

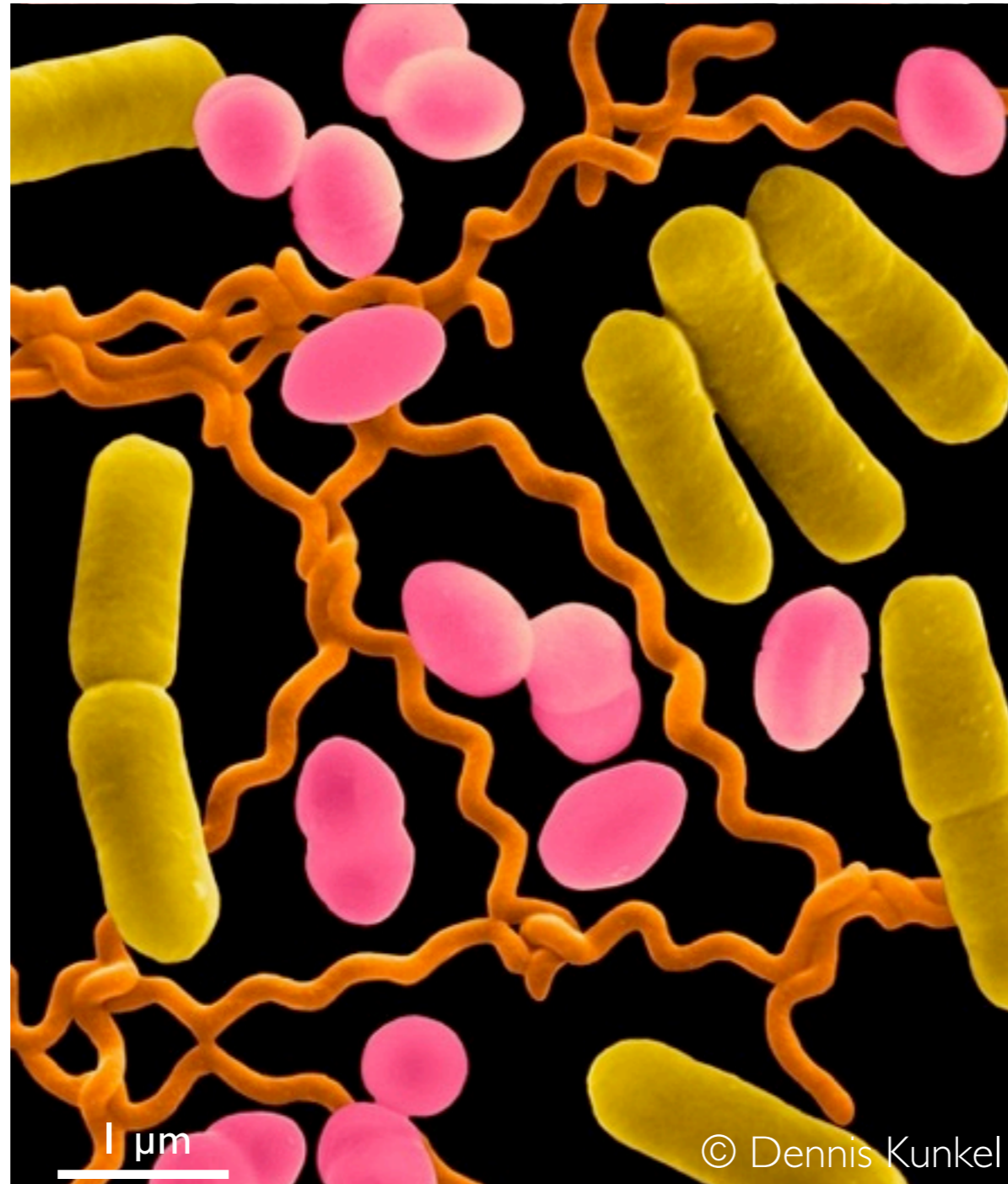


Evolution, competition and cooperation in bacterial populations

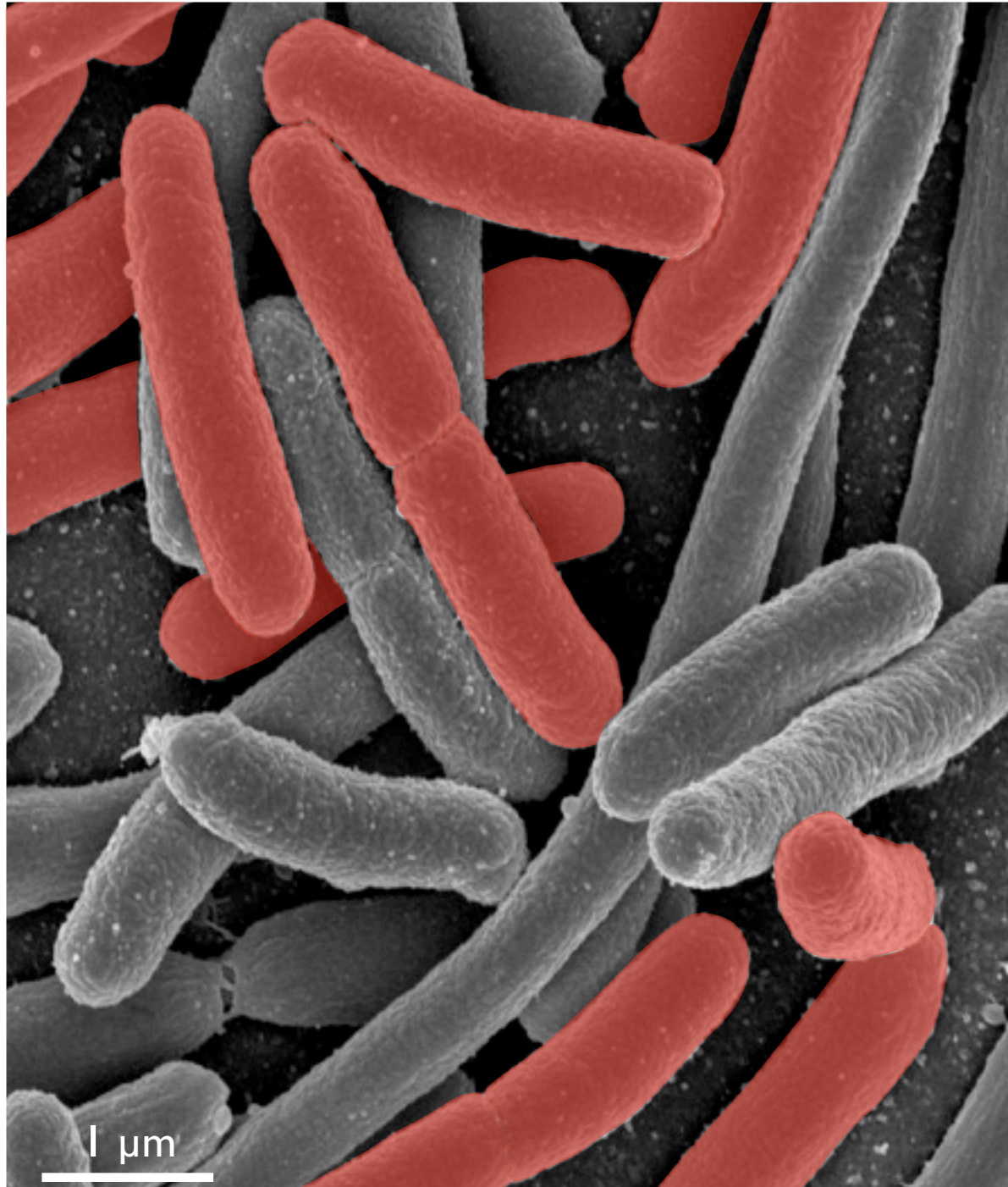
Thomas Julou, under the supervision of **David Bensimon**
Laboratoire de Physique Statistique – Ecole Normale Supérieure

