Antibiotic Resistance Plasmids and Spatial Structure

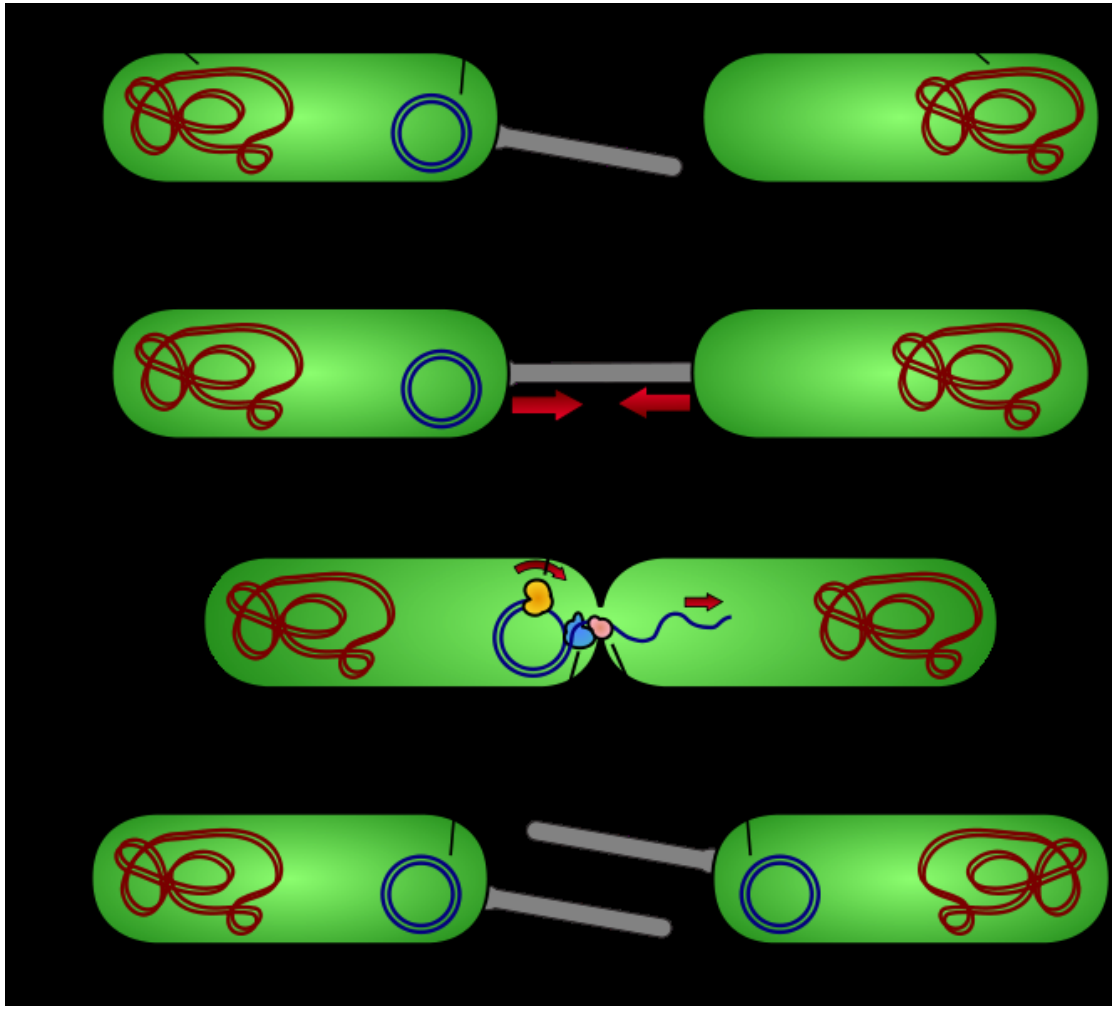
Steve Krone
University of Idaho
Department of Mathematics
Institute for Bioinformatics and Evolutionary Studies

CIRM, June 2012

Outline

1. Plasmids: circular, extra-chromosomal genetic elements common in bacteria
    - rapid spread of multi-drug resistance in bacteria
    - horizontal gene transfer
2. Features of (spatial) microbial populations ignored in most mathematical models. Key processes "stop"!
3. Pitfalls of using ODE-based estimates for spatial populations
    - plasmid transfer rates (similar to infection rates in epidemics)... IPS models

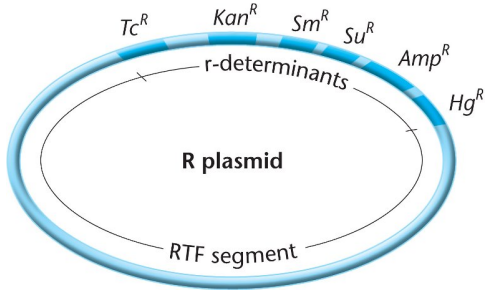
1. Plasmids



plasmid transfer from donor to recipient cell . . . Donor, Recipient, Transconjugant

Plasmid features

- Horizontal Gene Transfer (bacterial sex)
- rapid non-chromosomal spread of genes for simultaneous resistance to multiple antibiotics



* accumulation of resistance genes (antibiotics, heavy metals, ...)

