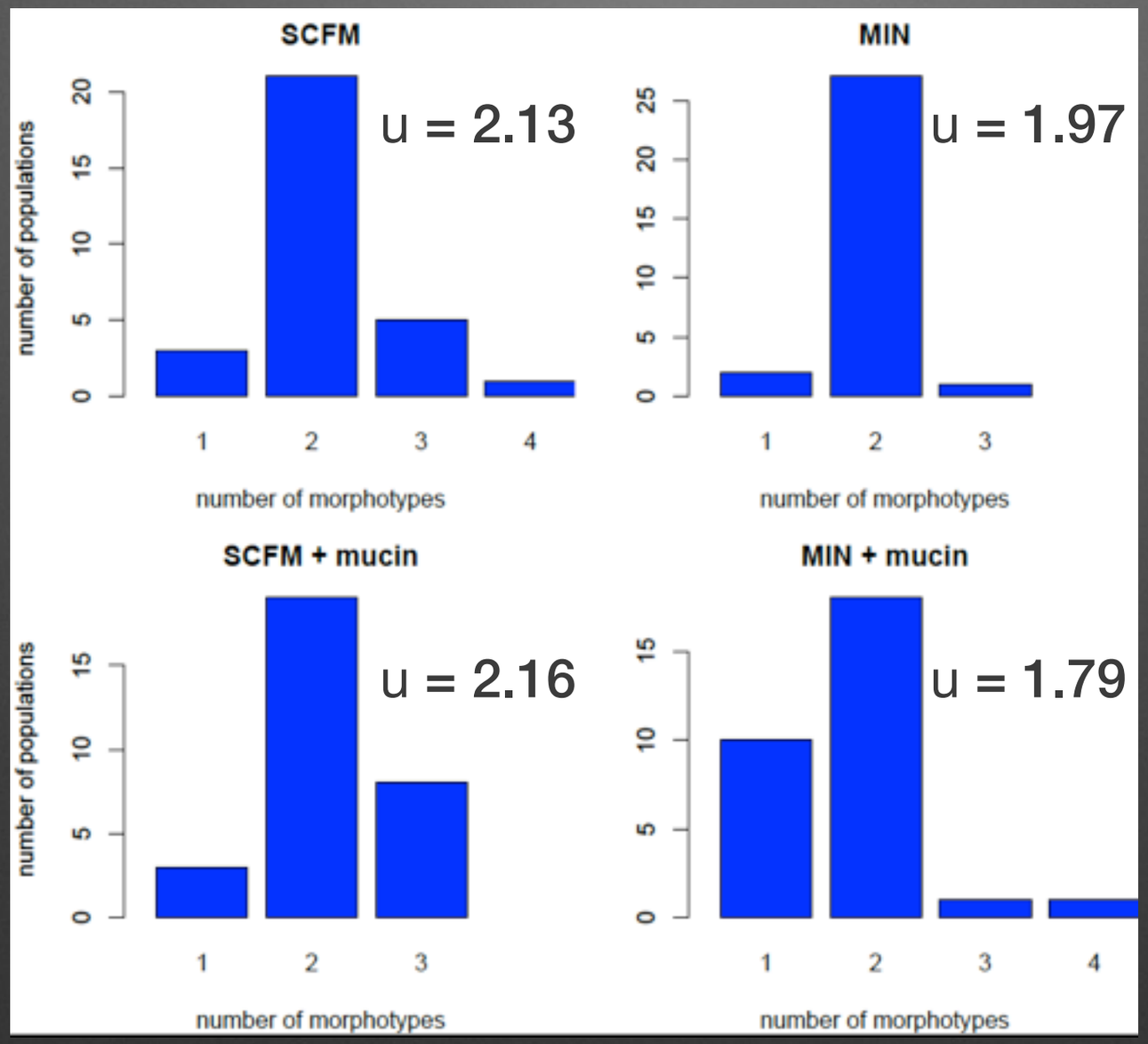
Phenotypes associated with patho-adaptation



