Active Antagonism: Reproducing Microorganisms and Fluid Flow

- Spatial population genetics of antagonistic organisms
- Fluid mechanics
- Active dynamics, fluid flows & antagonism: spinodal decomposition and nucleation in "Model A"

Simple Darwinian expectations fail...

Below threshold radius $R_c$ : droplet contracts
Above threshold radius $R_c$ : droplet expands

K1 toxin
K2 toxin

*S. cerevisiae* antagonistic strains

“Clash of Genomes”: Range expansions with neutrality, selective advantage, cooperation or competition

What happens at the interface?

Collision of two neutral E. coli strains with a heritable genetic markers on a hard agar plate....

Hernan Garcia, Rob Phillips & drn
Red and Green Bacterial Strains….
1. Could be neutral
2. Could have different doubling times
3. One or both could secrete amino acids useful to the other (mutualism)
4. One or both could secrete toxins that impede the other (competitive exclusion)
In a well-mixed environment:

Fisher equation for a pulled *genetic* wave

\[ f_G(t) = \frac{f_G(0)e^{st}}{1 + f_G(0)(e^{st} - 1)} \]

\[ \frac{\partial f_G(x,t)}{\partial t} = D \frac{\partial^2 f_G}{\partial x^2} + sf_G(1 - f_G) \]
Frequency-Dependent Selection for Mutualists

If $w_B$ and $w_Y$ are the number of blue and yellow offspring produced during one generation at a given point on the frontier... selective advantage $s \approx 2 \frac{w_B - w_Y}{w_R + w_Y}$

If $f(x,t)$ is the yellow fraction, describe mutualism by...

$$s(f) \approx s_0(f^* - f)$$

$$w_Y(x,t) = g + \beta(1 - f(x,t))$$

$$w_B(x,t) = g + \alpha f(x,t)$$

$$f^* = \frac{\beta}{\alpha + \beta}$$

$$\frac{\partial f(x,t)}{\partial t} = D \frac{\partial^2 f(x,t)}{\partial x^2} + s(f) f(1 - f)$$

$\alpha, \beta > 0 \rightarrow \text{Mutualism, } s(f) \approx s_0(f^* - f)$

"Evolutionarily Stable Strategy"

Range expansions for **mutualists** \((\alpha & \beta > 0)\)

*M. Müller, B. I. Neugeboren, drn and A. W. Murray, PNAS 111, 1037 (2014).*

**Mutualism:** survival requires exchanging amino acids, if leucine and tryptophan are not already present in the natural environment
Mutualists on various substrates

**CSM (Complete Synthetic Medium; abundant Leucine & Trptophan)** → Mutualism unimportant
\[ \alpha = \beta = 0 \]

**CSM-Leu-Trp (Leu, Trp missing)** → obligate mutualism
\[ \alpha, \beta > 0 \]

Experiments reveal that an “evolutionary stable strategy” is indeed reached independent of the initial condition…

Dynamics at the frontier described by an inflating version of Model A: K. Korolev, and drn, PRL 10, 88103 (2011)

\[
\frac{\partial f(x,t)}{\partial t} = D \frac{\partial^2 f(x,t)}{\partial x^2} - s_0 f(1-f)(f-f^*) + \sqrt{f(1-f)/N_{\text{eff}}} \zeta(x,t); \quad f^* = \frac{\beta}{\alpha + \beta}, \quad s_0 = (\alpha + \beta) / g
\]

\[
\frac{\partial f(x,t)}{\partial t} \approx D \frac{\partial^2 f(x,t)}{\partial x^2} - s_0 \Delta(f-f^*) + \sqrt{\Delta/N_{\text{eff}}} \zeta(x,t), \quad \Delta = f^*(1-f^*), \quad \sim \text{Model A, } T > T_c
\]
Now add fluid mechanics: Microorganisms on liquid substrates

Severine Atis
Bryan Weinstein
Andrew Murray
**Microorganisms grown on liquid but highly viscous substrates create their own flows (without pumps and syringes...)**

Hard Agar

Genetic demixing of yeast on a 1% hard agar YPD plate (viscosity $\eta = \infty$)

Liquid Media

Epoch of genetic demixing stretched out....