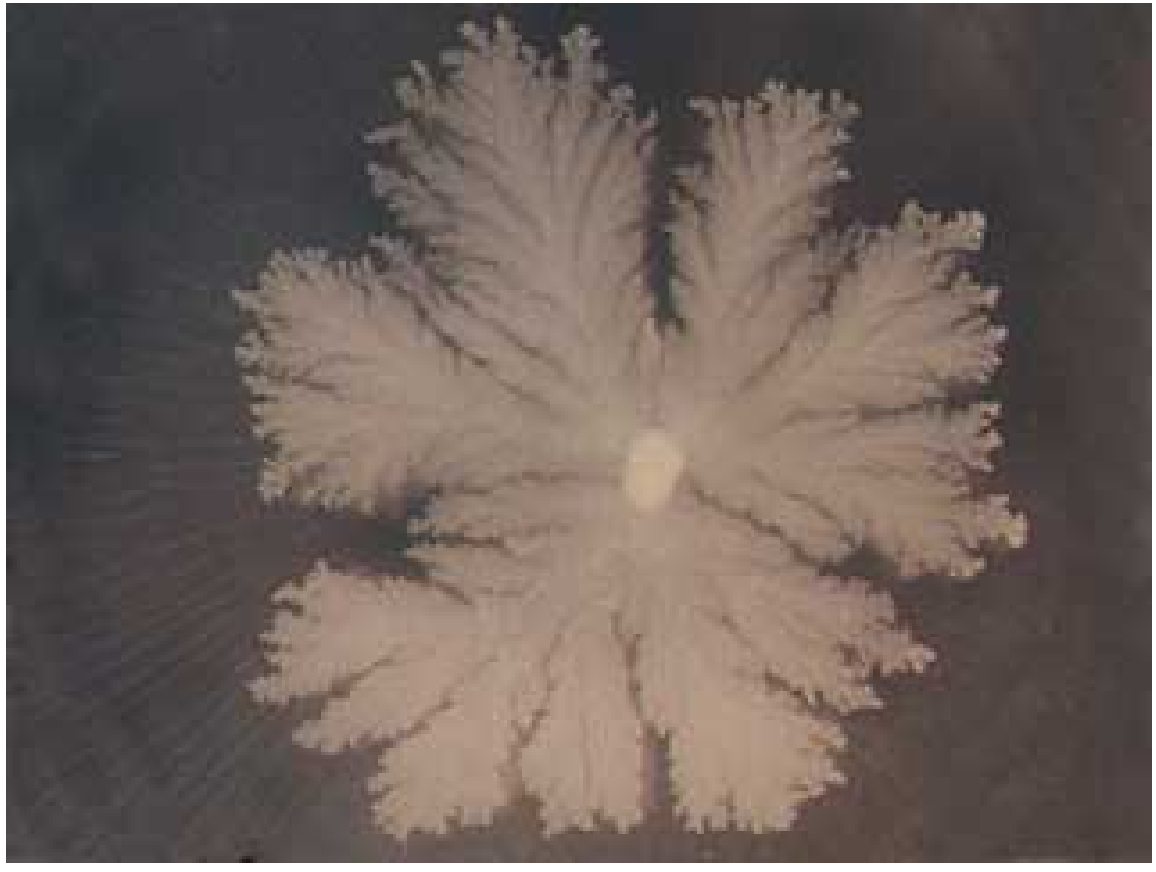
* co-selection (crisis of AB resistance getting out of hand)

- contact required for plasmid transfer
  - liquid: diffusion + attachment/detachment dynamics (mating aggregates)
  - spatial: attachment more stable $\Longrightarrow$ rapid transfer possible in certain spatial configurations; otherwise, wait for contact at "interfaces"
  - different dynamics (e.g., density dependence)
    *** IPS Simulation ***

2. Features of (spatial) microbial populations under-represented in mathematical models

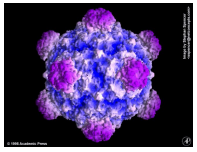
- Key processes stop (unlike what we expect from traveling wave phenomena)
- Limited spatio-temporal windows for ecology and evolution, but can be very intense.
- Then wait ... disturbance, migration, environmental change $\Longrightarrow$ initiate new round of active dynamics