Colony morphologies

Spatial structure

P > 0.05

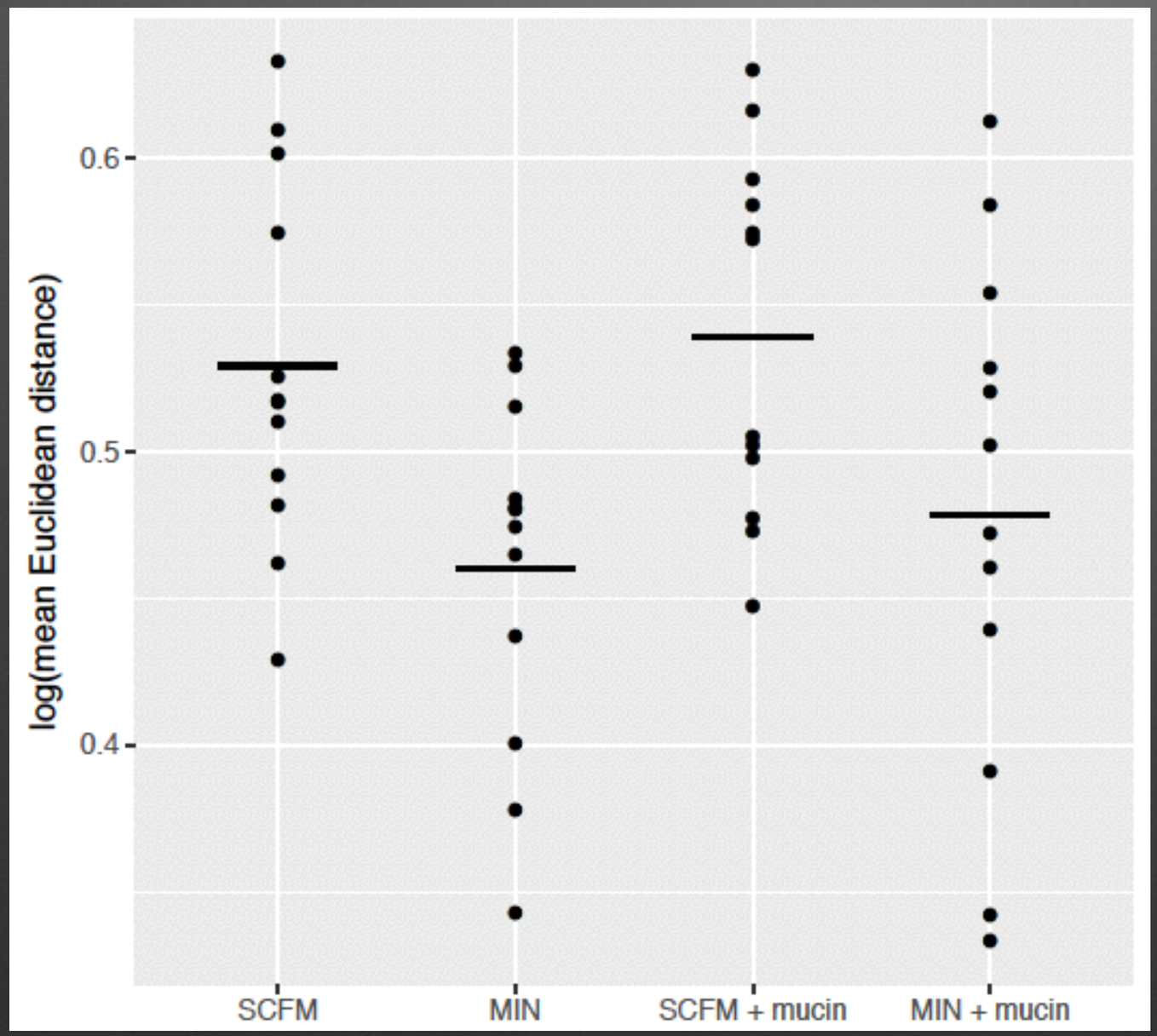


- pigmentation
- opacity
- iridescence
- surface texture
- margin
- halo
- autolysis
- SCV