PhD defence
June 9th 2011

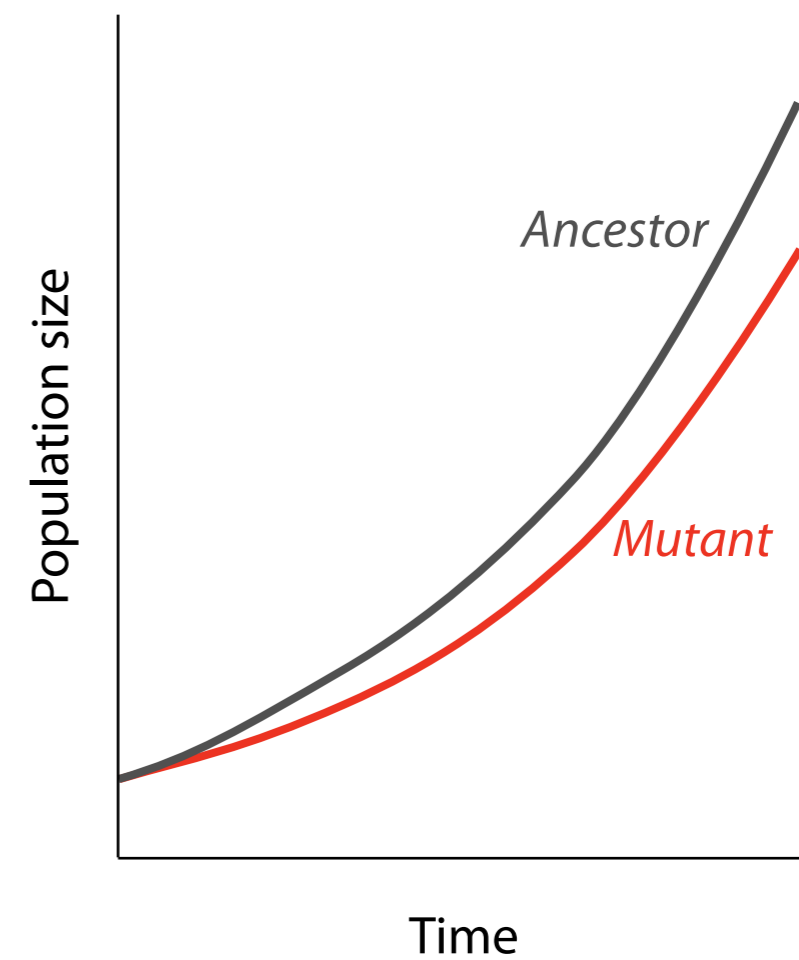
Diversity & Selection



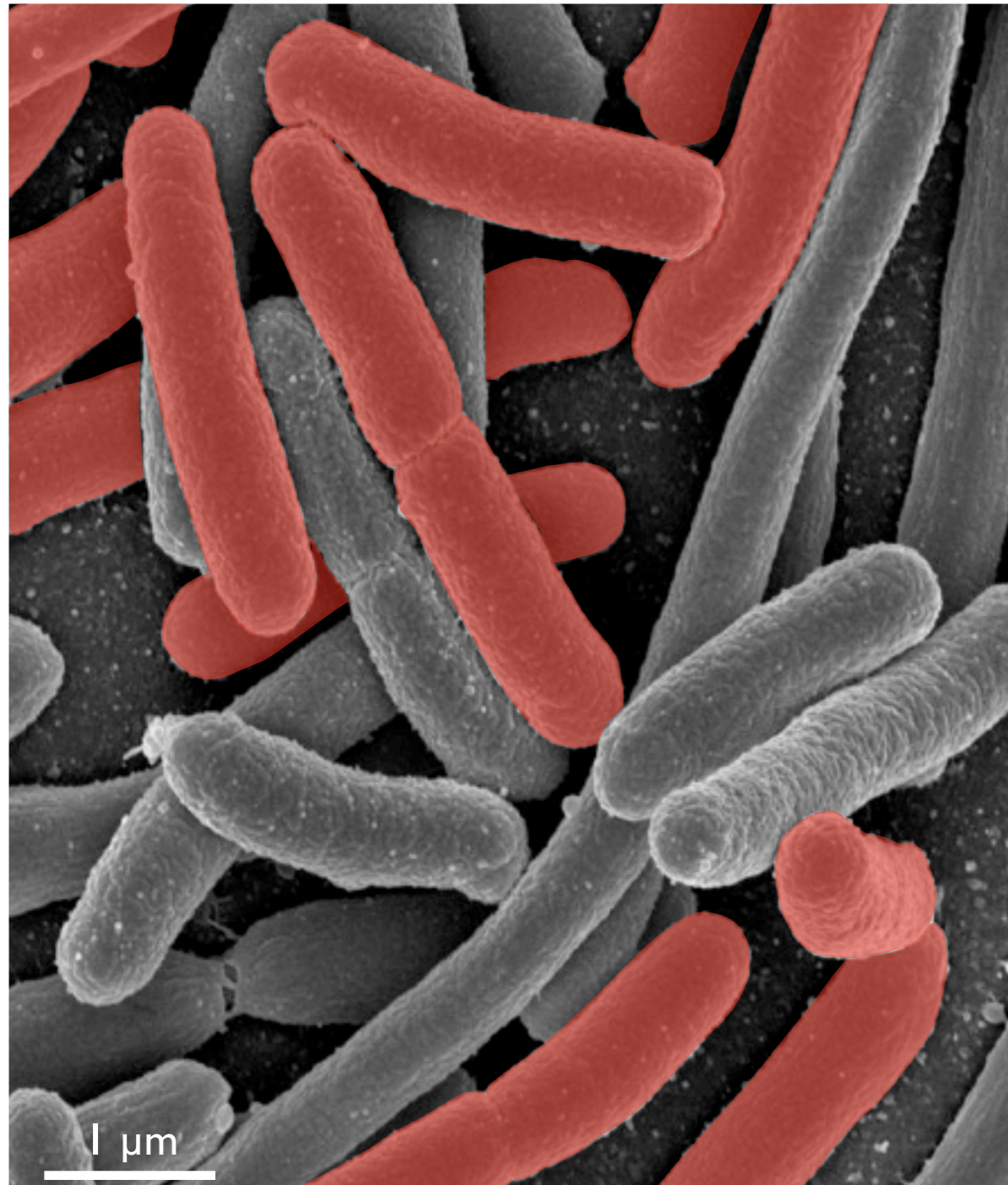
Diversity & Selection



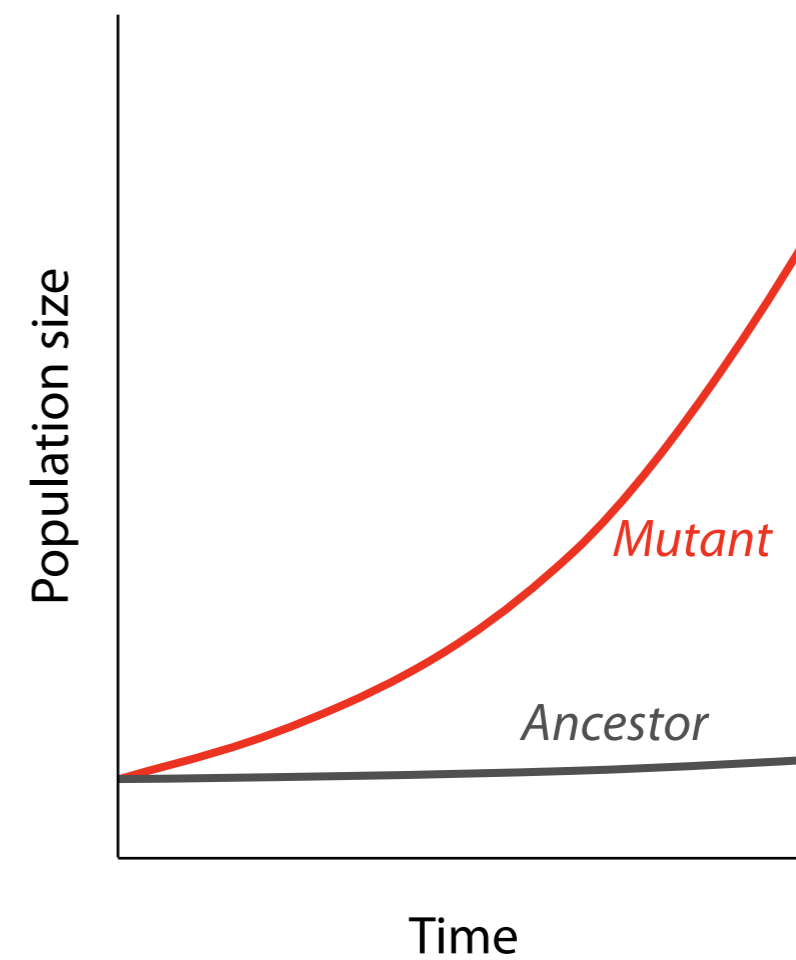
without antibiotics



Diversity & Selection



with antibiotics



Phenotypic variability



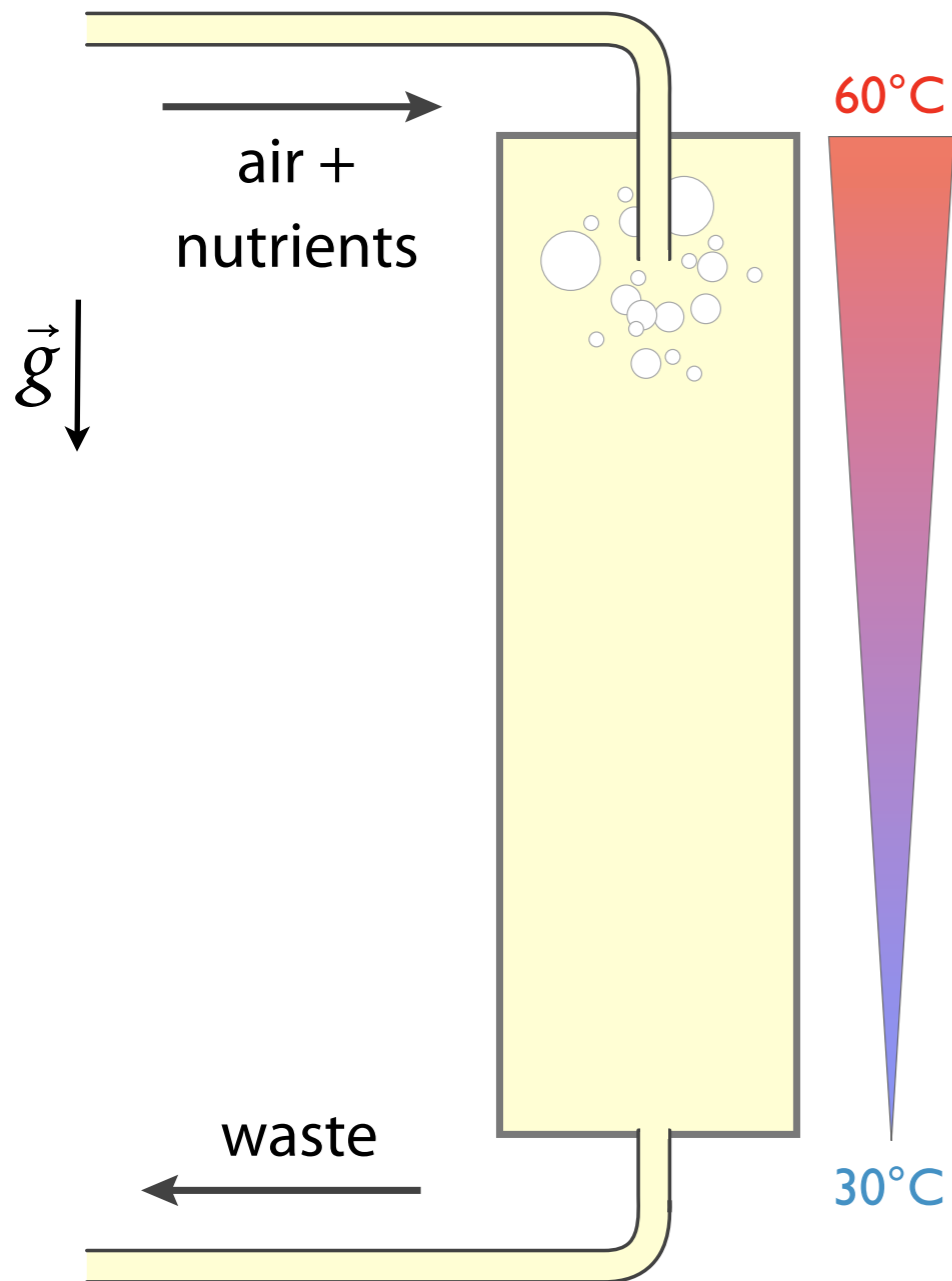
Outline

1. Evolution in a temperature gradient
2. Variability of siderophores in clonal populations

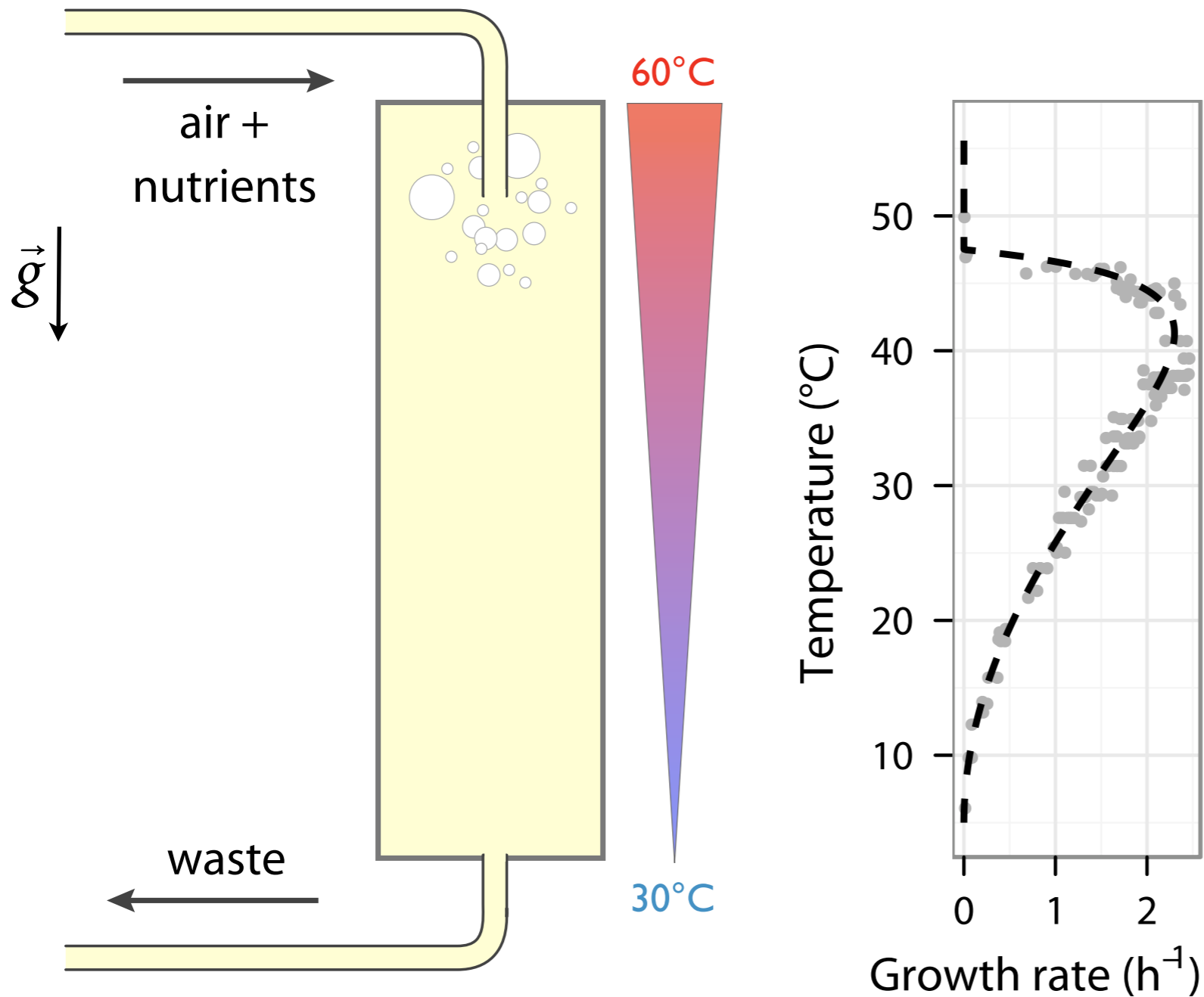
Outline

1. Evolution in a temperature gradient
2. Variability of siderophores in clonal populations

Evolution in a temperature gradient

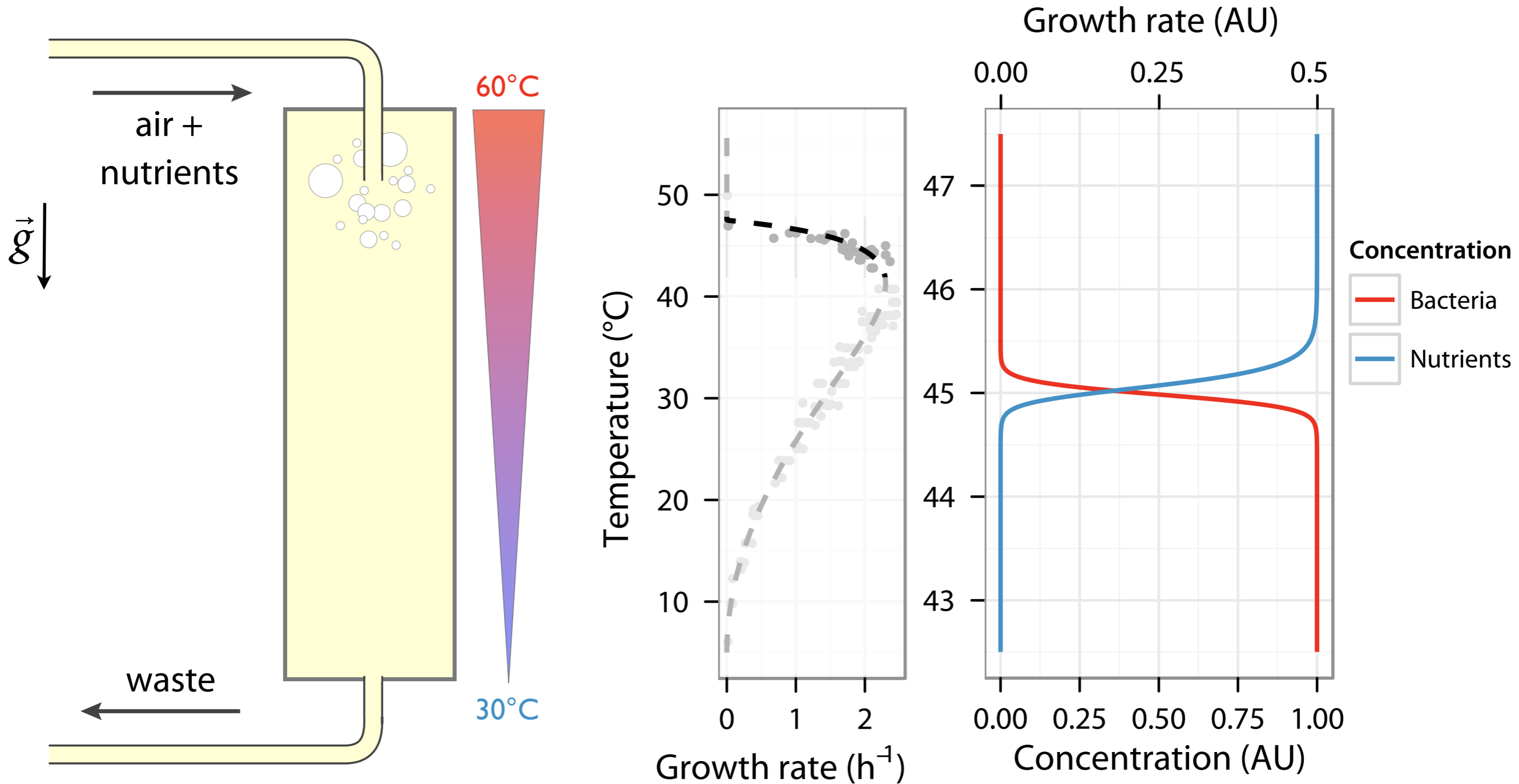


Evolution in a temperature gradient



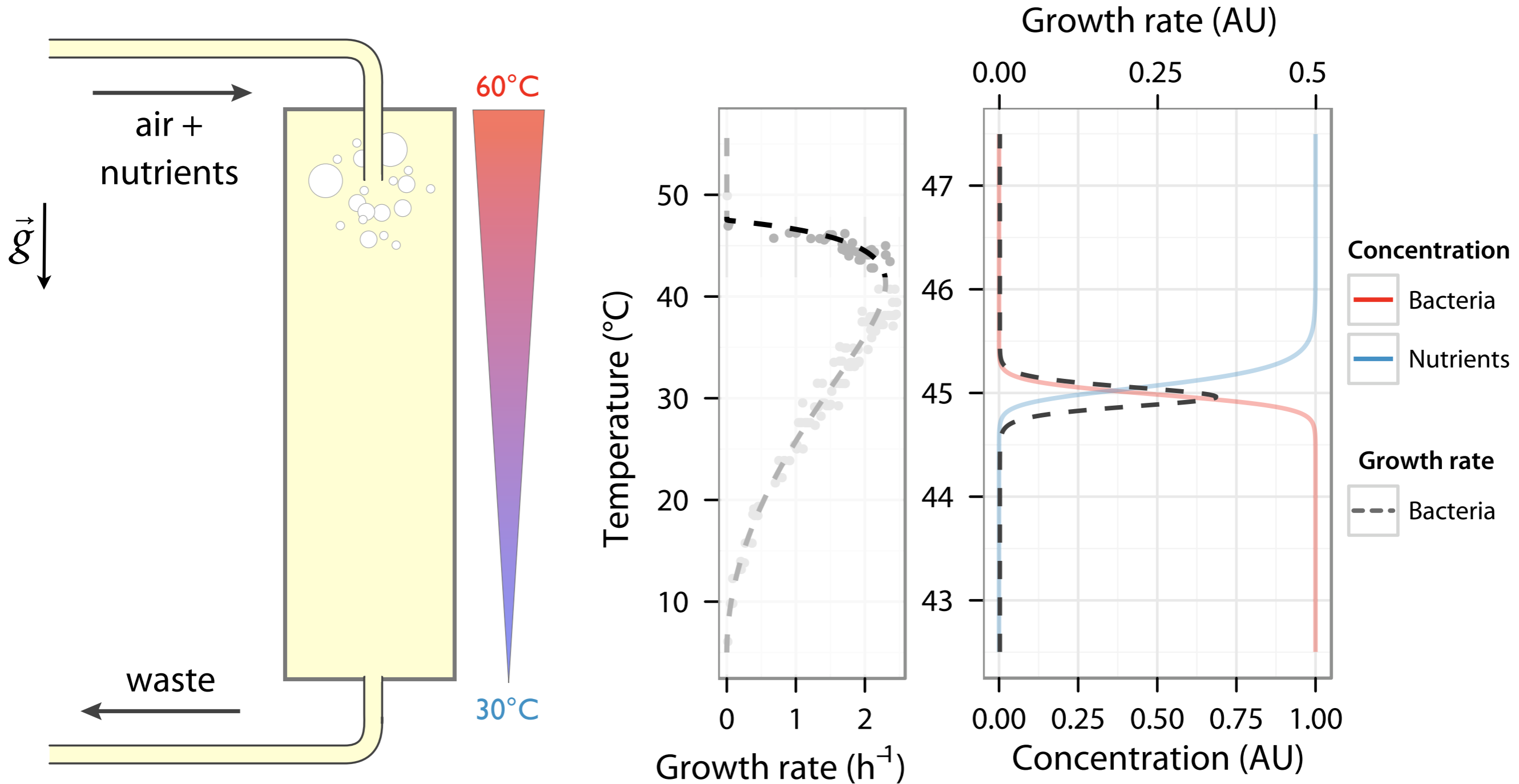
(Barber 1908; Rosso, *et al.* 1993)

Evolution in a temperature gradient



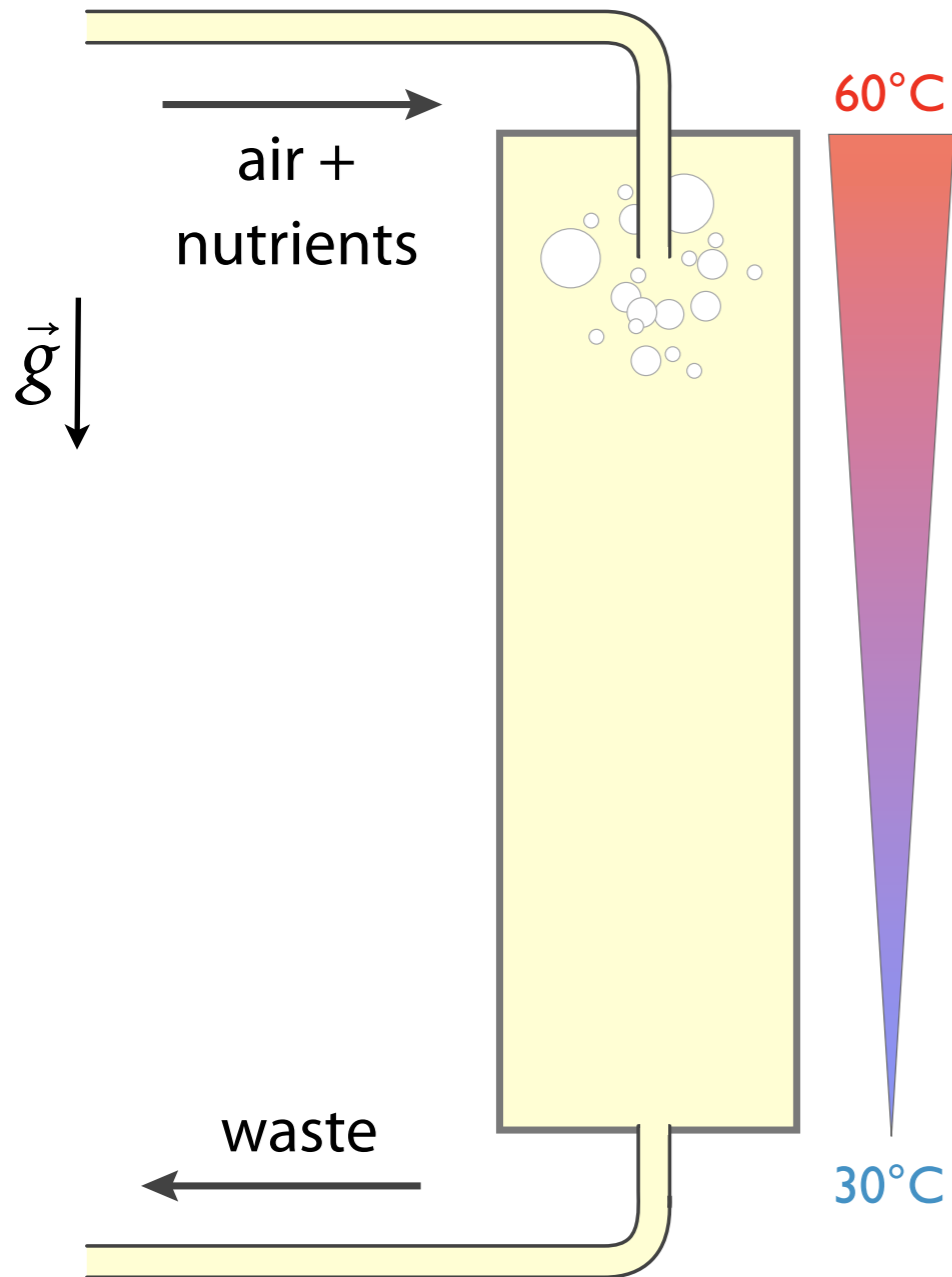
(Barber 1908; Rosso, *et al.* 1993)

Evolution in a temperature gradient



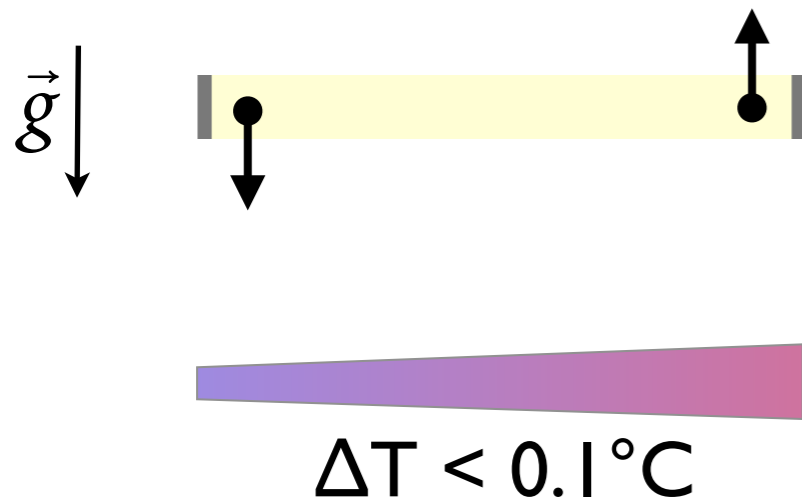
(Barber 1908; Rosso, *et al.* 1993)

Convection



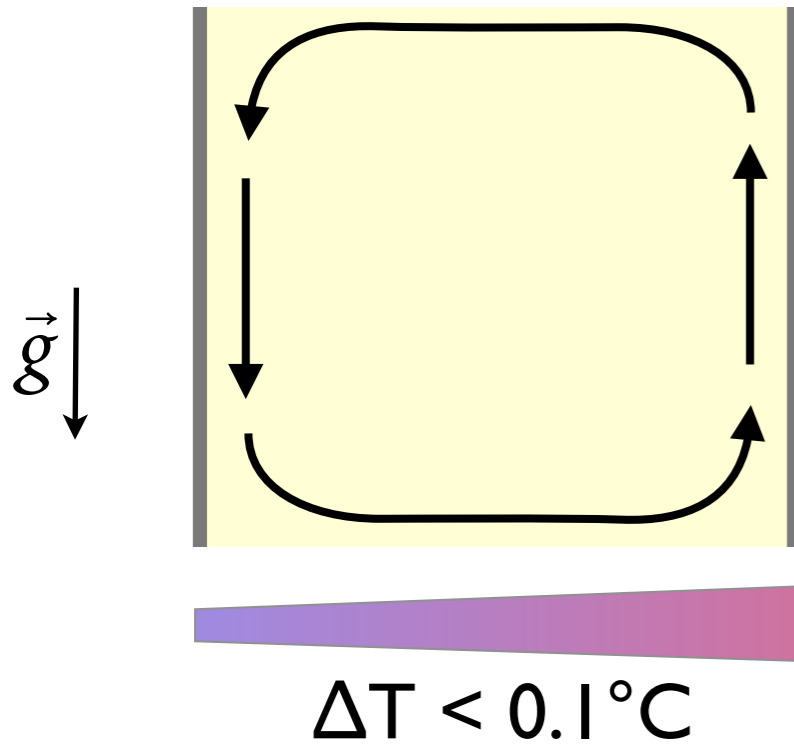
- Bacteria speed $\sim 1-10\mu\text{m/s}$
- Lateral heterogeneity of temperature
- Reduce convection intensity by
 - decreasing temperature heterogeneity
 - changing the chamber geometry
 - increasing the solution viscosity

Convection



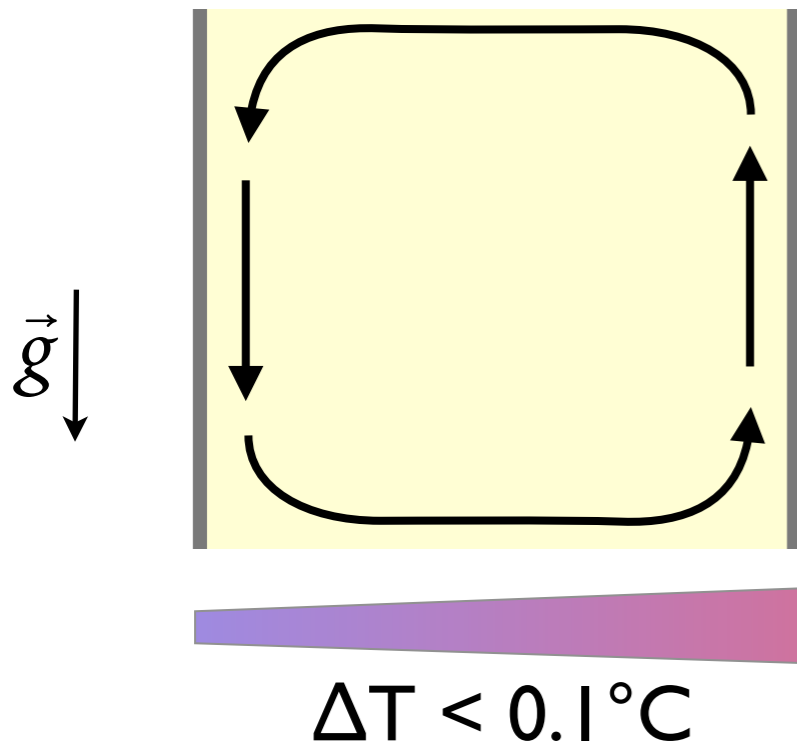
- Bacteria speed $\sim 1-10\mu\text{m/s}$
- Lateral heterogeneity of temperature
- Reduce convection intensity by
 - decreasing temperature heterogeneity
 - changing the chamber geometry
 - increasing the solution viscosity