Spatial heterogeneity in bacterial colonies



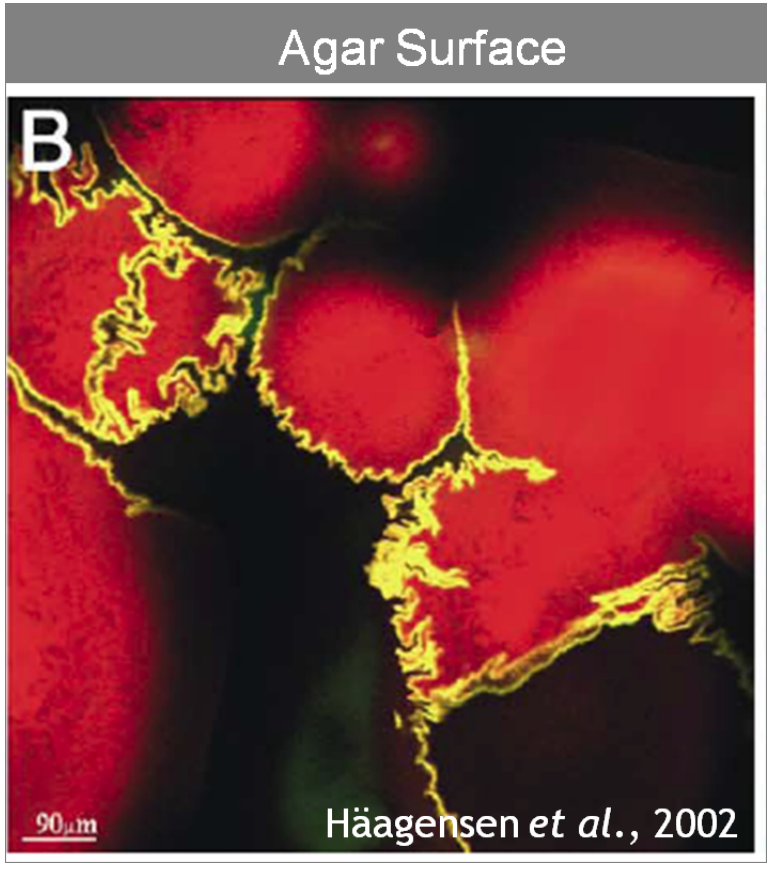
Fractal-like; peaks and valleys due to differential nutrient consumption/access

Phage plaques



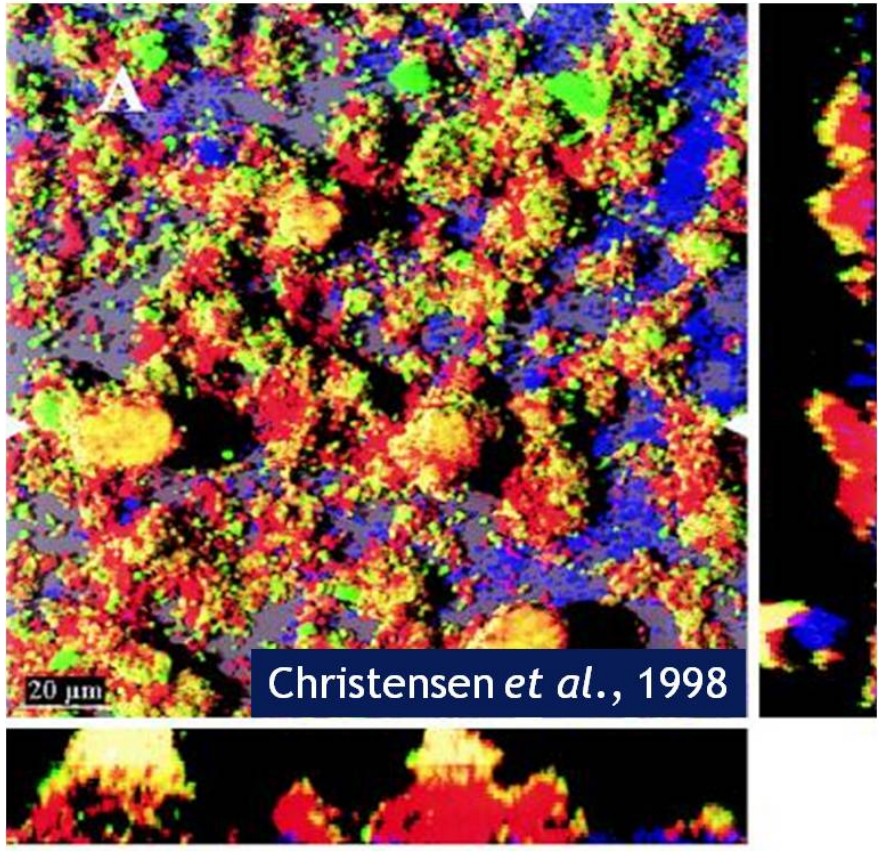
Constant rate of spread for several hours, then stop when host cells reach stationary phase.