Nutritional complexity

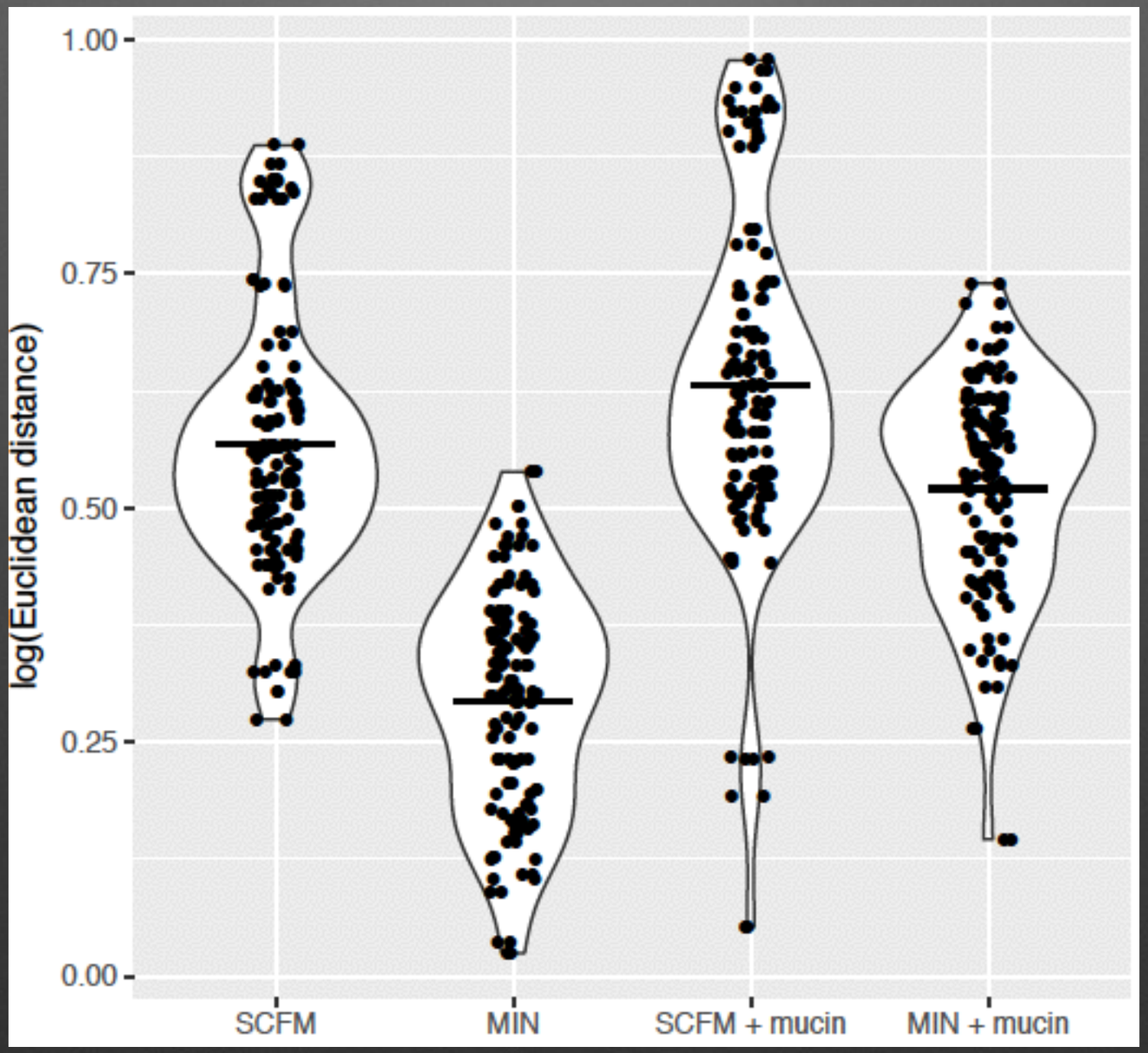
P < 0.05

Nutritional complexity drives diversification within populations

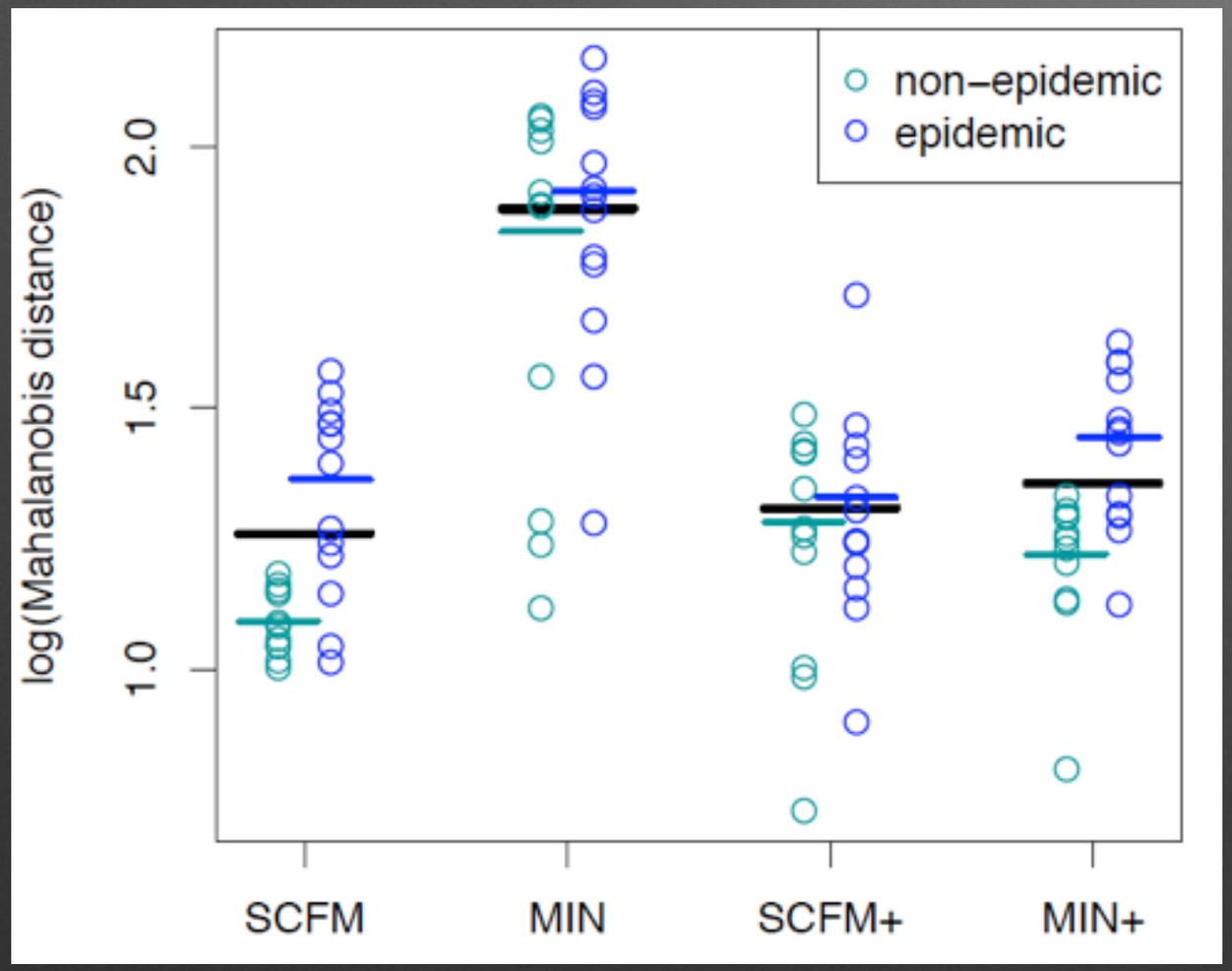


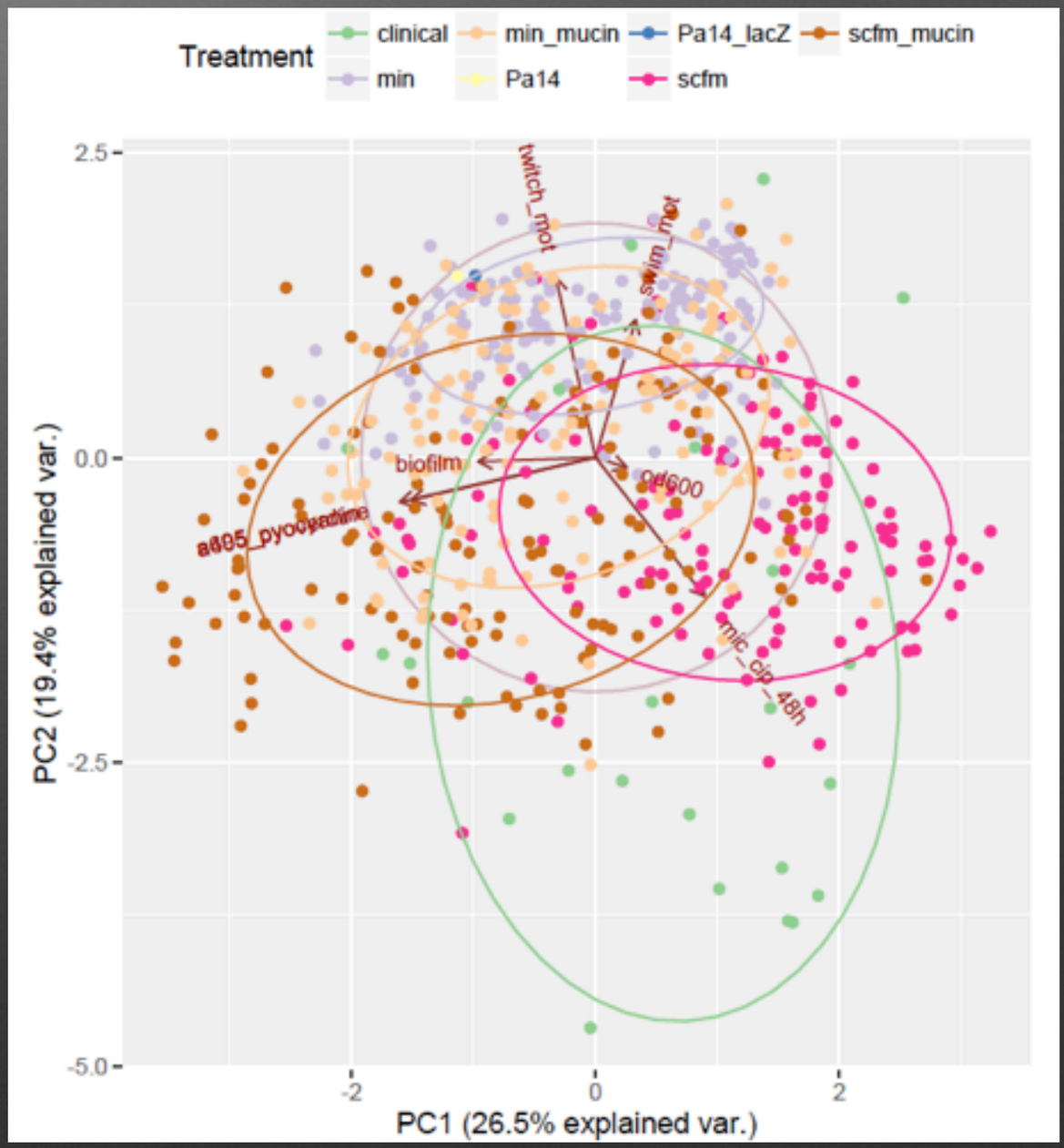
- growth rate in LB
- pyoverdine
- pyocyanin
- biofilm formation
- swim motility
- twitch motility
- antibiotic resistance (cip, cef, col, tob)

Nutritional complexity & spatial structure cause populations to diverge



Phenotypic similarity to clinical isolates from CF patients across Ontario



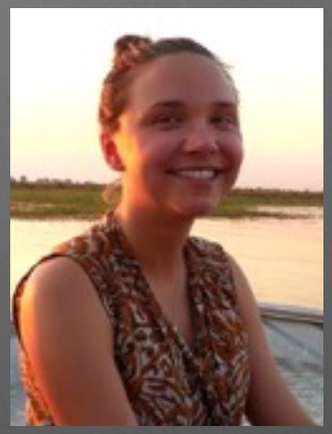


Insights into chronic infection from *in vitro* experiments

- Diversity evolves rapidly alongside patho-adaptation
- Nutritional complexity drives diversification within populations
- Spatial compartmentalization can promote divergence among populations
- *In vitro* diversification in CF-like conditions recapitulates *in vivo* diversity