Convection

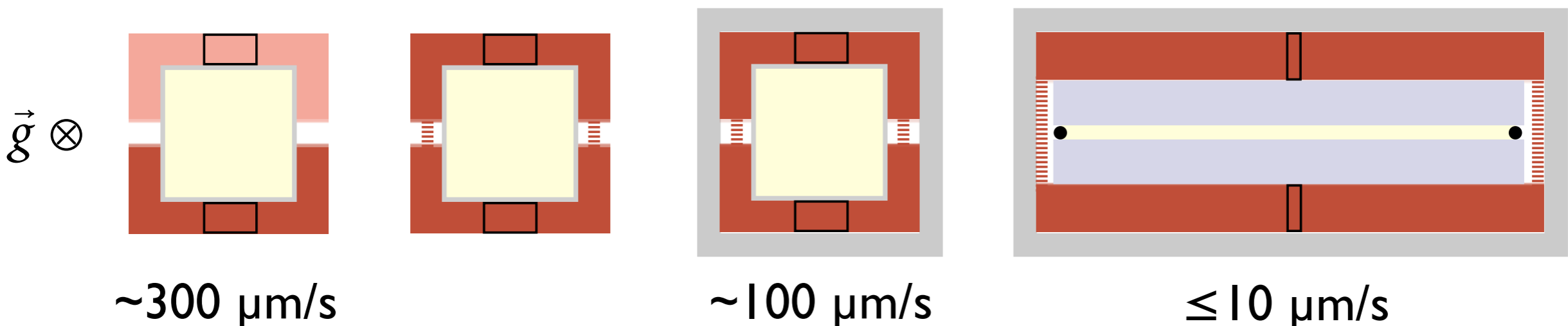


- Bacteria speed $\sim 1-10\mu\text{m/s}$
- Lateral heterogeneity of temperature
- Reduce convection intensity by
 - decreasing temperature heterogeneity
 - changing the chamber geometry
 - increasing the solution viscosity

Convection

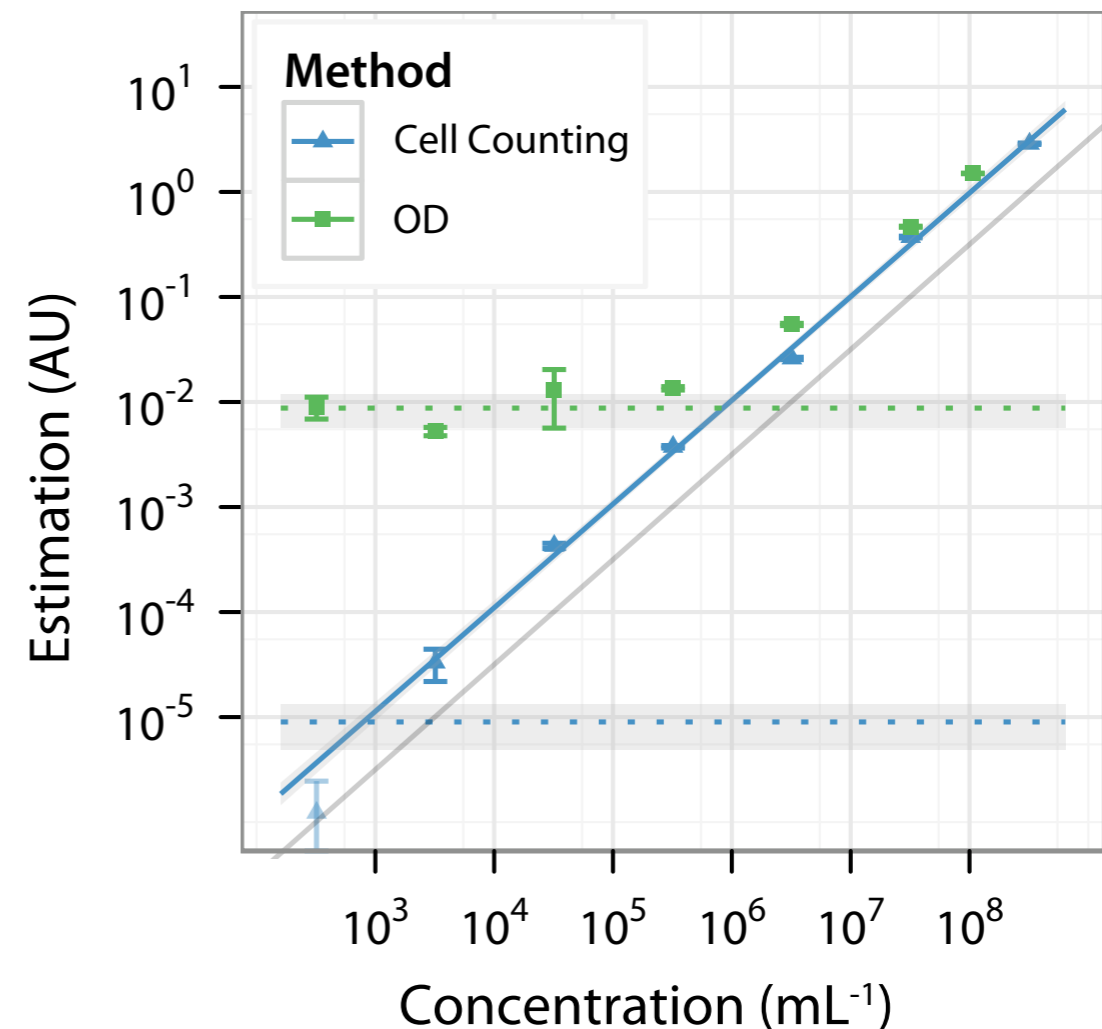
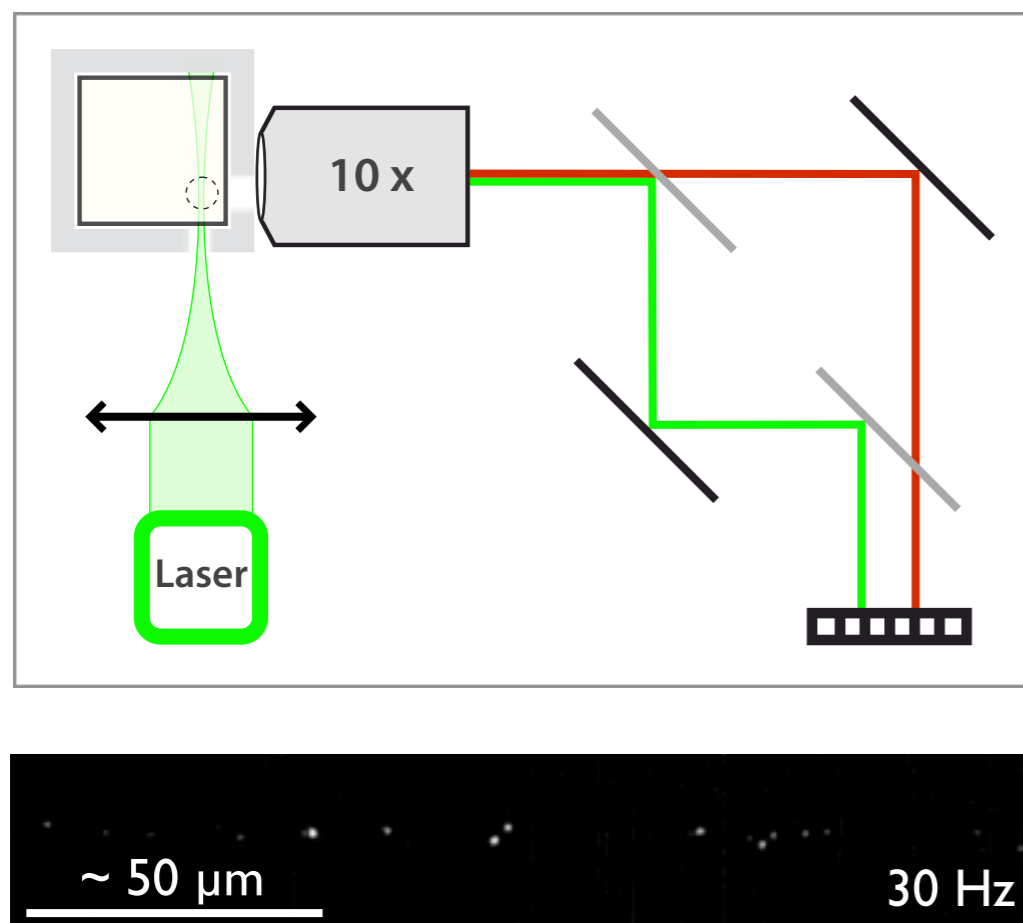


- Bacteria speed $\sim 1\text{-}10\mu\text{m/s}$
- Lateral heterogeneity of temperature
- Reduce convection intensity by
 - decreasing temperature heterogeneity
 - changing the chamber geometry
 - increasing the solution viscosity



Microbial population dynamics at low densities

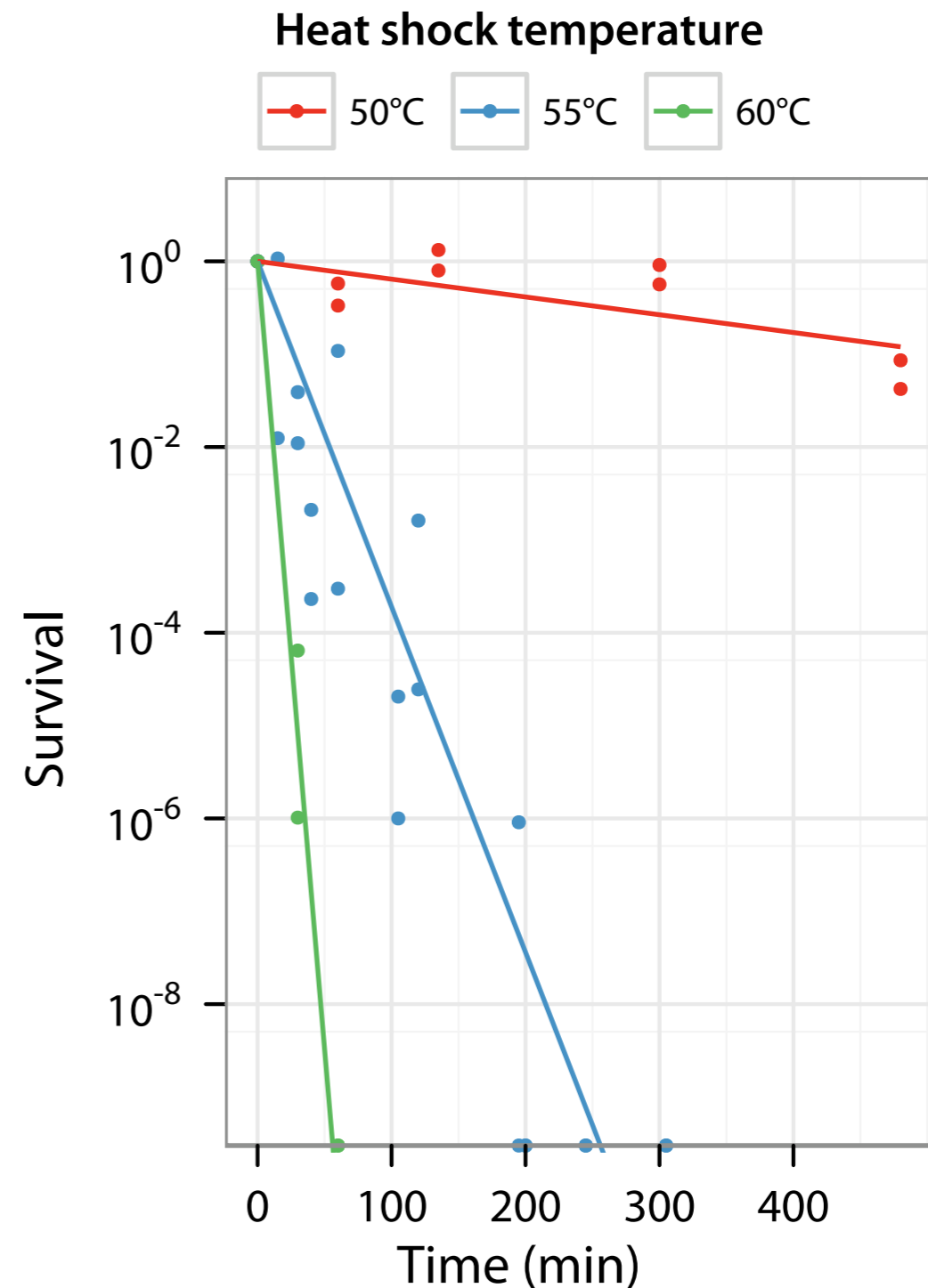
- Non invasive, continuous, cost-efficient measurement based on scattered light.
- Density estimation down to 10^3 mL^{-1} in liquid cultures.



Colonization of high temperature niches

Disentangling biological responses:

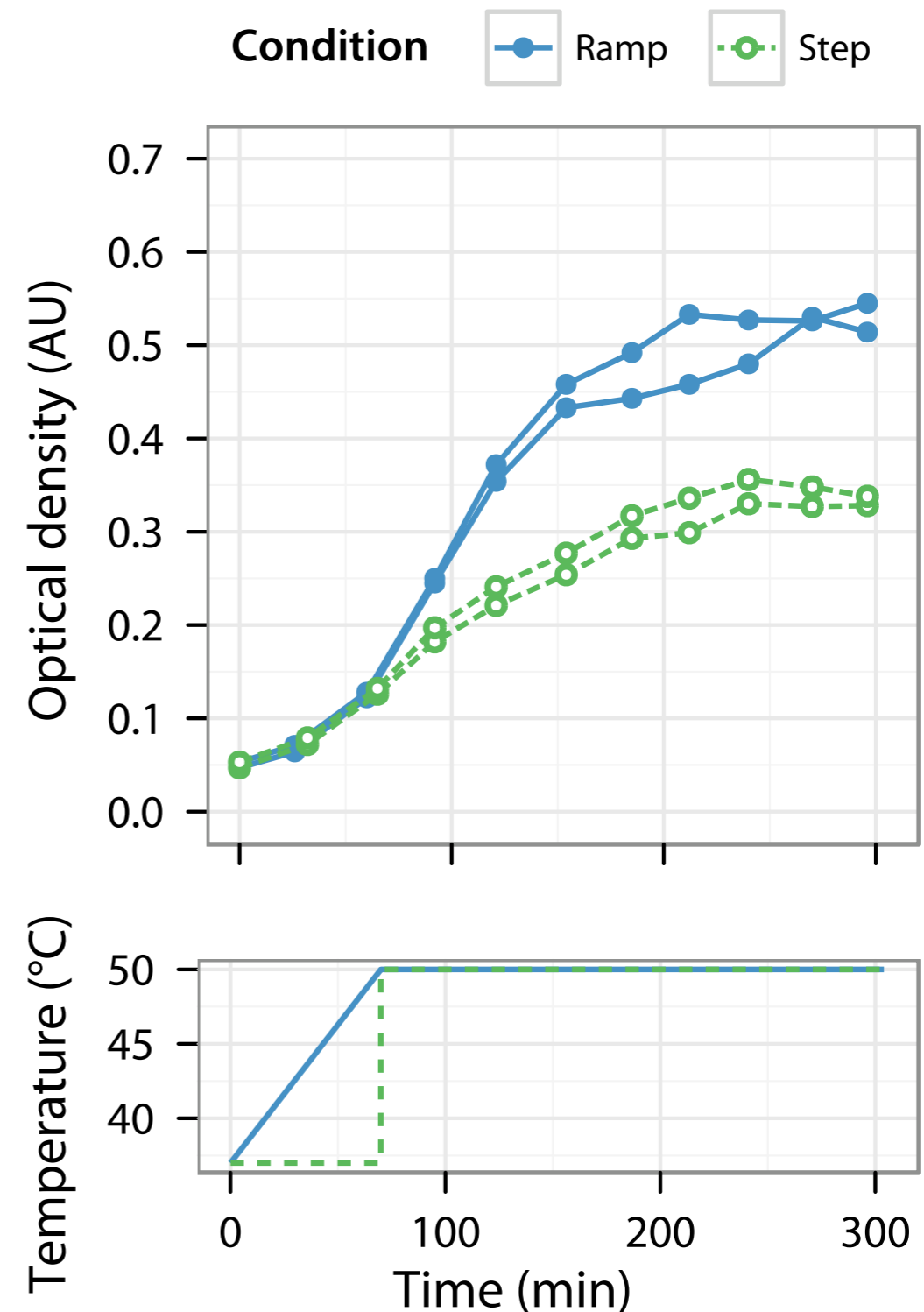
- Effect of high temperatures on growth and **survival**



Colonization of high temperature niches

Disentangling biological responses:

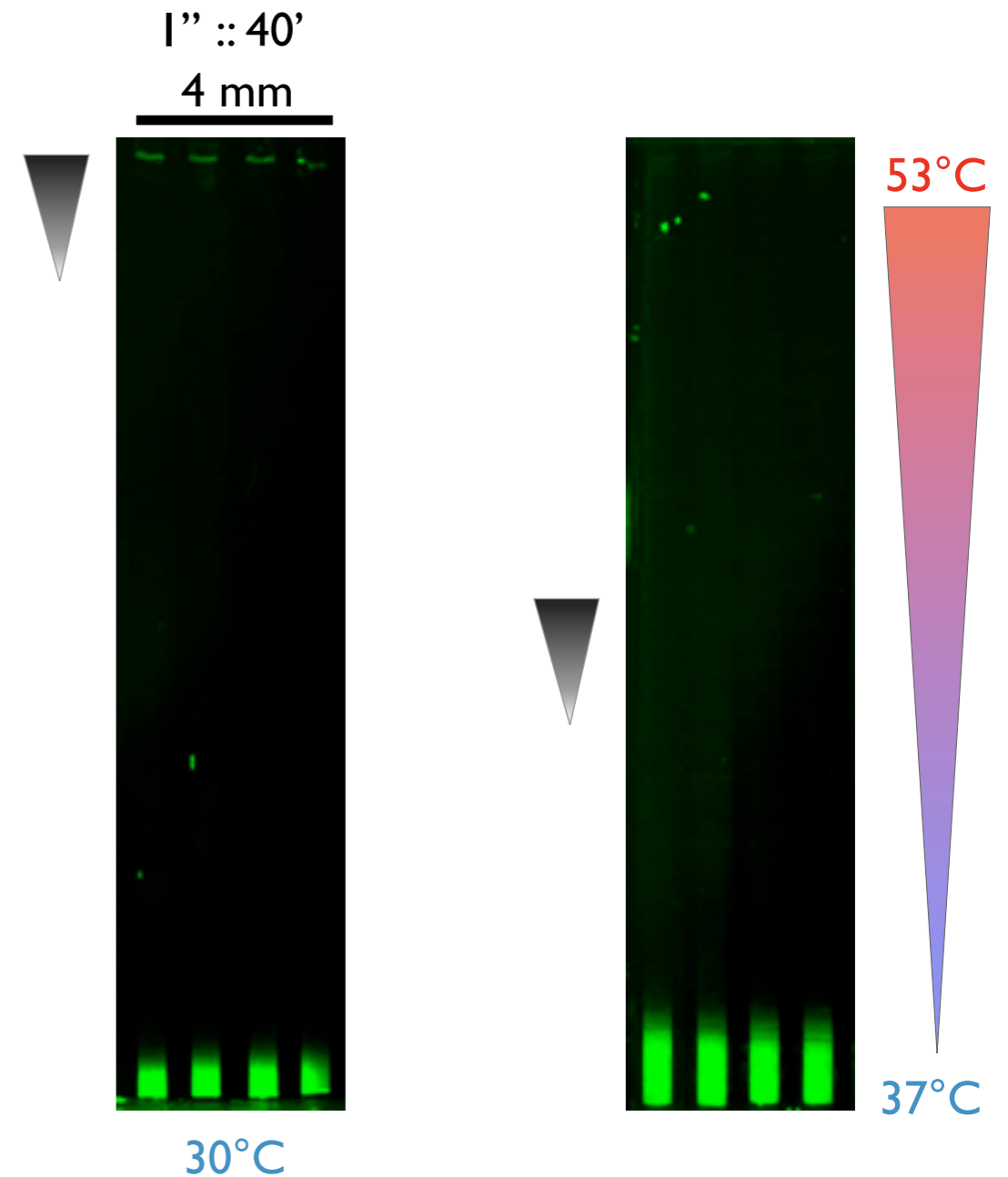
- Effect of high temperatures on growth and **survival**
- Impact of **acclimation** on growth and survival



Colonization of high temperature niches

Disentangling biological responses:

- Effect of high temperatures on growth and **survival**
- Impact of **acclimation** on growth and survival
- Role of **chemotaxis** and **thermotaxis**



in collaboration with Jonathan Saragosti (Curie Institute)