Limited plasmid transfer on agar plates



No infectious wave of transfer!

Limited plasmid transfer in biofilms



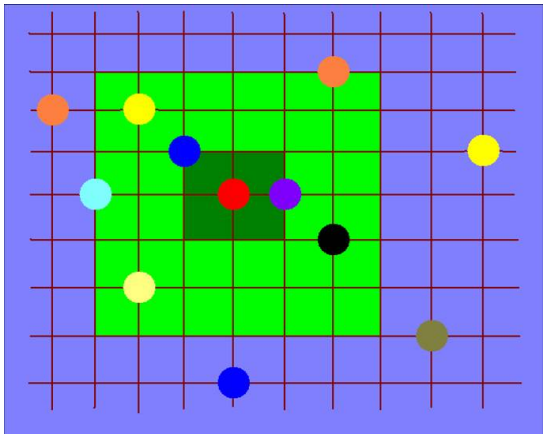
Very little plasmid transfer inside biofilm.

Stochastic spatial models–IPS

- Explicitly model

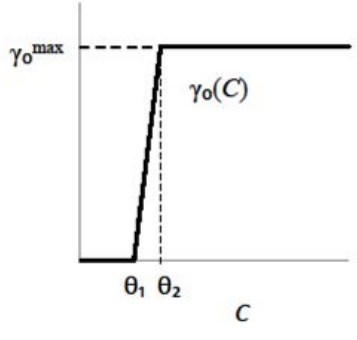
    1. discrete spatial structure: $\mathbb{Z}^2$ (2D or "2+" D)
    2. Each site can be in several different states (vacant, donor, recipient, transconjugant cells, nutrient, antibiotic, ...)
    3. nutrient consumption/"diffusion" (crucial)
    4. randomness & spatial structure down to individual cell level

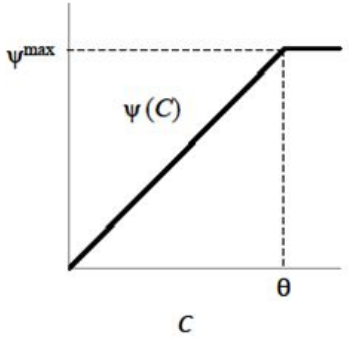
2D model features



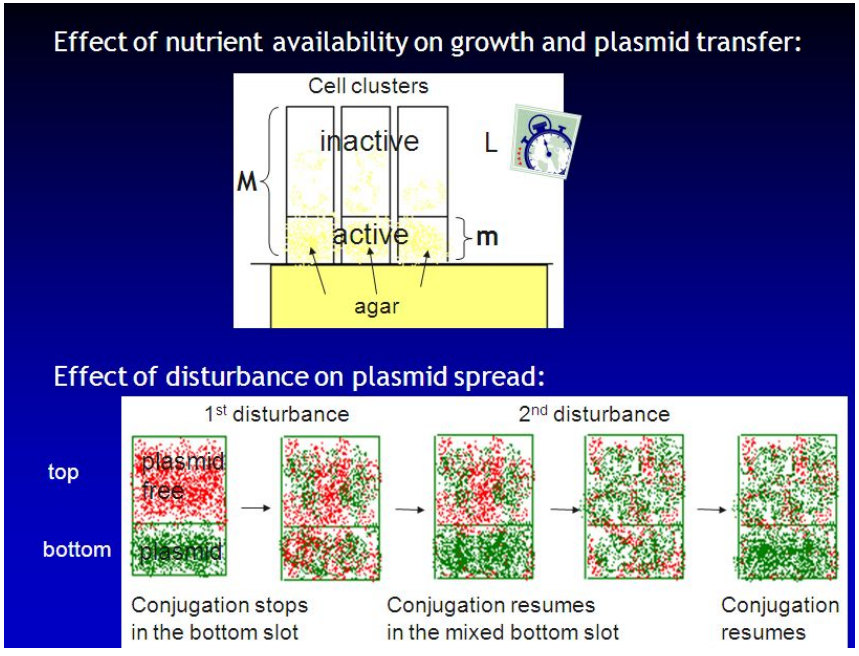
*interaction ranges for growth, death, plasmid transfer, nutrient diffusion, antibiotic diffusion ...

*nutrient-dependent plasmid transfer and growth rates





adding some 3D structure



Over 2D lattice, add several layers: M cells per site allowed with m_1 in 1st layer, m_2 in 2nd layer, ...