Thanks to…

Aaron Hinz
Post-doc



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NSERC CRSNG

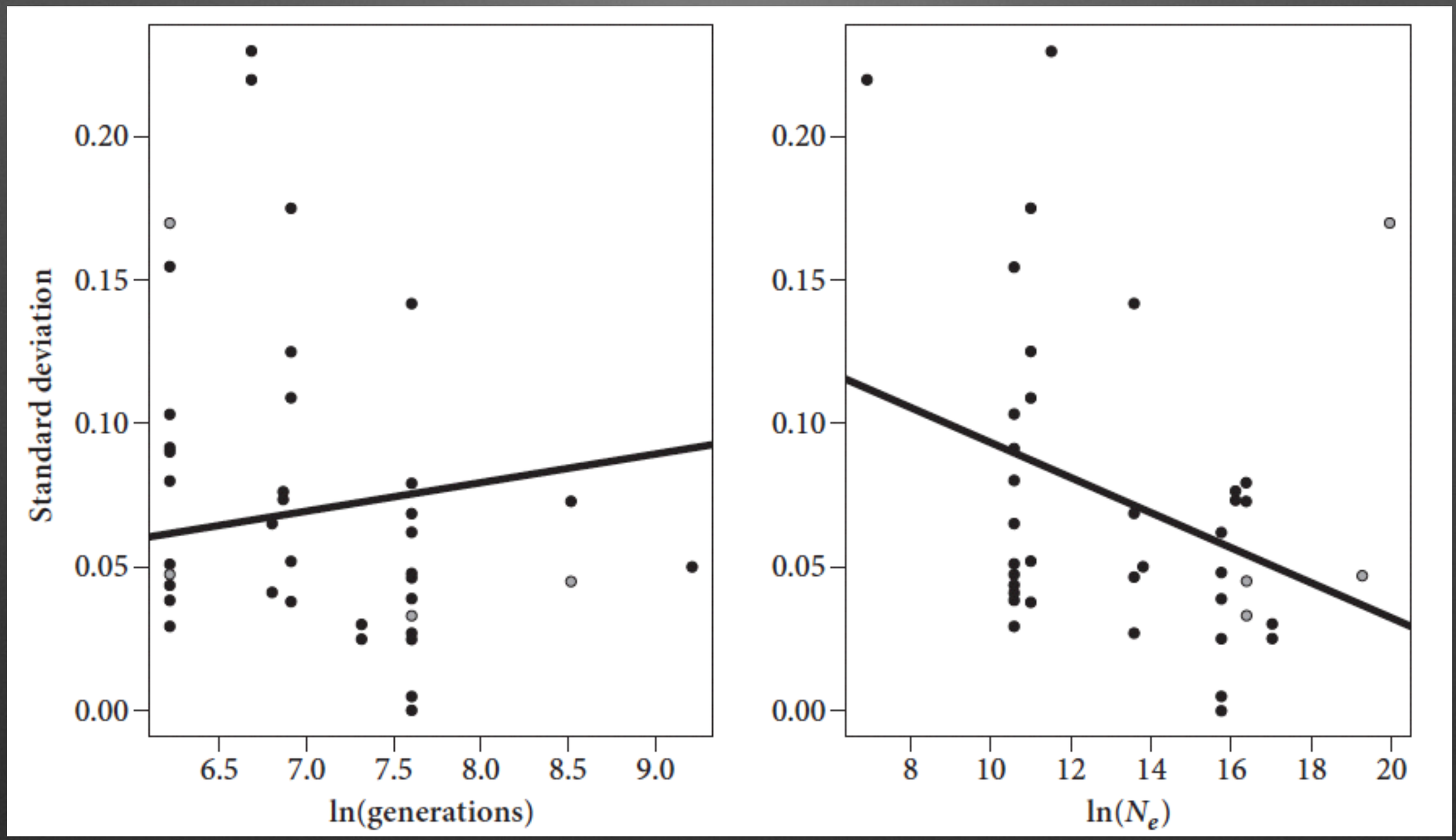
CIHR IRSC
Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