Overview

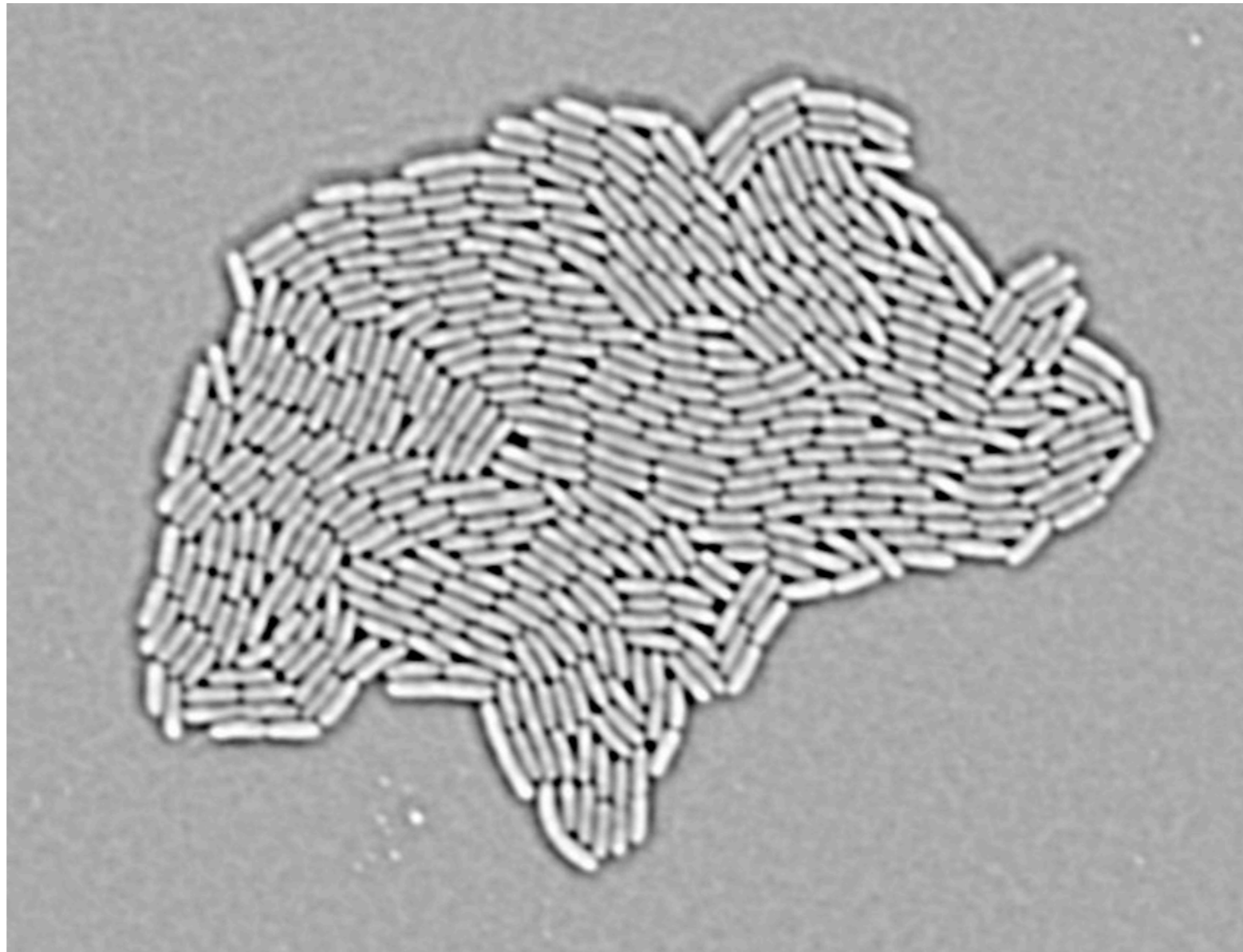
- Development of a setup in which bacteria evolve resistance to high temperature.
Characterization of a convective flow, and setup improvement to reduce it under $10\mu\text{m/s}$.
- Development of a concentration measurement method for bacterial cultures as diluted as 5000mL^{-1} .
- Characterization of biological responses involved in the colonization of high temperature niches.

Outline

1. Evolution in a temperature gradient
2. Variability of siderophores in clonal populations

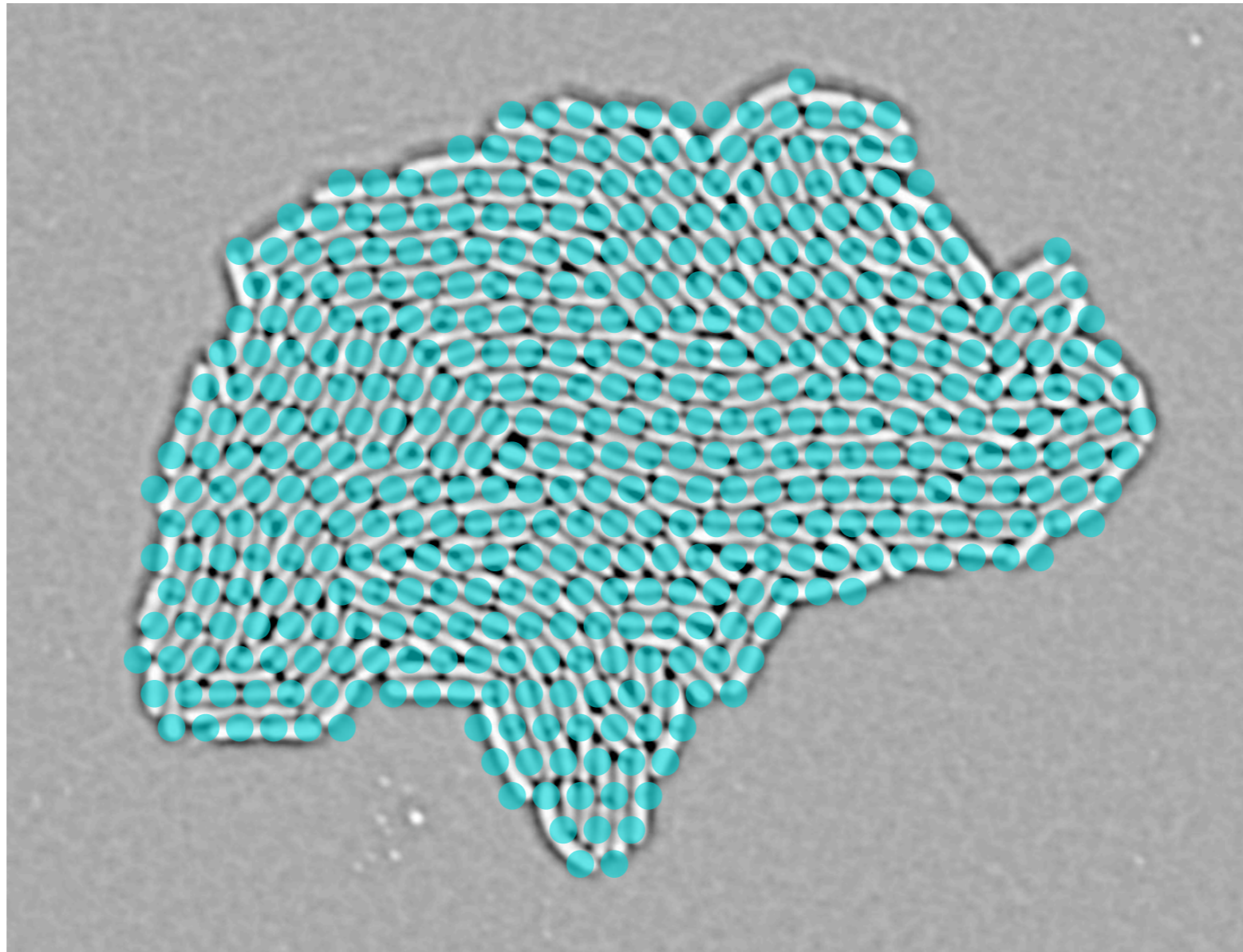
Public goods & Cooperation

- Conflict between selection at the level of the individual and at the level of the group



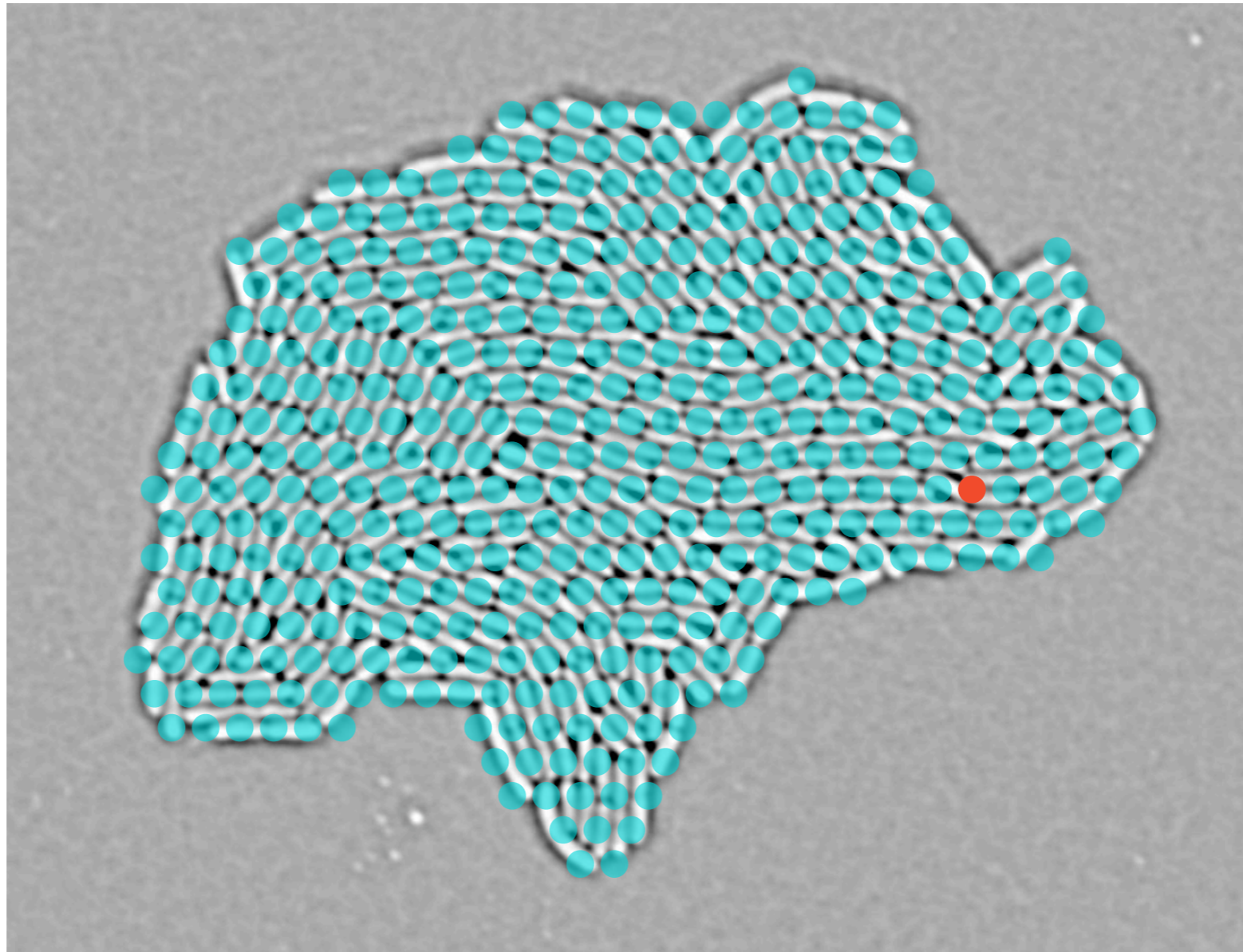
Public goods & Cooperation

- Conflict between selection at the level of the individual and at the level of the group



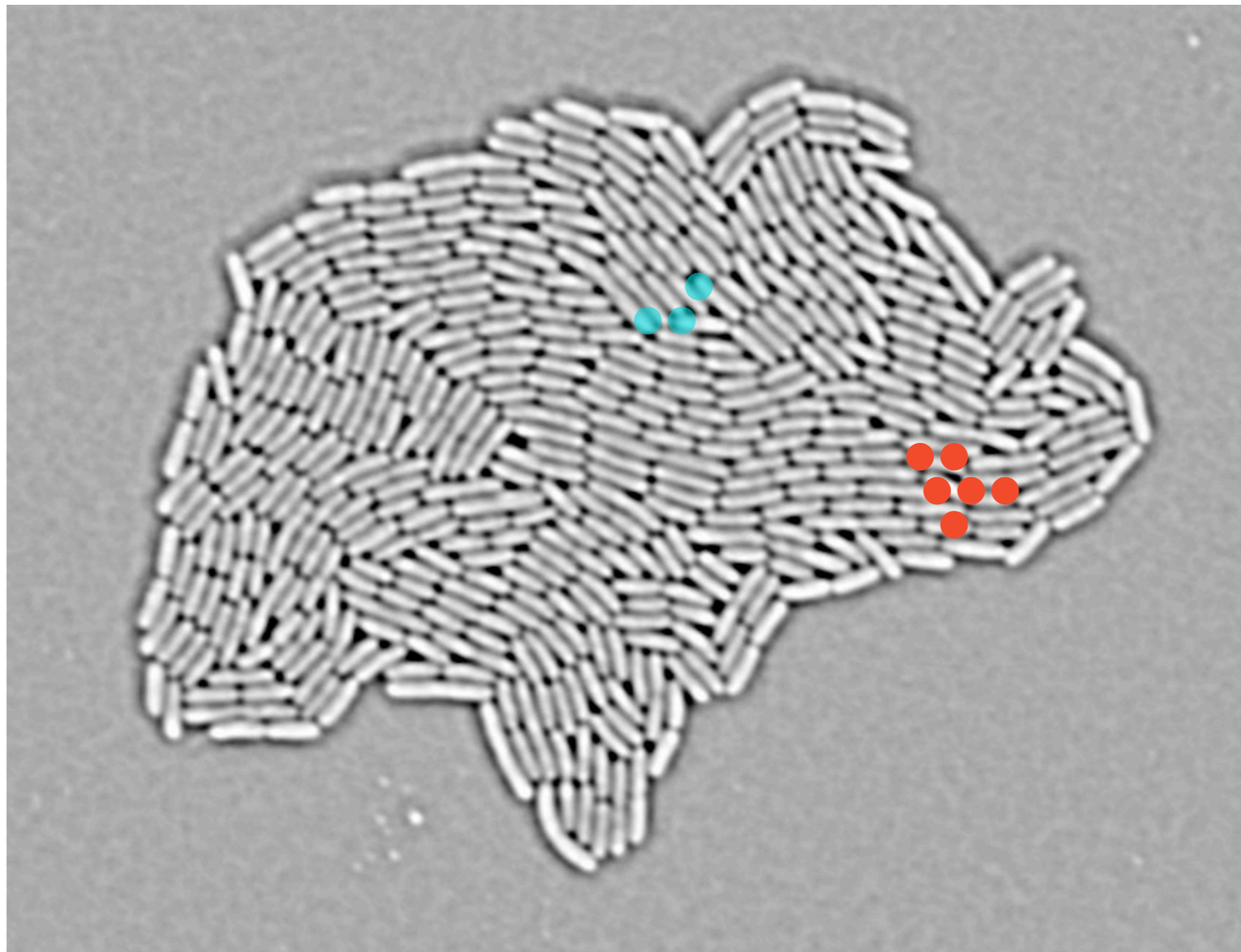
Public goods & Cooperation

- Conflict between selection at the level of the individual and at the level of the group



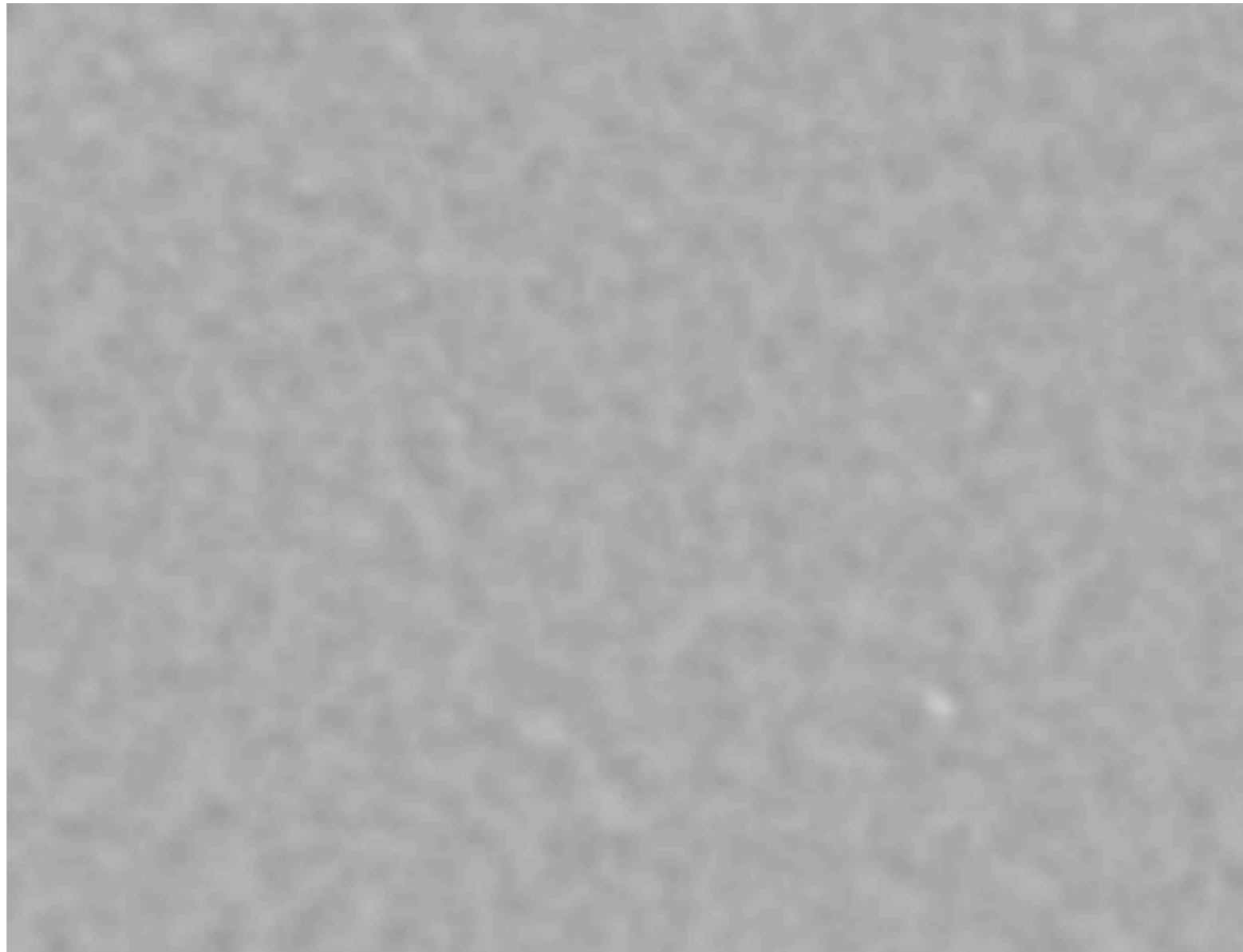
Public goods & Cooperation

- Conflict between selection at the level of the individual and at the level of the group



Public goods & Cooperation

- Conflict between selection at the level of the individual and at the level of the group