Each layer has its own nutrient-dependent growth rates

Growth in lower layers can push up into next layer

"coupled map lattice" with coupling parameter for amount of interaction/spread between neighboring sites

local rates

p_g ... coupling parameter for growth (prob that offspring is sent to neighboring site)

p_c ... coupling parameter for plasmid transfer

$n^w_{R,i}$ ... number of R's within focal site at level i

$n^{nbr}_{R,i}$ ... number of R's at 8 neighboring sites at level i

$f^w_V = (M - n^w_R - n^w_D - n^w_T)/M$ ... fraction of vacant "space" at focal site

$f^{nbr}_V = (8M - n^{nbr}_R - n^{nbr}_D - n^{nbr}_T)/8M$ ... fraction of vacant "space" at neighboring sites

rate at which focal site produces new R:

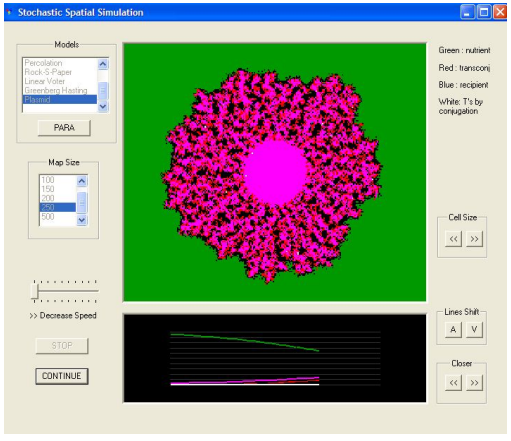
$$\psi_R[(1-p_g)f_V^w + p_g f_V^{nbr}]\ n_R^w$$

rate of production of new T's by focal site:

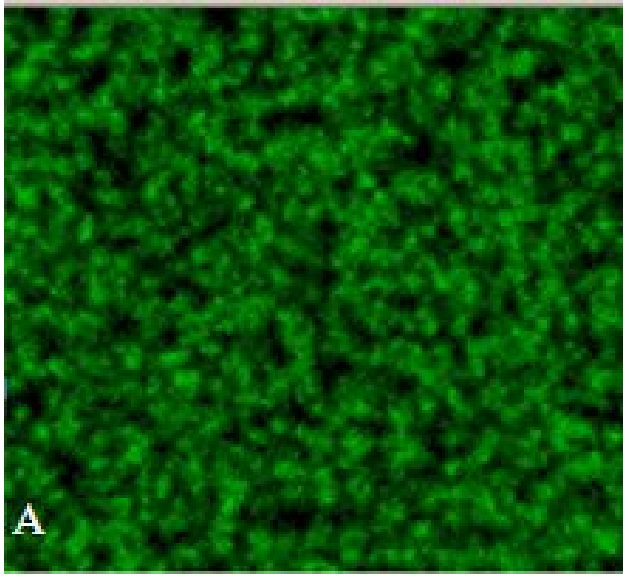
$$\psi_T[(1-p_g)f_V^w + p_g f_V^{nbr}]\ n_T^w$$