uOttawa

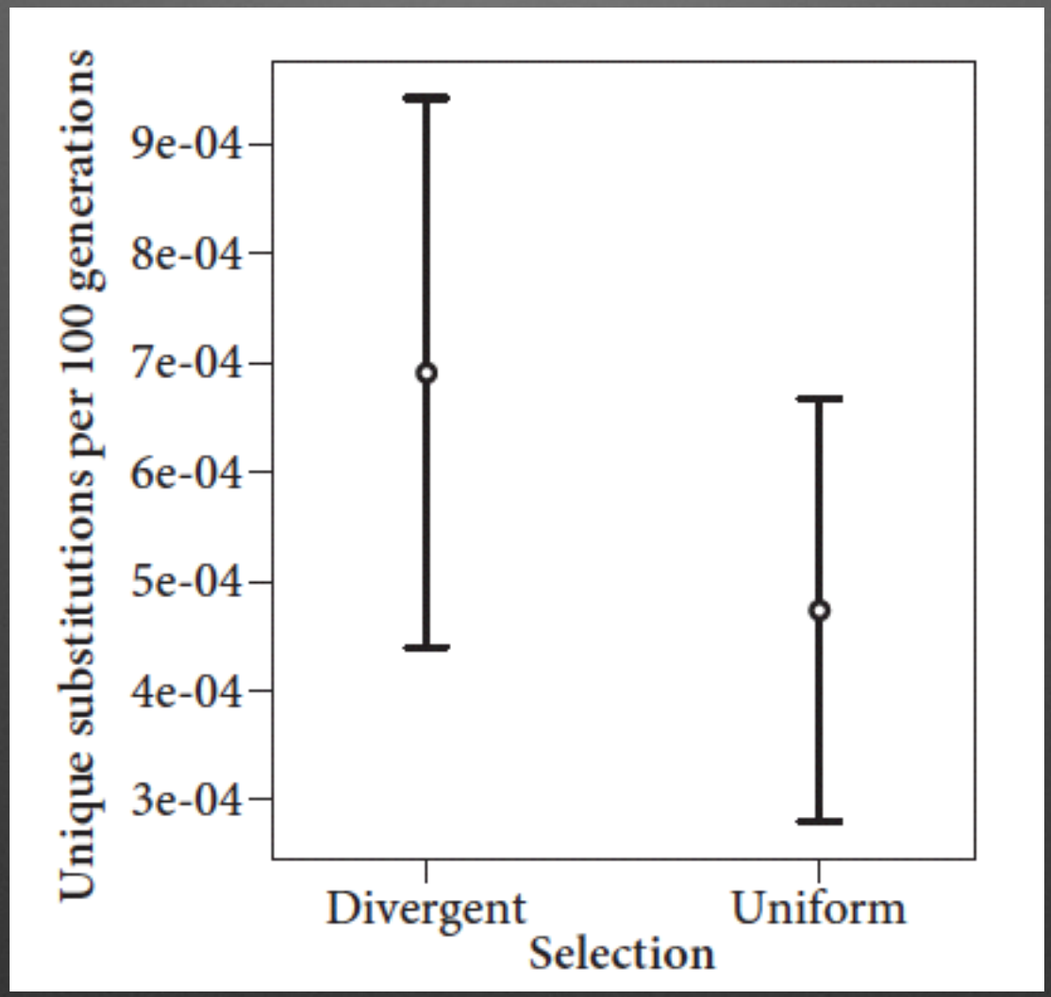
Cystic Fibrosis Canada

jpiamr
Joint Programming Initiative on Antimicrobial Resistance

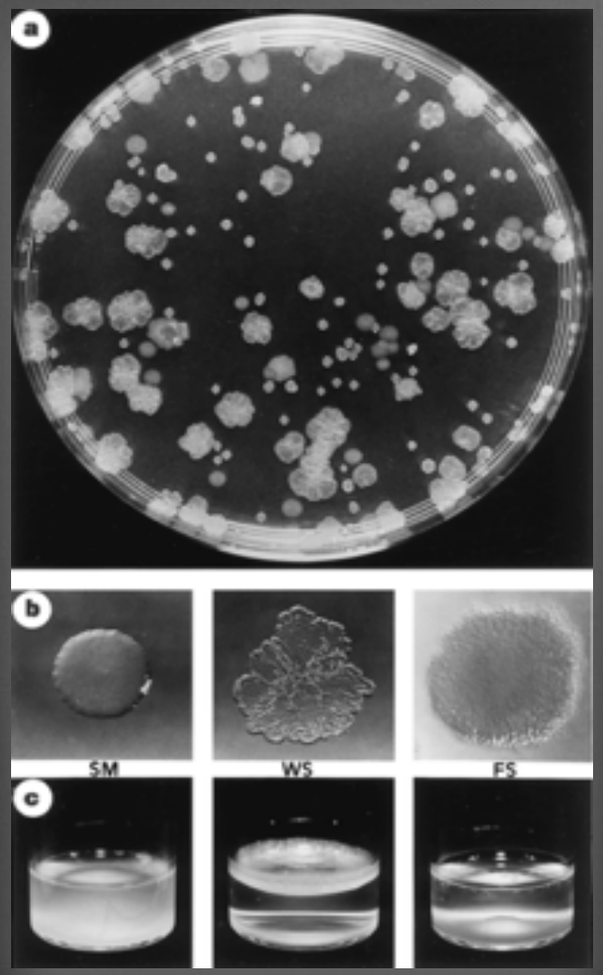
Replicate populations diverge in fitness in a common environment