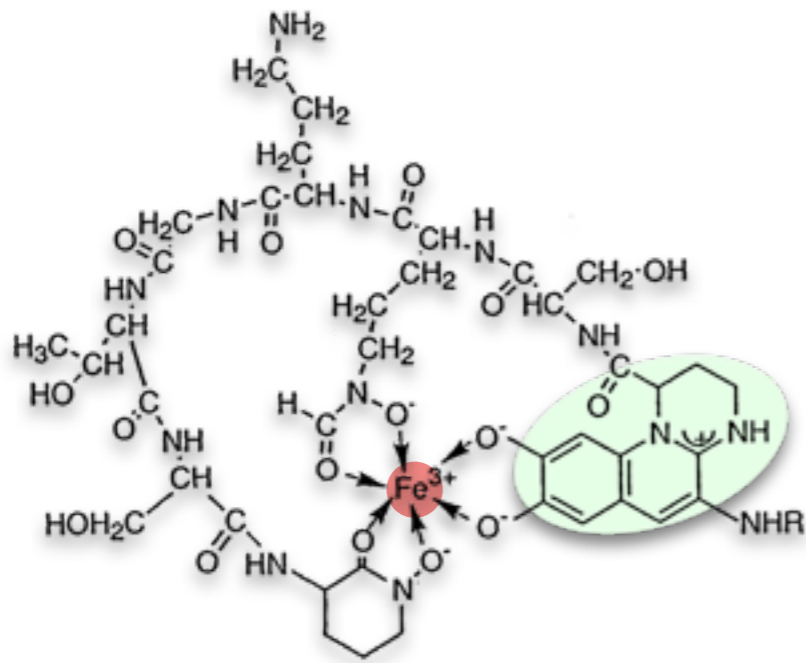


The struggle for iron

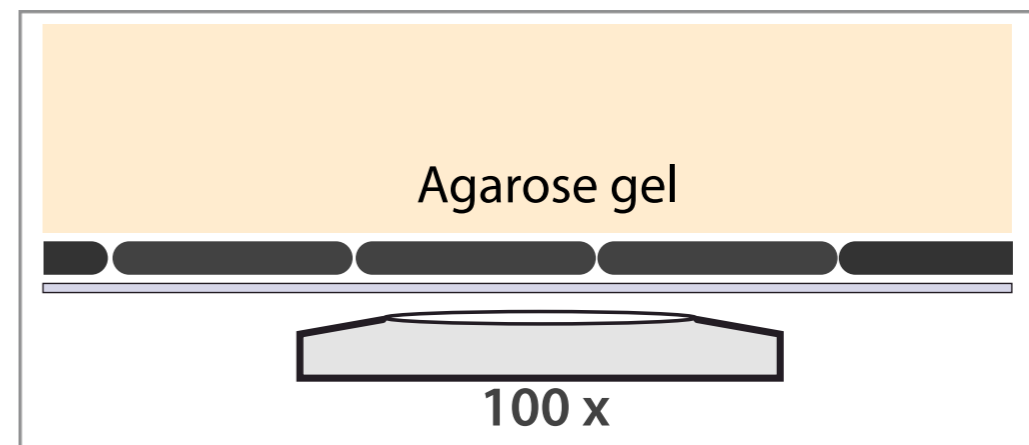
- Iron is widely used as enzyme cofactor and is essential for cellular metabolism $[\text{Fe}]_{\text{bacteria}} = 10^{-6}\text{M}$
 - Nucleic acids maintenance and replication
 - Gene expression
 - Energy production (electron transfer chain)
- Soluble iron is at very low concentration in nature (due to O_2)
 $\text{Fe}^{3+} + 3 \text{HO}^- \rightleftharpoons \text{Fe}(\text{OH})_3$ $[\text{Fe}]_{\text{free}} = 10^{-9} - 10^{-19}\text{M}$
- Bacteria growth depends on their ability to concentrate iron: Siderophores are secreted molecules with very high iron affinity

Siderophores in *Pseudomonas aeruginosa*

- Pyoverdine is the major siderophore in *P. aeruginosa*
 $K_a \text{ pyoverdine} \approx 10^{32} \text{ M}^{-1}$
- Pyoverdine is a public good
(improving growth and secreted)
- Pyoverdine is naturally fluorescent



(Del Olmo, *et al.* 2003)

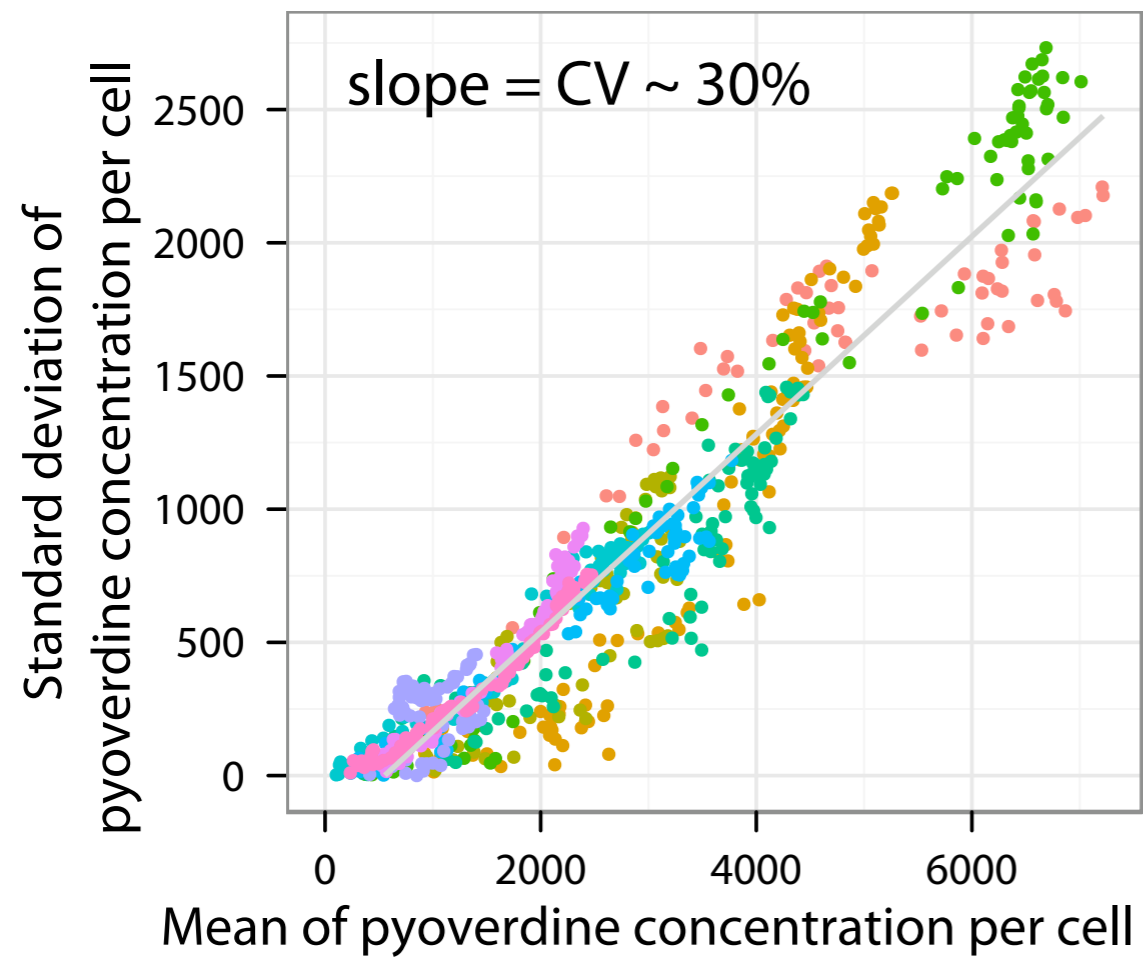


Dynamics of pyoverdine levels

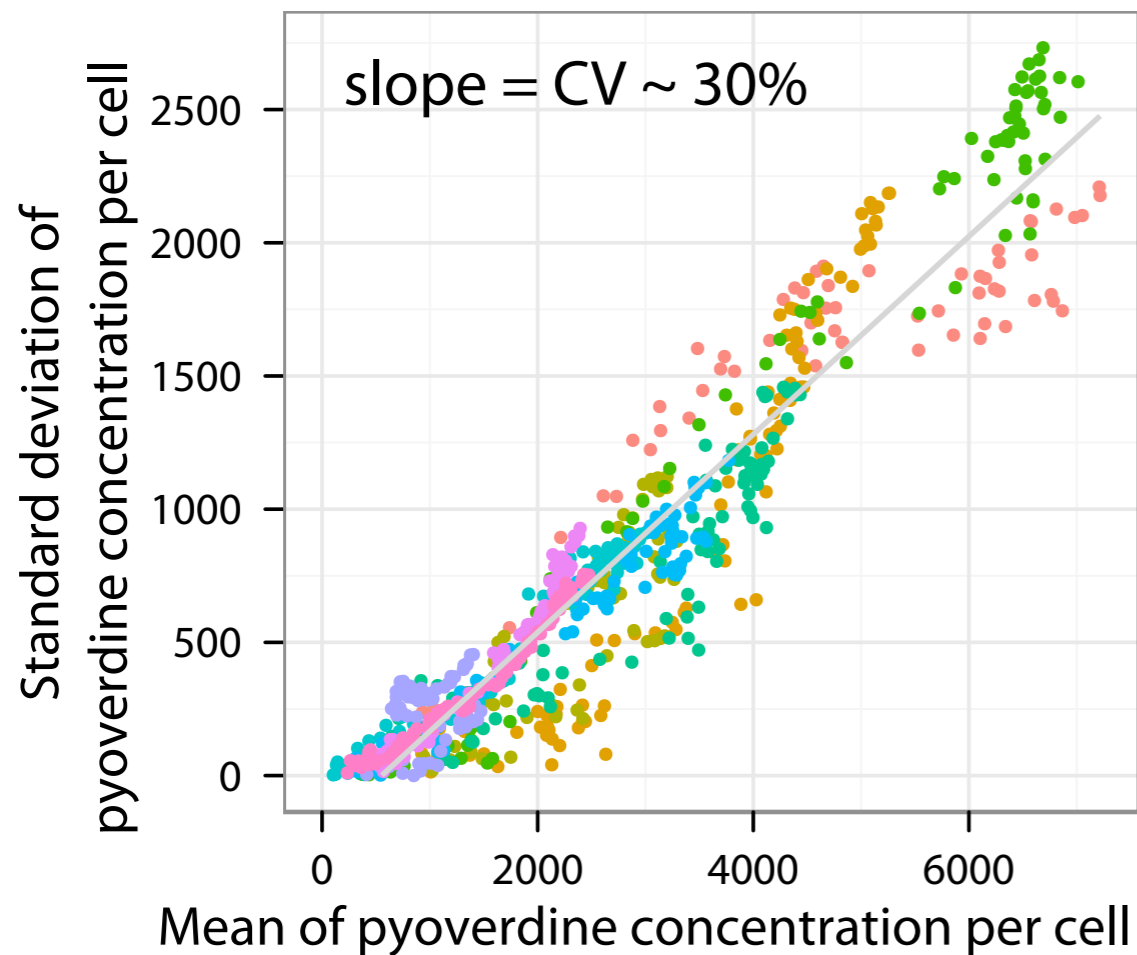
1" :: 75'

5 μ m

Pyoverdine distribution in a clonal population



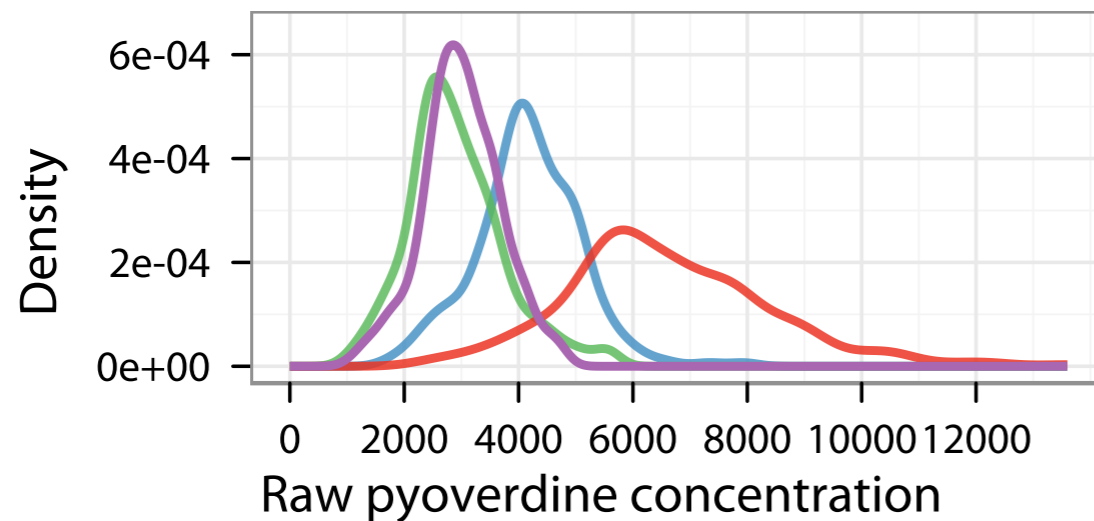
Pyoverdine distribution in a clonal population



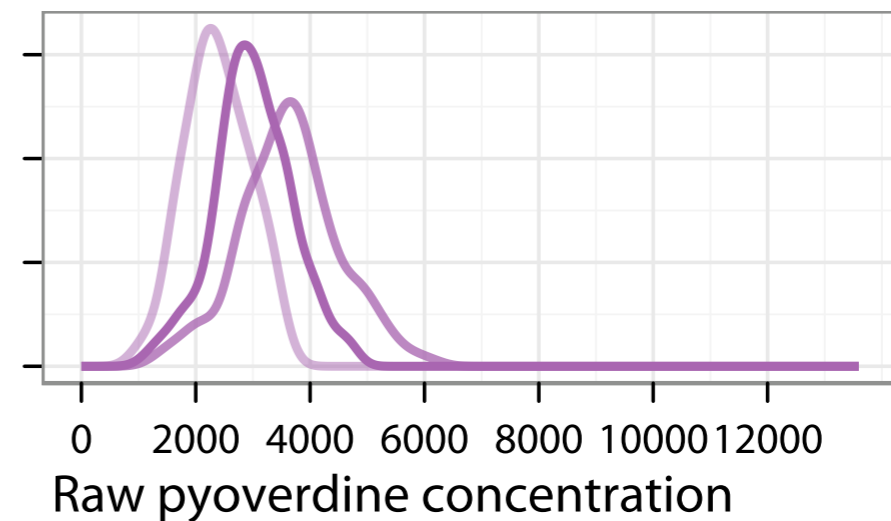
- Number of pyoverdines per cell
~ 500 to 5000
- Large number N of molecules
mean $\propto N$ and $sd \propto \sqrt{N}$
- Expected coefficient of variation
 $CV = sd / \text{mean} \propto 1/\sqrt{N}$
~ 1 to 5%

Pyoverdine distribution in a clonal population

Different movies

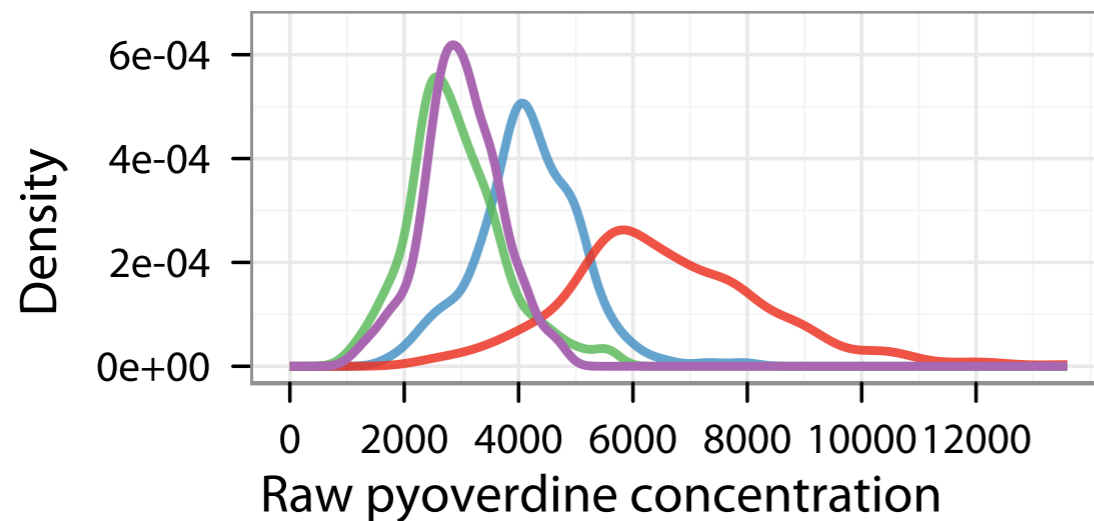


Different frames

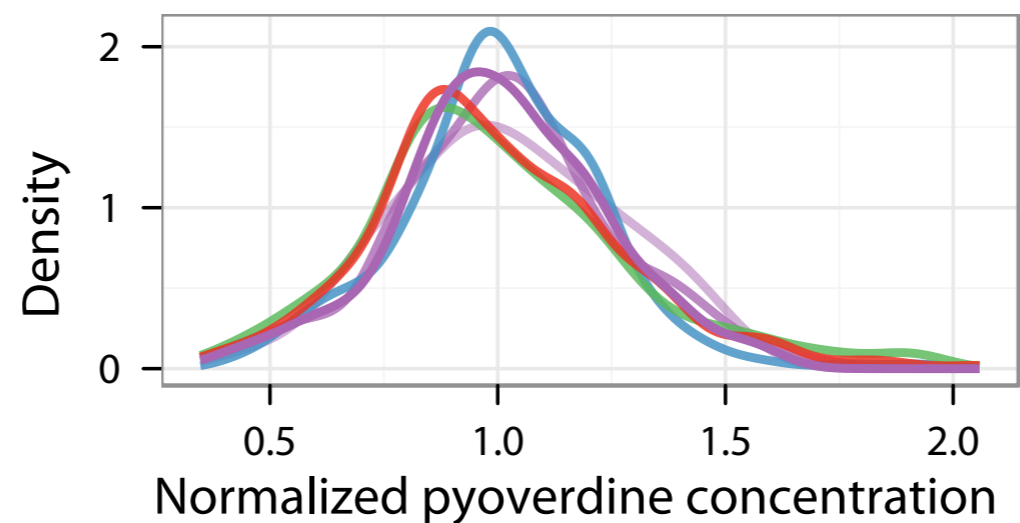
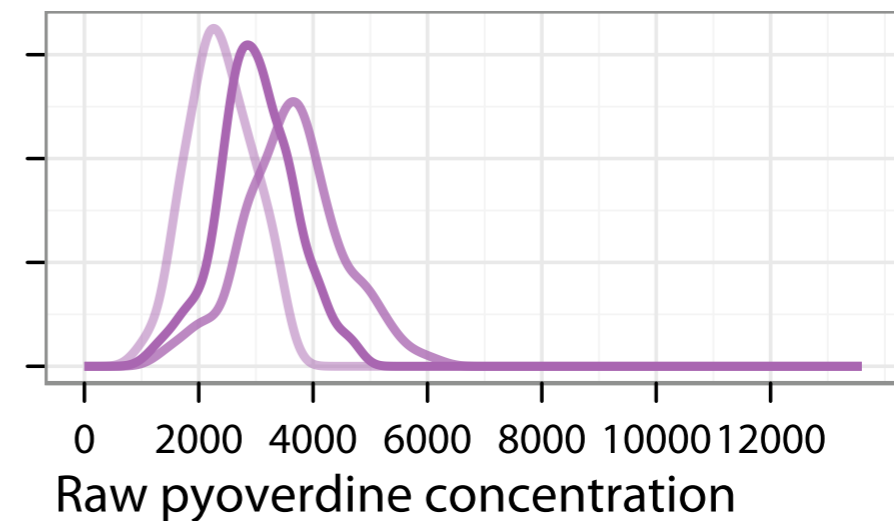