$$+(\gamma_T n_T^w + \gamma_D n_D^w)[(1-p_c)f_R^w + p_c f_R^{nbr}]$$

examples

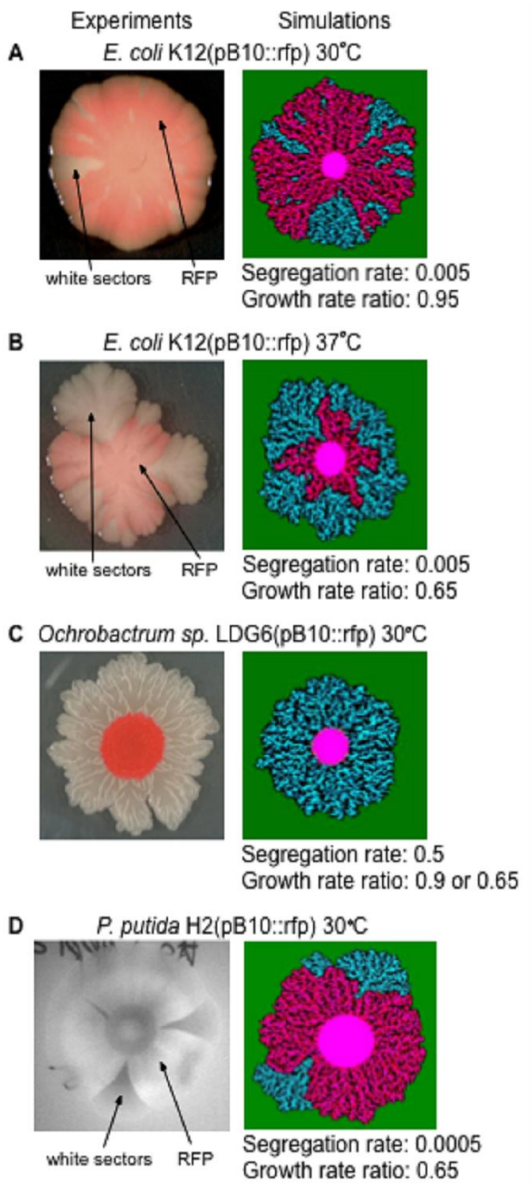


colony expansion on agar plate



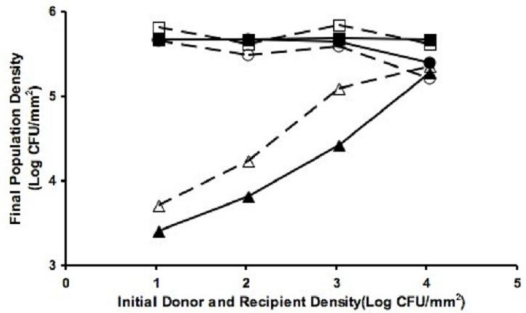
biofilm

plasmid-free sectors



IPS model used to predict/explain

- factors influencing plasmid invasion (when initially rare)
- segregation and clonal sectors
- lack of invasive waves of plasmid transfer
- density dependent plasmid transfer that is only present in spatial cultures



3. Pitfalls of using ODE-based estimates of plasmid transfer rate for spatial populations

- Estimation of plasmid transfer rate ... important for understanding spread of antibiotic resistance genes, etc.
- role similar to infection rate in epidemiology ("horizontal" spread), but estimation confounded by "vertical" spread.
- Current methods based on ODE models (or no models at all!)
- very different interpretations of transfer rates in liquid and spatial settings

Simple ODE for liquid batch culture

$$
\begin{aligned}
\dot{R} &= \psi_{_R} R - R(\gamma_{_T} T + \gamma_{_D} D) \\
\dot{D} &= \psi_{_D} D \\
\dot{T} &= \psi_{_T} T + R(\gamma_{_T} T + \gamma_{_D} D) \\
\dot{C} &= -e\,(\psi_{_R} R + \psi_{_D} D + \psi_{_T} T)
\end{aligned}
$$

- R=recipients, D=donors, T=transconjugants, C=nutrient
- $\psi = \psi(C)$ growth rate, $\gamma = \gamma(C)$ conjugation rate
- typical values for liquid γ: $10^{-8} - 10^{-14}$

"Endpoint Estimate" of plasmid transfer rate

$$\gamma_{max} = \psi_{max} \cdot \frac{1}{N_1 - N_0} \ln\left(1 + \frac{T_1 N_1}{R_1 D_1}\right)$$

- $N = R + D + T$
- subscripts: 1 ... final time (endpoint), 0 ... initial time
- γ_{max} = max transfer rate, ψ_{max} = max growth rate
- Other commonly used indicators of plasmid transfer efficiency: $T/N, T/R, T/D$ ... differ by orders of magnitude; not rates in any model

Derived by assuming simple ODE holds AND that

$$\psi_R = \psi_D = \psi_T = \psi_{max} \cdot \frac{C}{C + K}$$

and

$$\gamma_D = \gamma_T = \gamma_{max} \cdot \frac{C}{C + K}$$

- same Monod form of nutrient dependence!
- Most of these assumptions are wrong, but simulations of model suggest endpoint estimate fairly robust to changes in assumptions (for liquid systems).