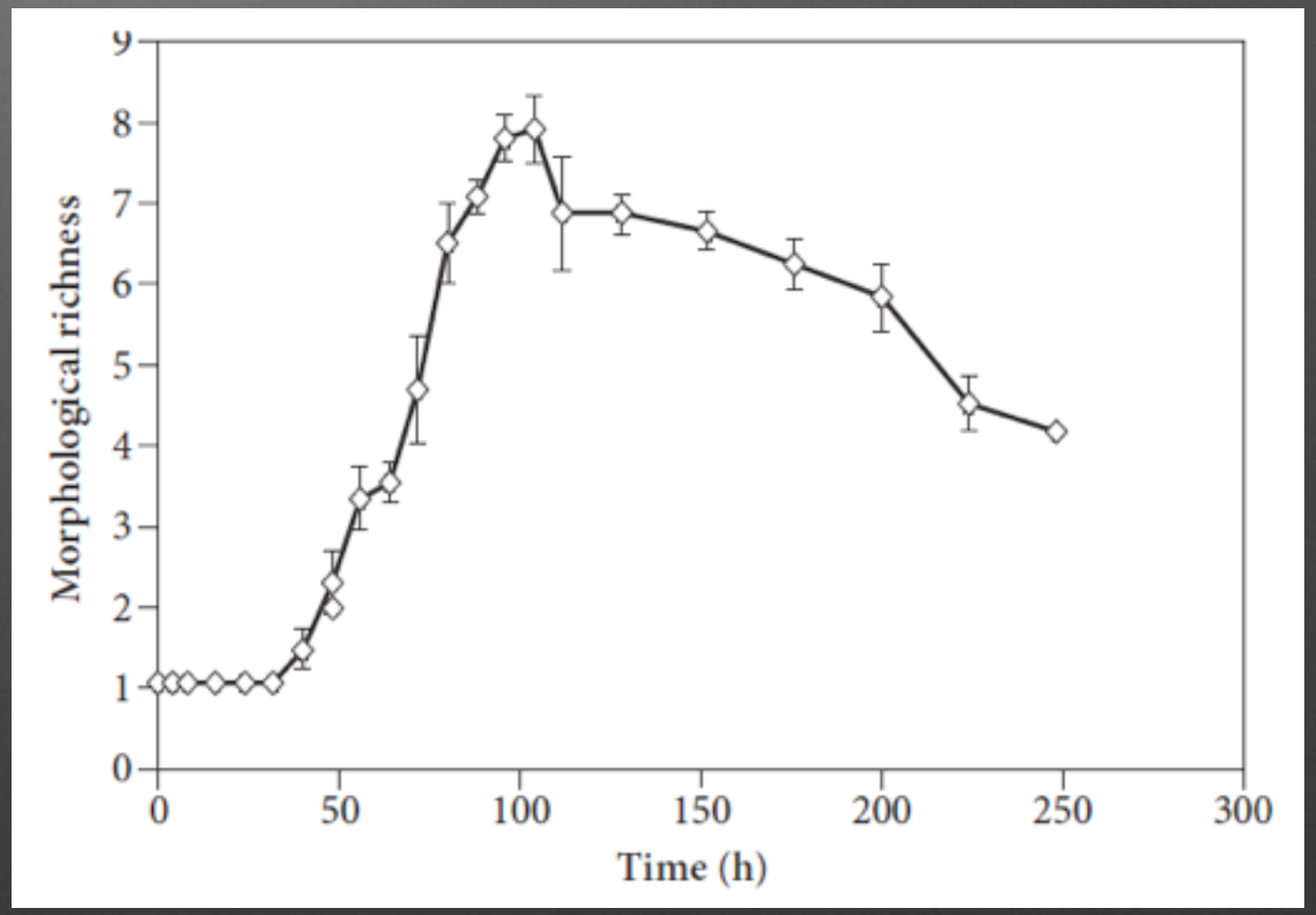
Number of substitutions in phage experiments



Dynamics of diversity



Rainey & Travisano *Nature* 394, 69-72 (1998)



Meyer et al (2011) *Proc R Soc* 278: 392-398