Pyoverdine distribution in a clonal population

Different movies

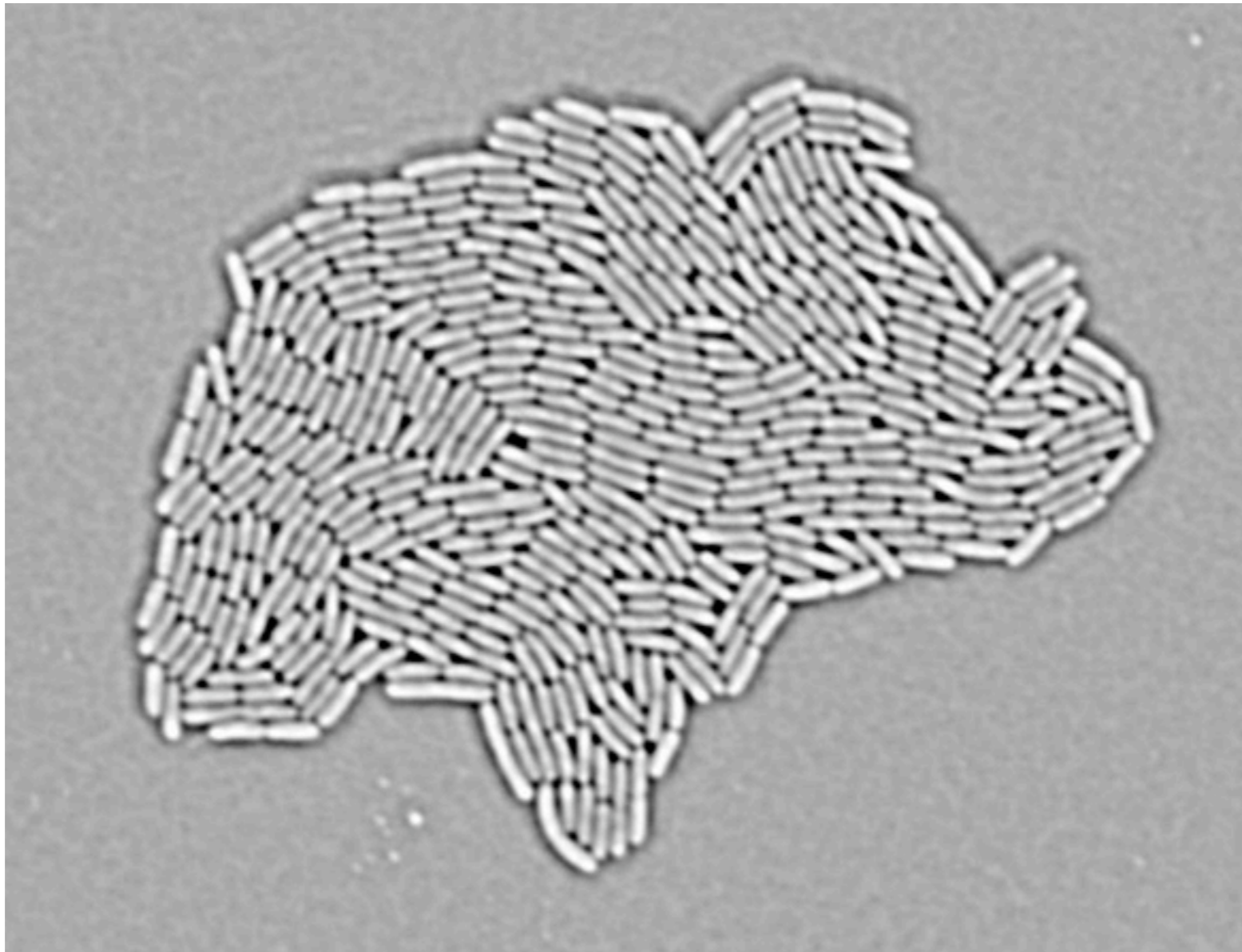


Different frames



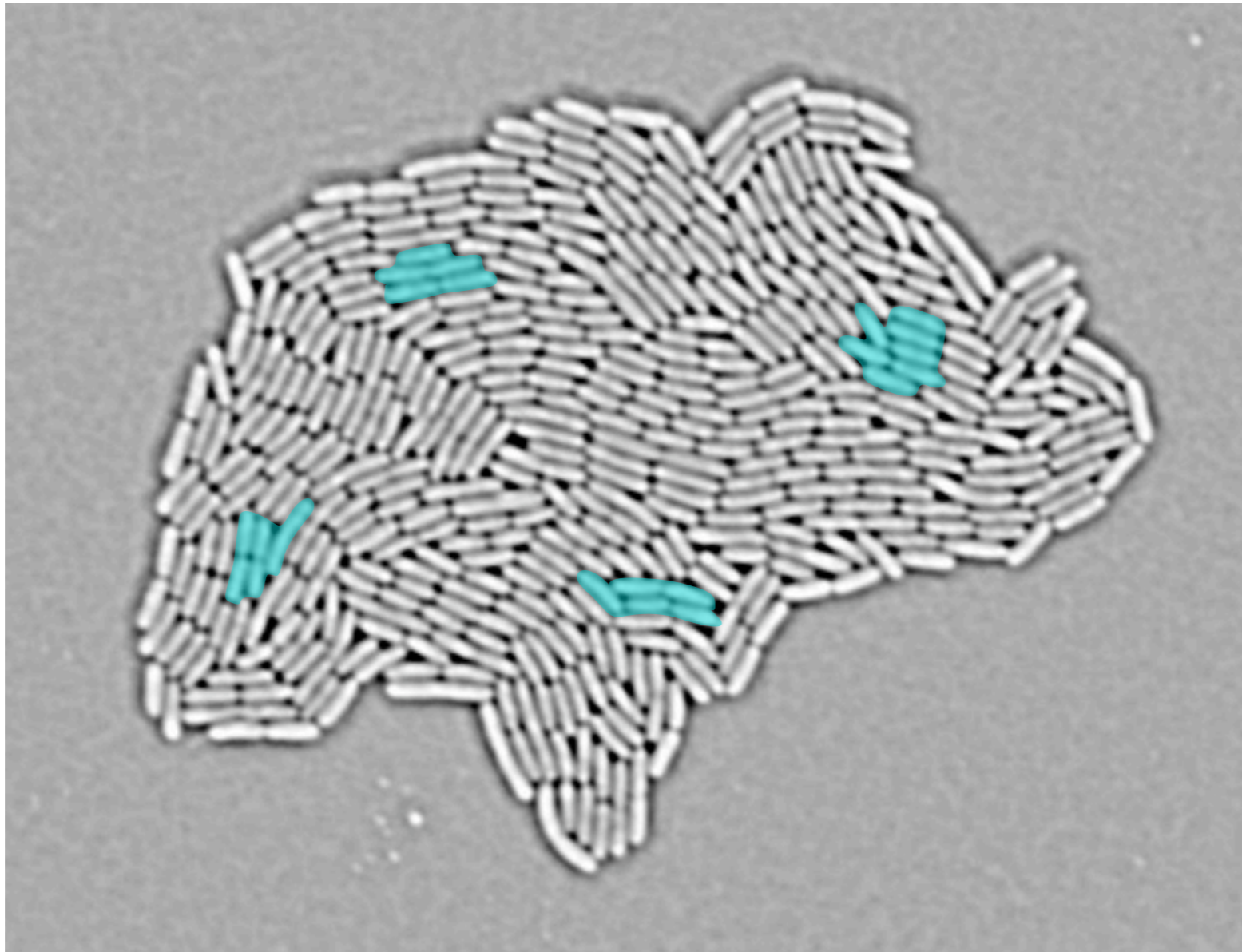
Cooperation & Phenotypic variability

What distribution do you expect for a cooperative trait in a clonal population?



Cooperation & Phenotypic variability

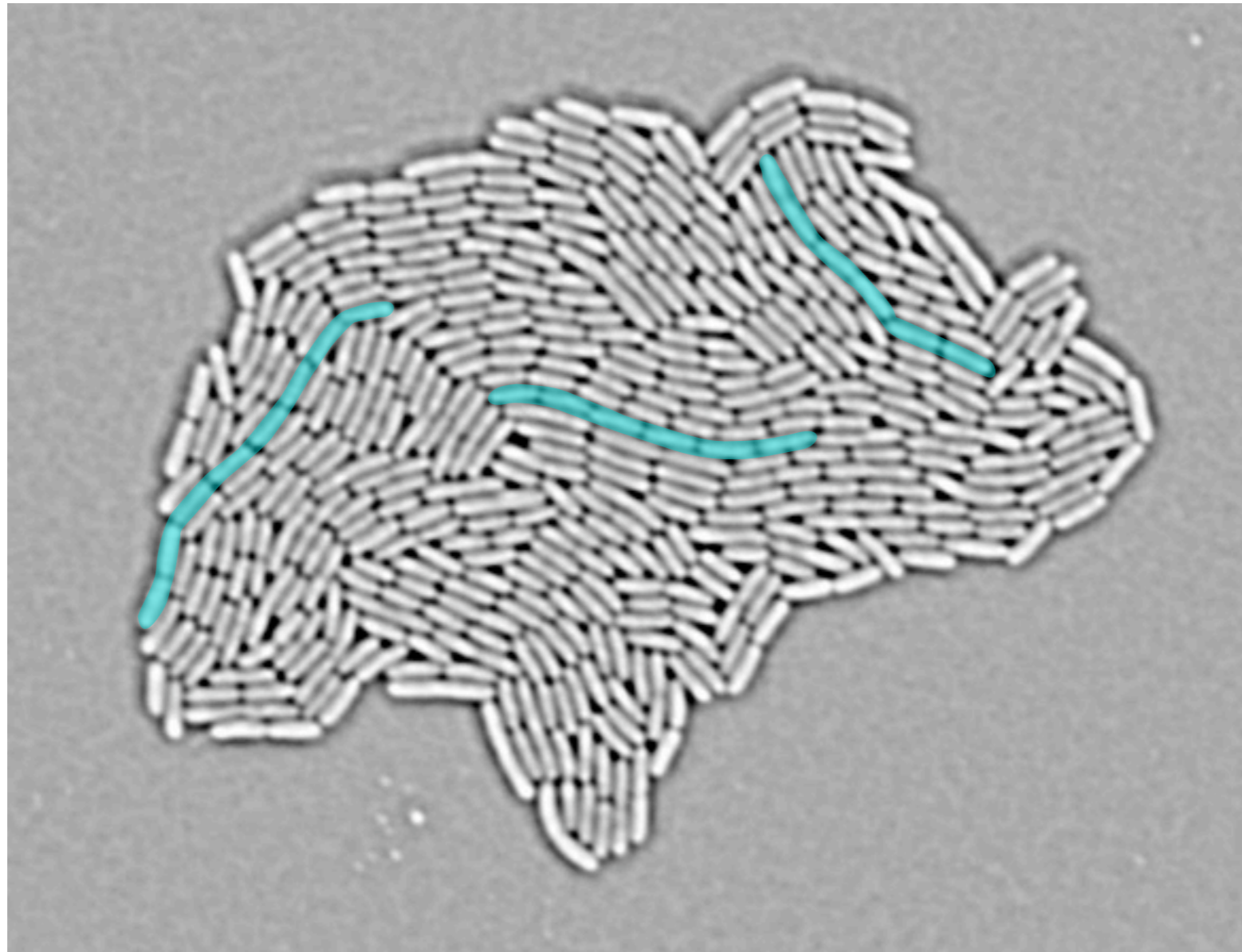
What distribution do you expect for a cooperative trait in a clonal population?



I. Neighbours
reciprocity

Cooperation & Phenotypic variability

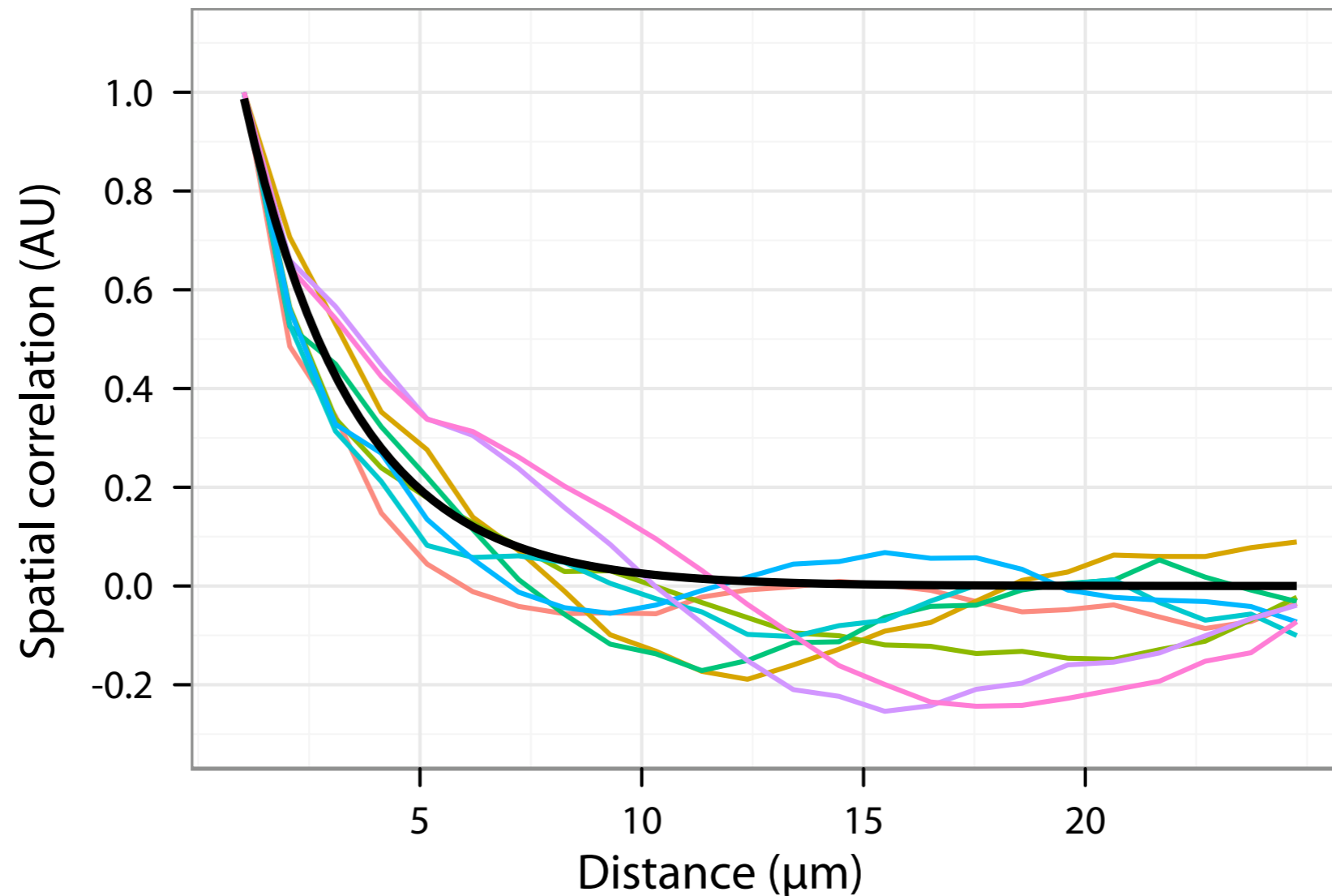
What distribution do you expect for a cooperative trait in a clonal population?



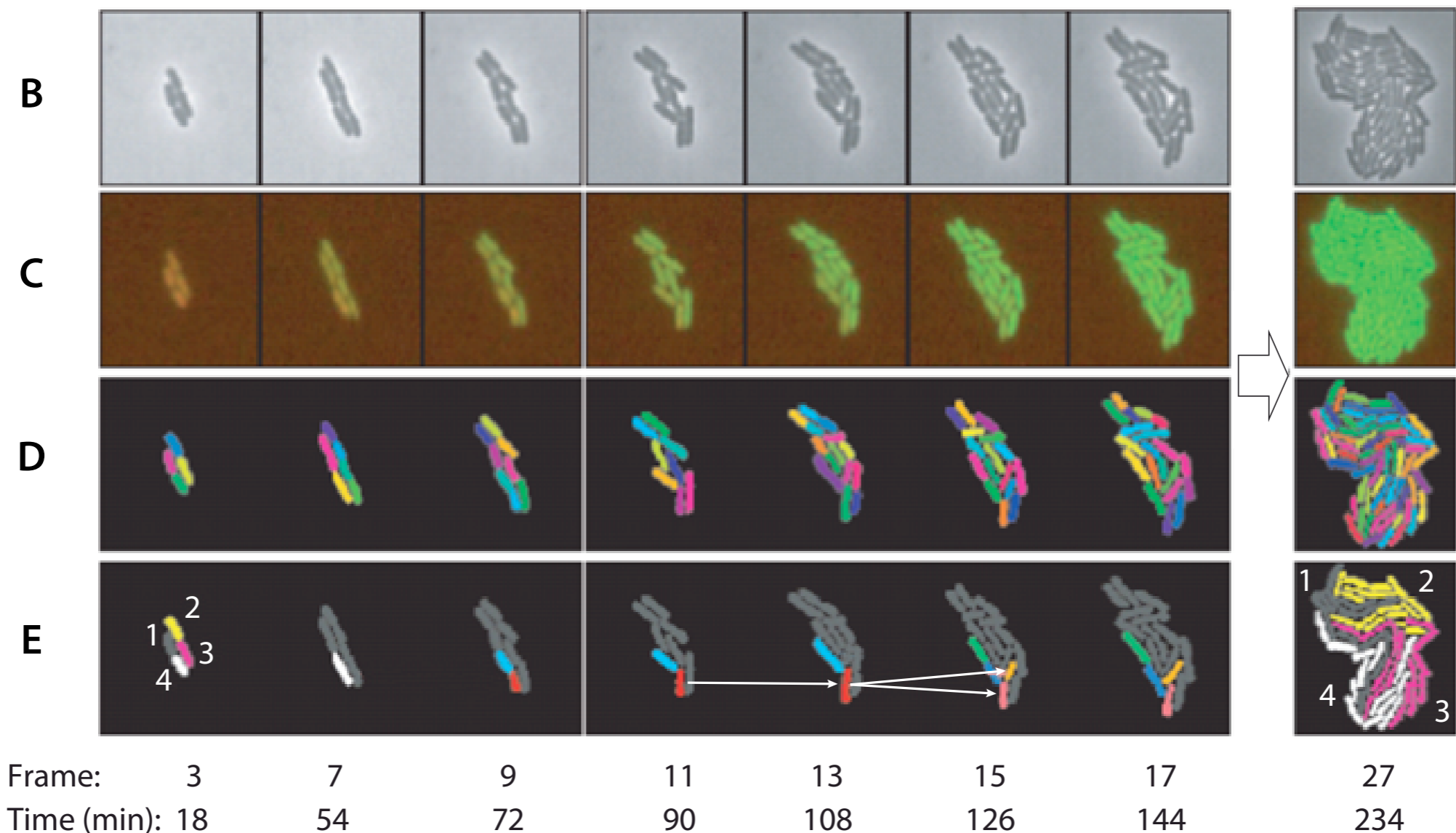
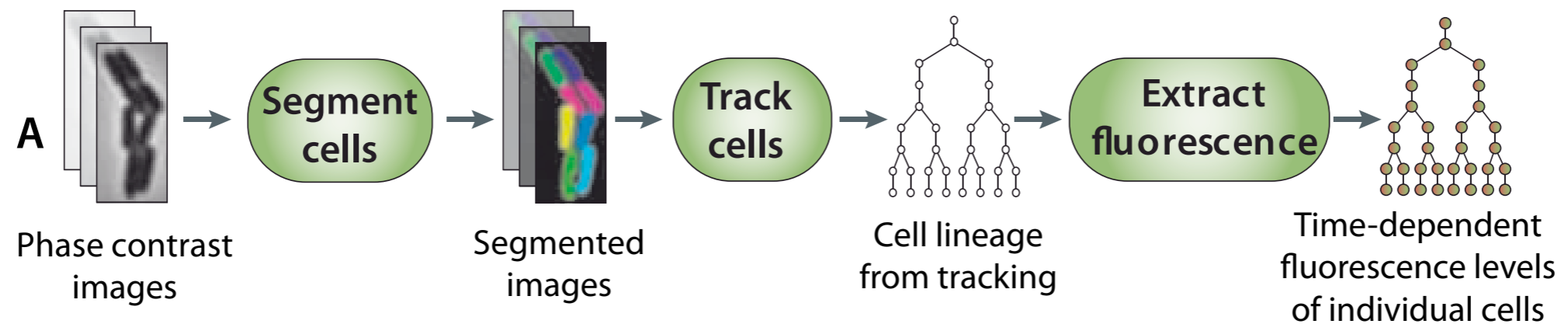
1. Neighbours reciprocity
2. Heritability

Spatial structure

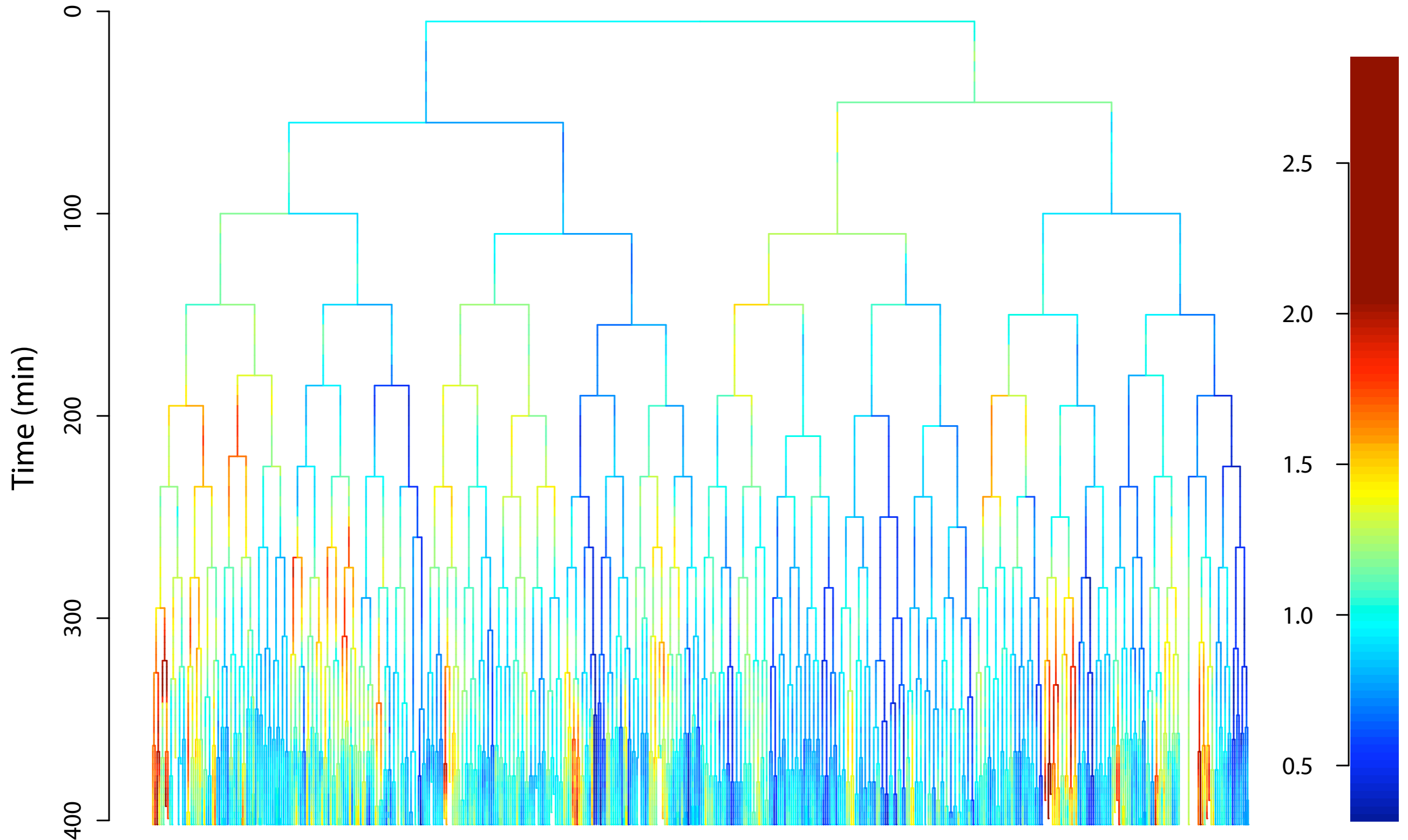
- Spatial correlation of average fluorescence per cell on one image (at stage 300 cells)
- Correlation characteristic length = $2.5\mu\text{m}$ \sim bacteria length