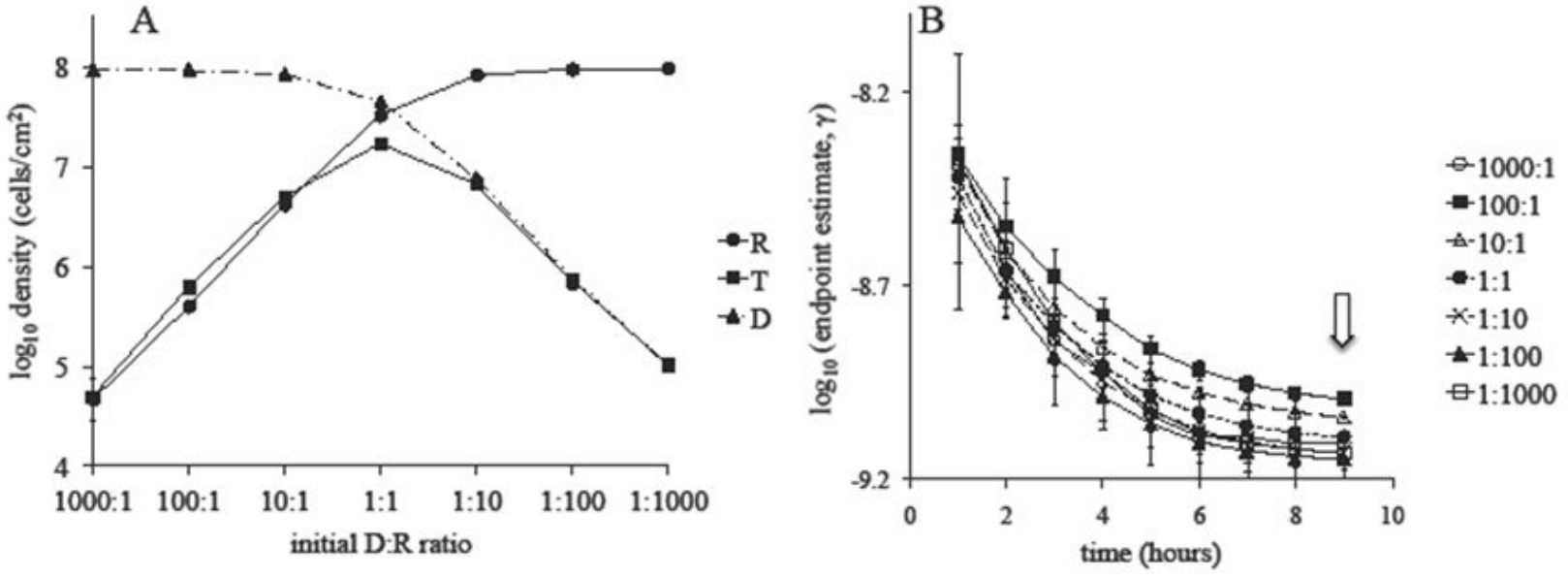
Issues

- Endpoint estimate has been applied to spatial populations (where transfer rate should be of order 1 instead of 10^{-9})
- Does it mean anything?
- Does it provide consistent information?
- Check using IPS simulations with known transfer rate

Scaling to compare spatial experiments and IPS simulation

- To compare, equate carrying capacities: Expts: $\overline{N}$ cells/ml Simulations: $L \times L$ grid $\implies$ L^2 cells/grid
- 1 cell/lattice $= \overline{N}/L^2$ cells/ml
- Ex) $\overline{N} = 10^9$ cells/ml and 1000×1000 grid $\implies$ lattice-based cell densities should be multiplied by 10^3 to obtain equivalent liquid density.
- Produces typical values

Simulation–D/R ratio effect

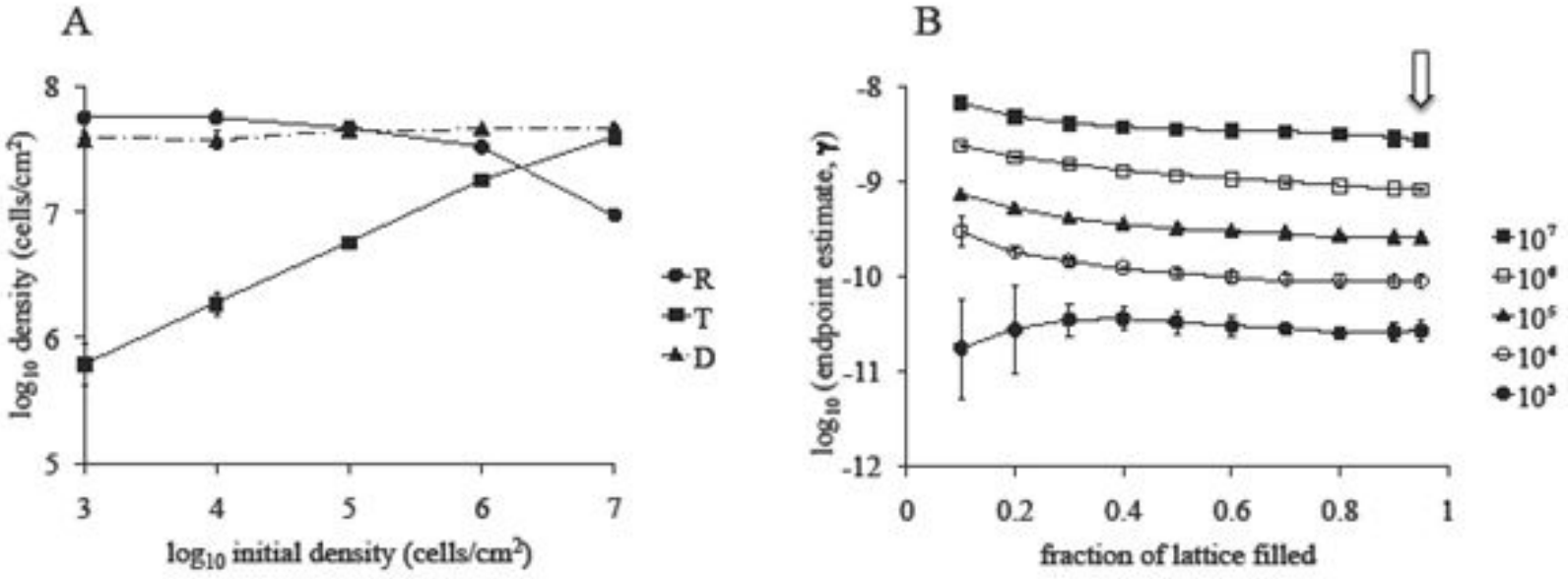


Left: T,D,R (stationary phase) densities as function of initial D:R ratio

Right: Endpoint estimates for different ratios

Conclude: Endpoint estimate not sensitive to initial ratios of D, R (and not dependent on time of sampling)

Simulation–density effect

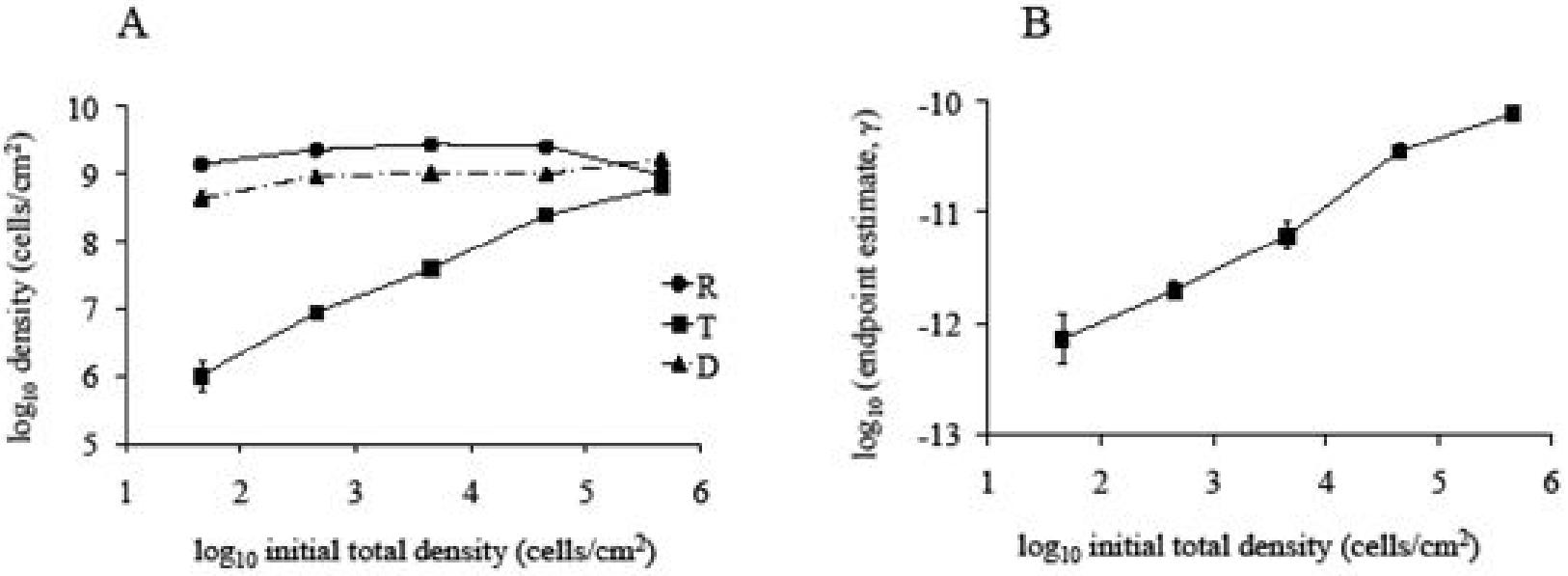


Left: T,D,R (stationary phase) densities as function of initial cell density (with D/R=1)

Right: Endpoint estimates for different initial densities

Conclude: Endpoint estimate is very sensitive to initial cell densities (and not dependent on time of sampling)

Experiment–density effect



Same effect of initial density on final transconjugant density for plasmid pB10; similar to results of Simonsen (1990) for a different plasmid

Conclusions about Endpoint estimate

- Only makes sense for spatial populations as a kind of "effective" plasmid transfer rate for some "equivalent" well-mixed population. This can only apply over short time periods, if at all.
- Does not provide consistent estimates of γ over different experimental conditions
- Need new methods for estimating spatially relevant parameters in microbial systems

Thanks!

Collaborators: Eva Top, Xue Zhong, Jarek Krol, Renie Lu, Jason Droesch

NIH $$