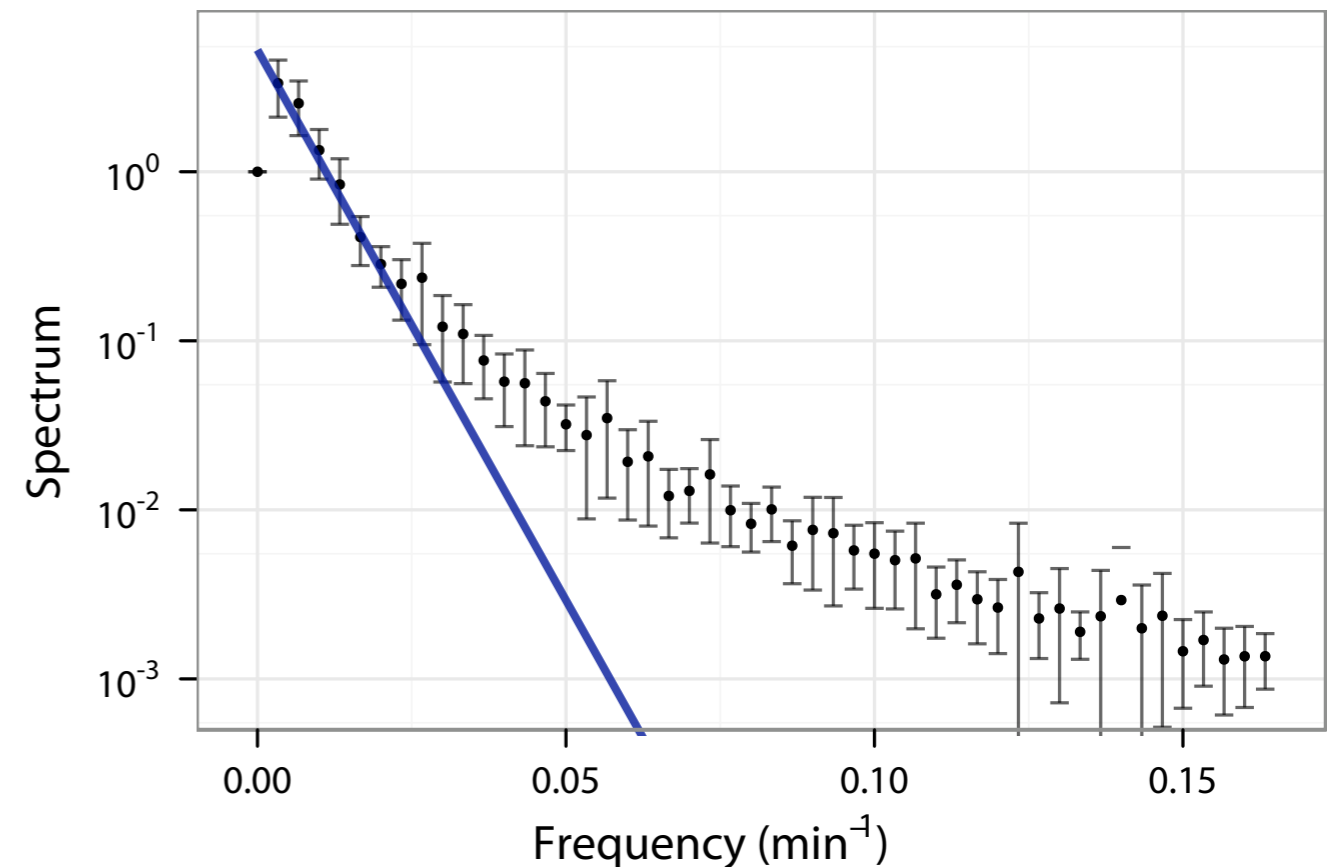
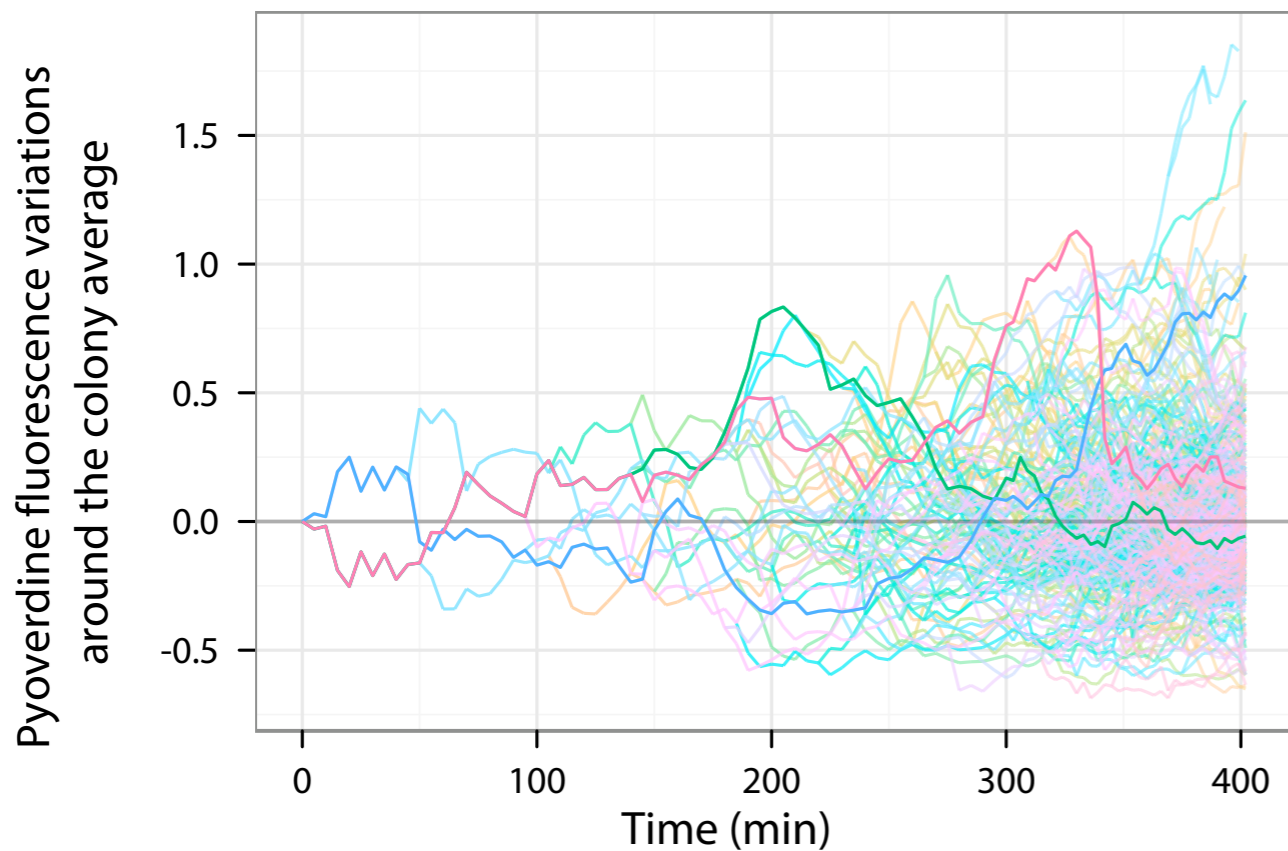
Single cell methodology



Heritability



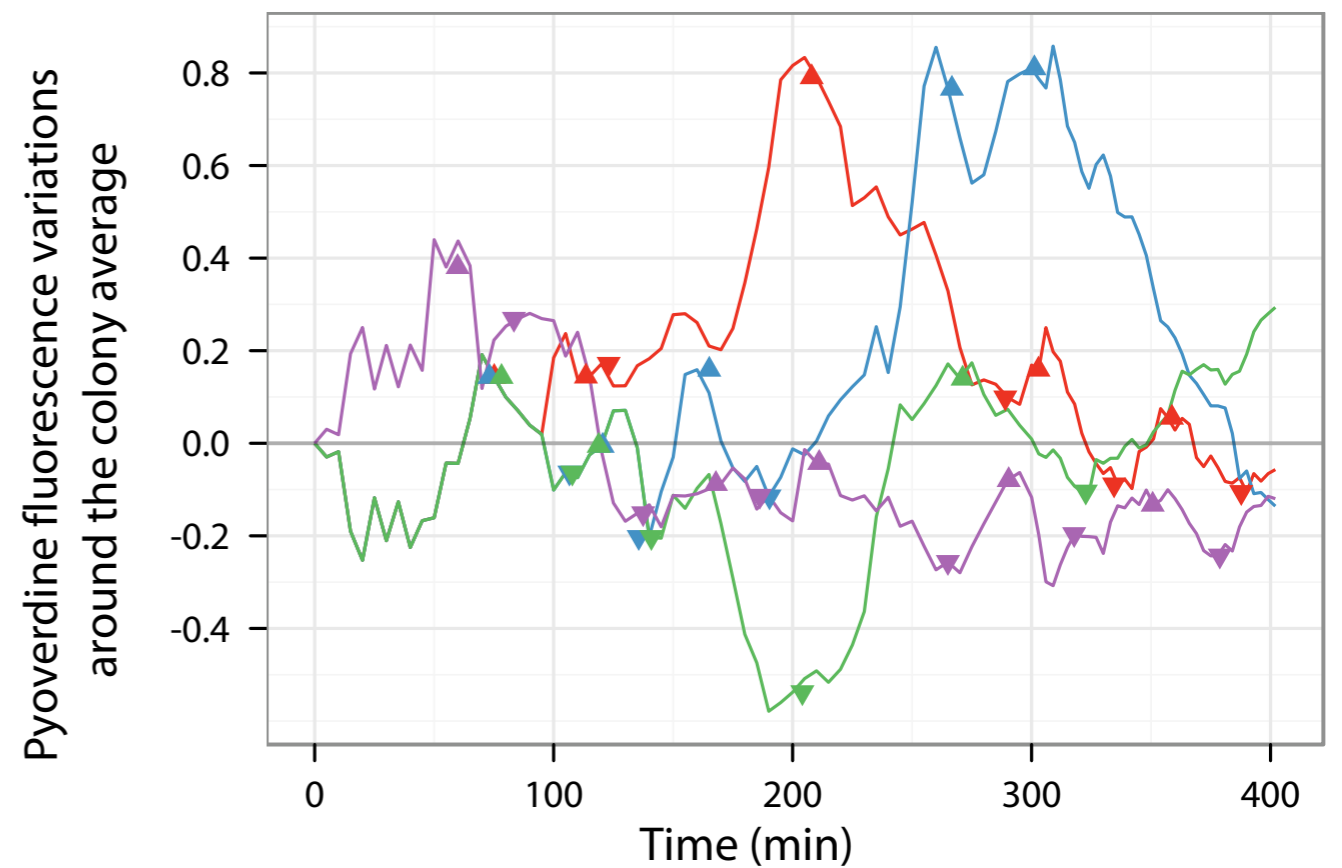
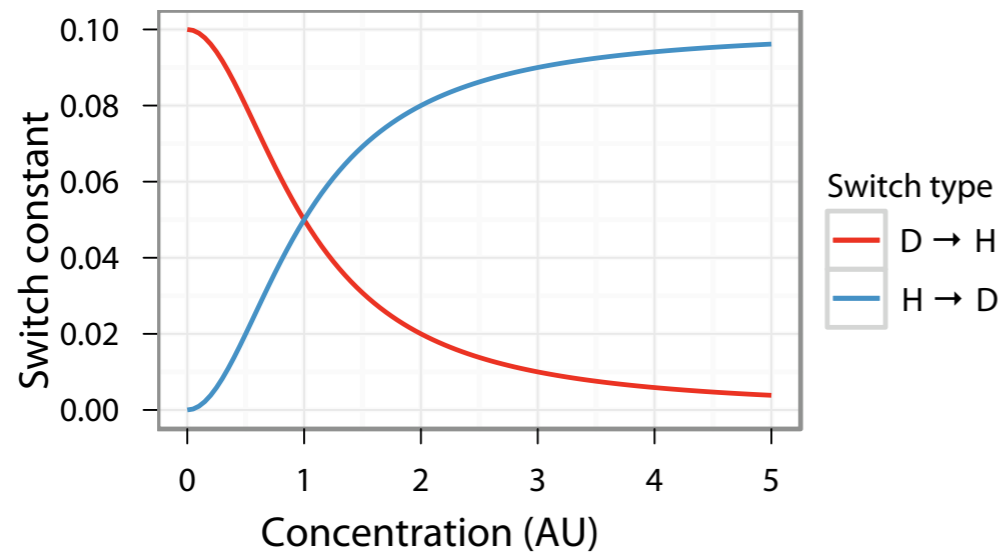
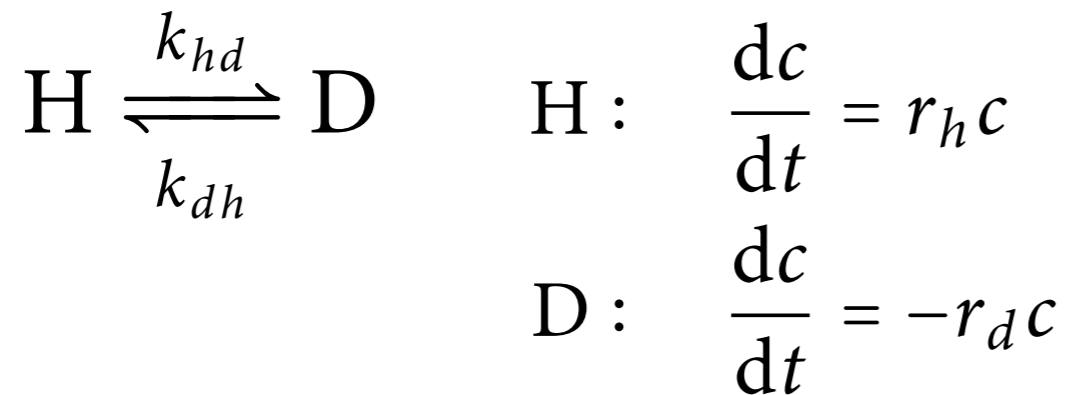
Dynamics of pyoverdine concentrations



- Fluorescence fluctuations along the branches
- No characteristic timescale of fluctuations
- Decorrelation time $\approx 120' \sim 2.5$ generations

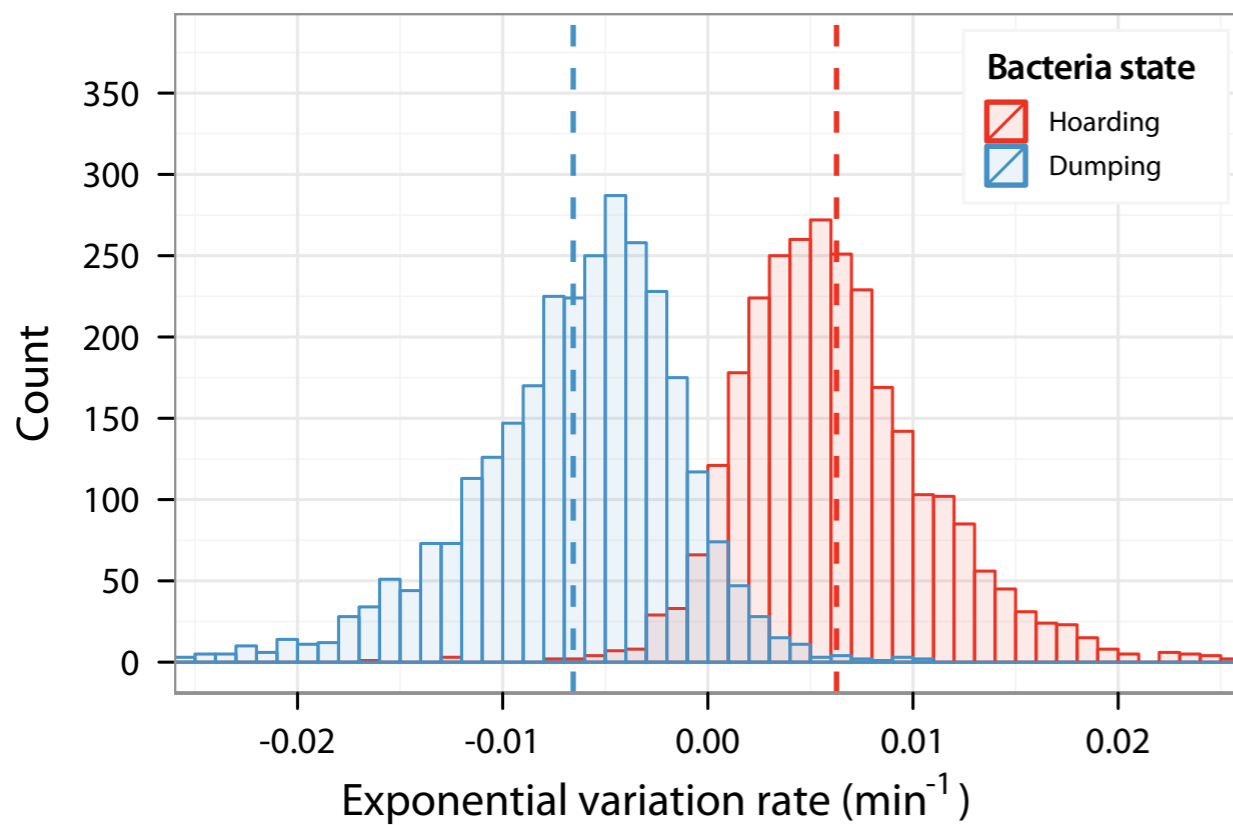
Modeling pyoverdine dynamics

A two-states model with a stochastic phenotypic switch governing pyoverdine metabolism (H: *hoarding* – D: *dumping*)

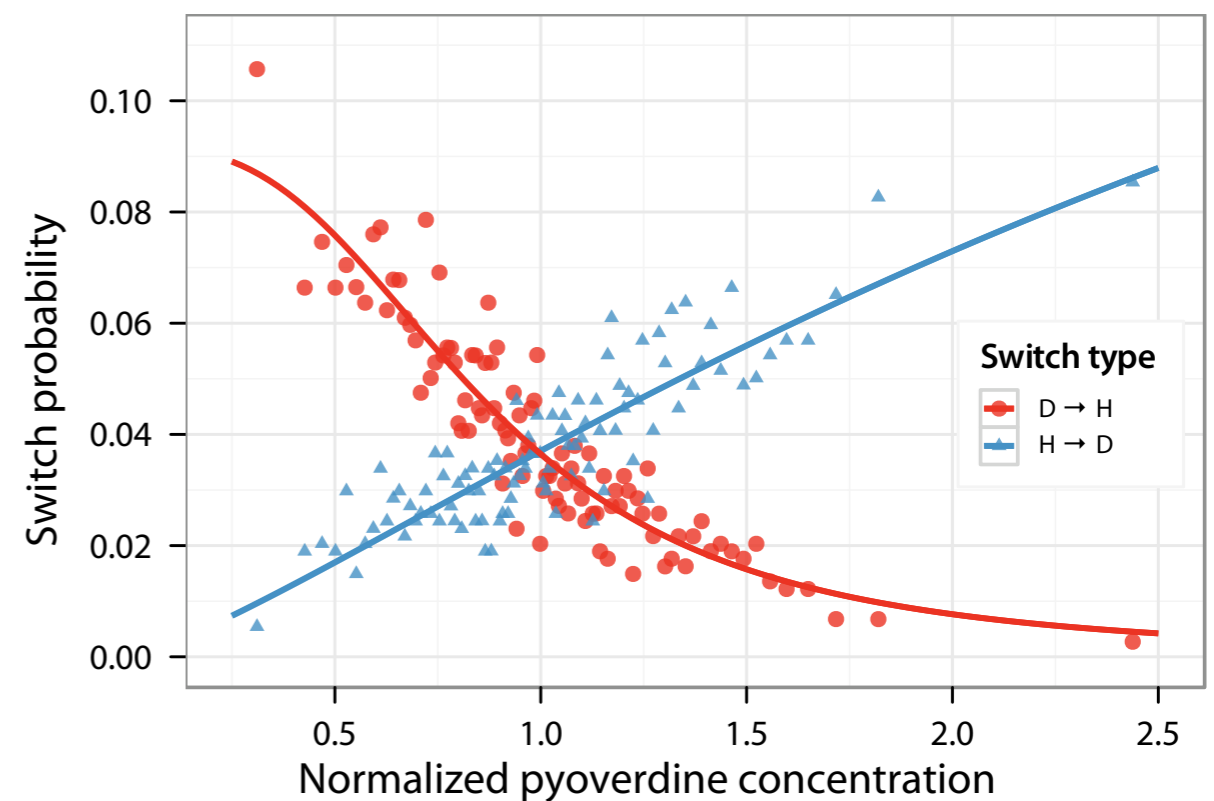


Modeling pyoverdine dynamics

Variation rates, r

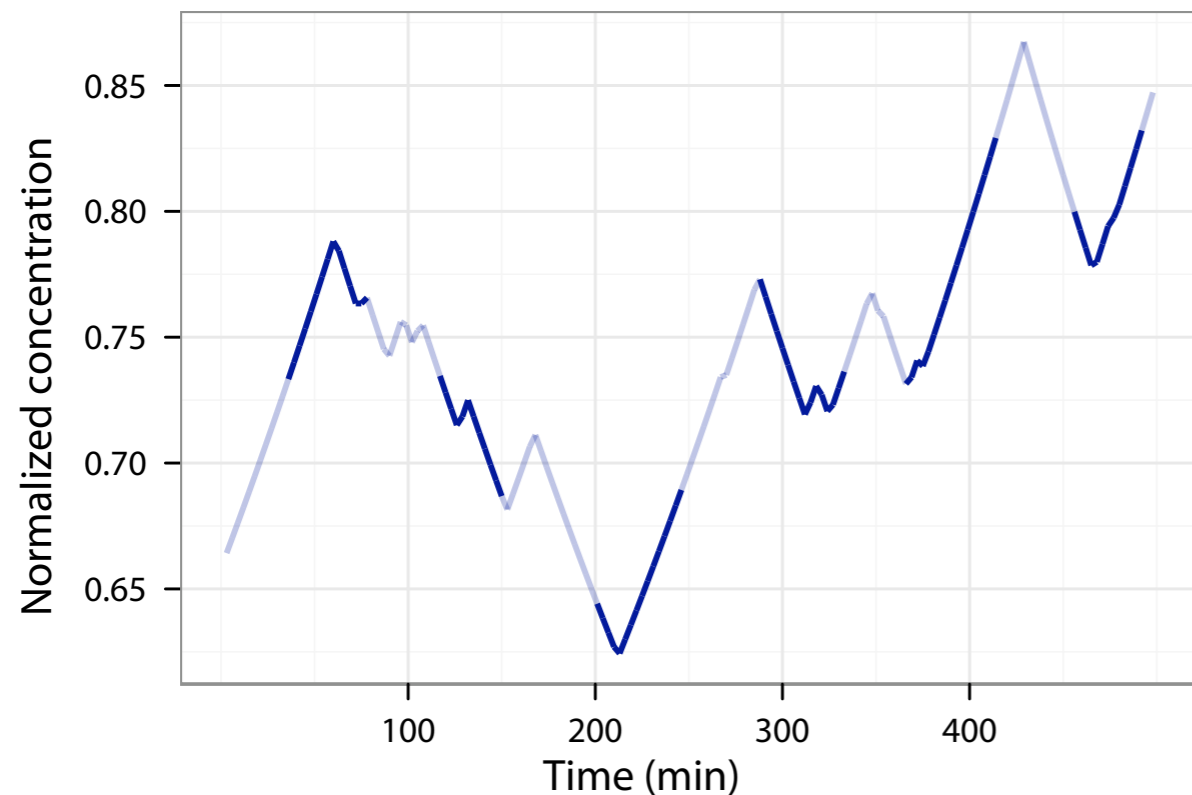


Switch constants, k

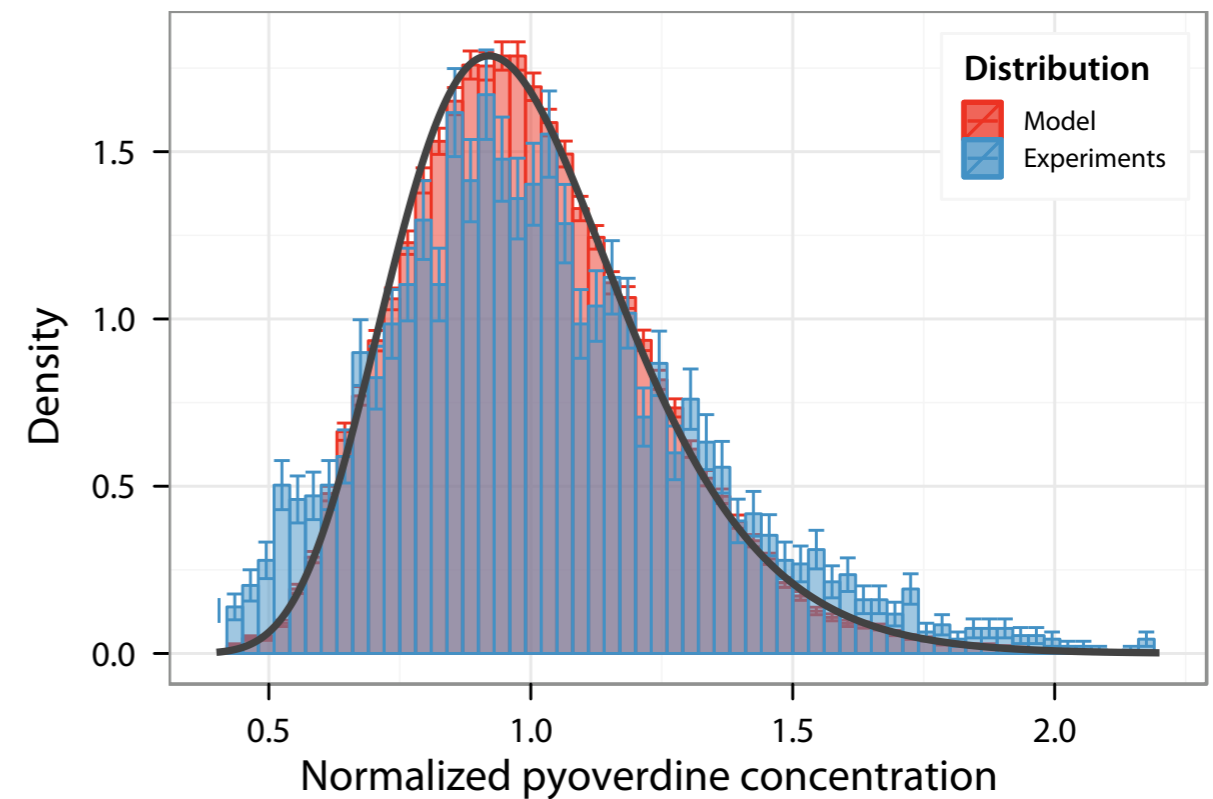


Modeling pyoverdine dynamics

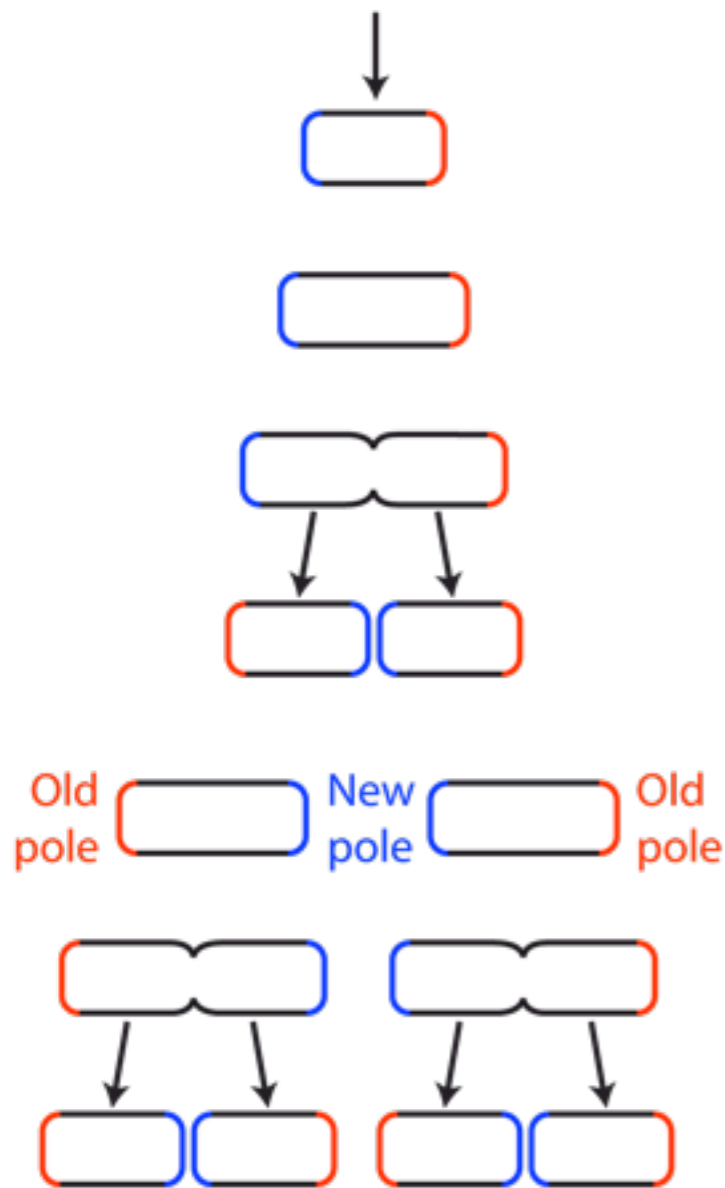
Simulated time series



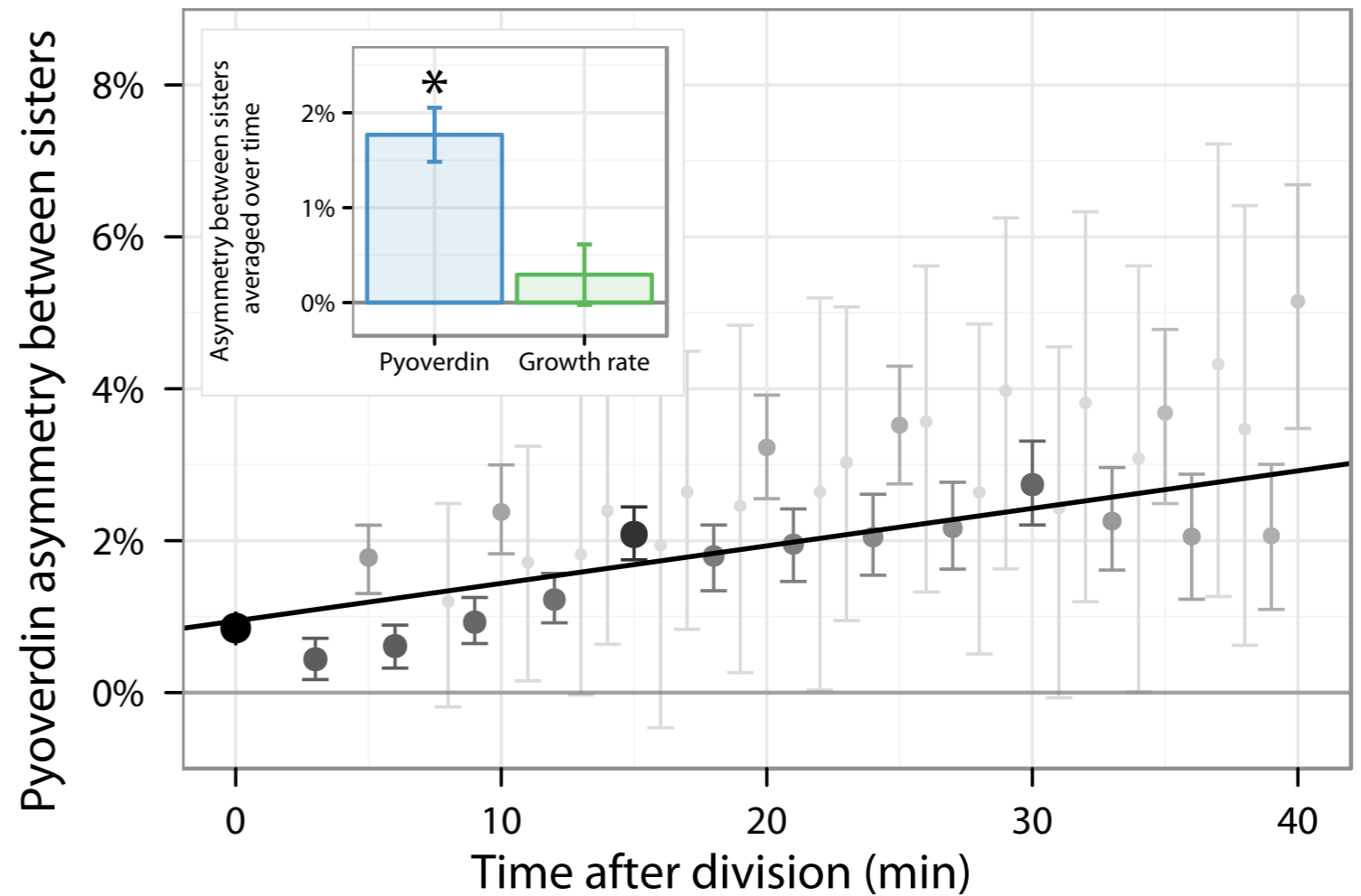
Distribution



Asymmetry between sisters

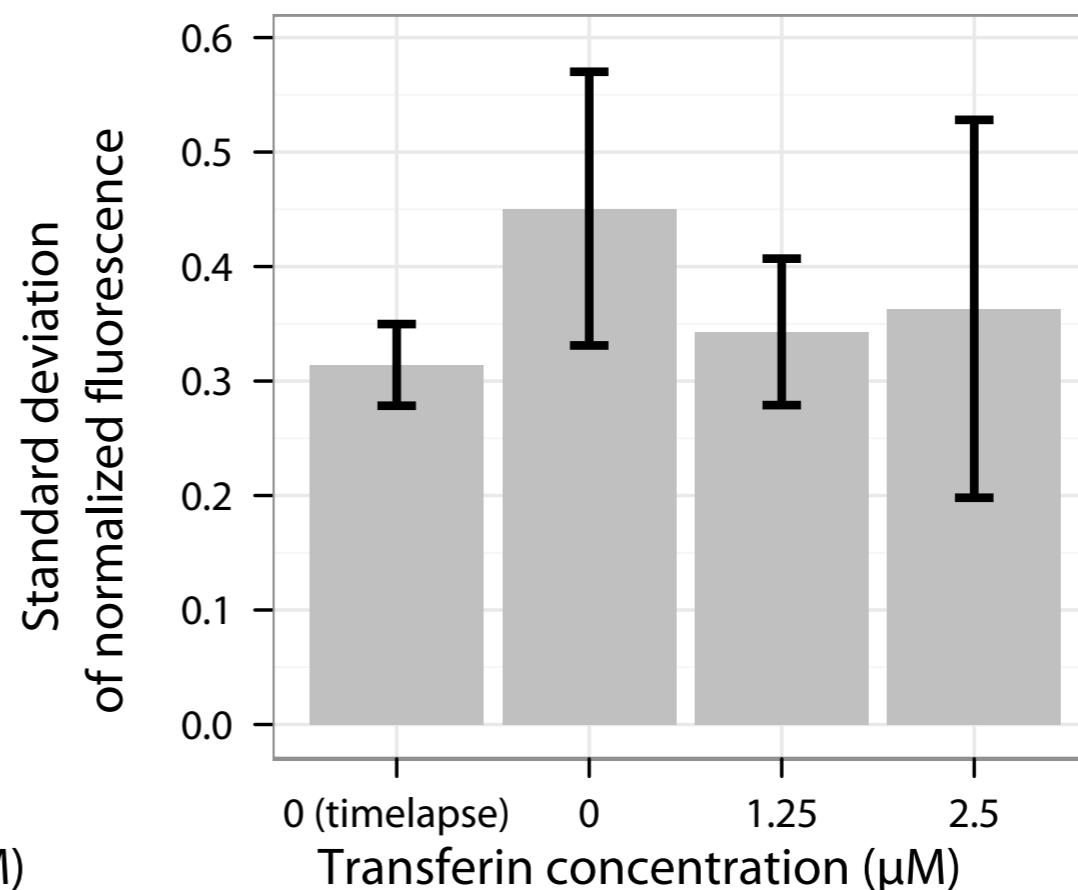
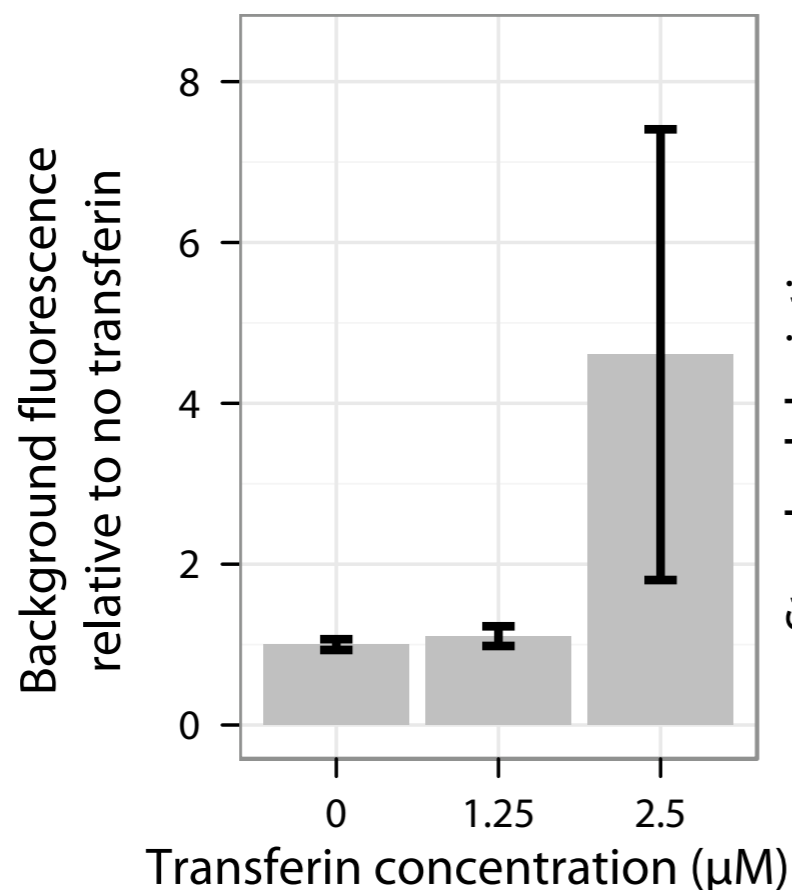


(Stewart *et al.* 2005)



Variability in iron-limited conditions

- Transferin is a human iron chelator (lower affinity than pyoverdine)
- Variability of pyoverdine is similarly high (~30%)



Overview

- Unexpected non trivial siderophore distribution (i.e. public good distribution in iron limited conditions) described in a clonal population.
- Moderate role of spatial structure and heritability.
- High variability interpreted as the result of a phenotypic switch.
- Asymmetric accumulation of pyoverdine between sisters.

One more thing...

Population control using laser ablation



Acknowledgments

- **ABCD Lab's** members
- **Frontiers in Life Science** graduate school's students and teachers

Collaborators

- Jonathan Saragosti, Pascal Silberzan & Axel Buguin (Curie Institute)
- Isabelle Schalk & Laurent Guillon (Strasbourg University)
- Thierry Mora (LPS ENS)

Acknowledgments

...and you for your attention!