Yeast on a liquid but highly viscous YPD (yeast/peptone/dextrose) media with 3% cellulose ($\eta \approx 600$ Pa-s)

<table>
<thead>
<tr>
<th>Cellulose % (w/v)</th>
<th>Viscosity (Pa-s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>2.0</td>
<td>51 ± 6</td>
</tr>
<tr>
<td>2.2</td>
<td>81 ± 9</td>
</tr>
<tr>
<td>2.4</td>
<td>120 ± 10</td>
</tr>
<tr>
<td>2.6</td>
<td>340 ± 50</td>
</tr>
</tbody>
</table>

(the viscosity of water is $\eta \approx 10^{-3}$ Pa-s; our viscosities are $10^4 - 10^5$ times larger)

The colony metabolism generates flows that dilate the growing cell mass radially!
After inoculation, reproducing microorganisms on liquid substrates can behave like gases, liquids or solids….

At very early times, the yeast cells exhibit gas-liquid phase separation.

Coarsening or “spinodal decomposition”….
Deformations of features inside colony in a liquid-like regime consistent with a dilational flow ($\eta = 600 \text{ Pa-sec}$)
Colony features dilate as if inscribed on an inflating balloon.

Simple model of 2d colony dynamics:

$$\frac{\partial \rho_{2d}}{\partial t} + \vec{\nabla} \cdot (\rho_{2d} \vec{v}_{2d}) = \alpha_1 \rho_{2d}, \quad \rho_{2d} = \text{cell density}$$

$$\alpha_1 = \text{growth rate} \rightarrow \vec{\nabla} \cdot \vec{v}_{2d}(r) = \alpha_1; \quad \text{assume overdamped liquid-like colony dynamics:}$$

$$0 \approx -\vec{\nabla} p_{2d} - \gamma \vec{v}(\vec{r}) ; \quad \gamma = \eta_s / hH; \quad \vec{v}_{2d}(\vec{r}) \approx \frac{\alpha_1 r\hat{r}}{2} \quad \text{dilational velocity field}$$

The first three images have the same scale bar = 100 µm. The final picture, with scale bar 500 µm, shows the same feature at the much larger colony scale.
Enhancing the radial flow field... (moderate substrate viscosity $\eta \approx 450$ Pa-s)

Liquid-like fingering instabilities

Dynamics of a single finger: Rayleigh-Plateau type instability with growth?
Now add antagonism: Pushed genetic waves generated by antagonistic interactions

- For pushed genetic waves, a “critical nucleus” is required to excite the wave to get it started
- Can we detect the existence of a critical nucleus with killer yeast or bacteria strains?
- Spinodal decomposition of antagonists?
Antagonistic microbial interactions

Diffusive antagonism

Bacteriocins or peptidic toxins
Image from https://geneticliteracyproject.org

Local antagonism

Type VI secretion systems
Image from Brunet et al (2013), Cell Reports 3(1)


Diffusive antagonism

Killer yeast viruses
Saccharomyces cerevisiae
Killer yeast (*S. cerevisiae*) genetic engineering

Express the toxin-encoding gene from the genome of *S. cerevisiae* under the control of titratable, inducible promoters, add fluorescent proteins to distinguish the two strains.

*Galactose metabolic pathway knocked out; galactose acts only as an inducer....*
Consider an allele fraction \( f \equiv f(x, t) \) at position \( x \) and time \( t \). Then:

\[
\partial_t f = D_s \nabla_x^2 f + \frac{f(1-f)}{\tau_g} \left[ (\alpha + \beta) \left( \frac{1}{2} - f \right) + \frac{\alpha - \beta}{2} \right] + \sqrt{\frac{2f(1-f)}{N\tau_g}}
\]

\[
= D_s \nabla_x^2 f - \frac{1}{\tau_g} \frac{dU(f)}{df} + \sqrt{D_g f(1-f)} \xi \quad \text{with number fluctuations:}
\]

\[
\langle \xi(x, t) \rangle = 0 \quad \text{and} \quad \langle \xi(x, t) \xi(x', t') \rangle = \delta(t-t')\delta(x-x').
\]

\( D_s = \) spatial diffusion constant \( \sim \) motility

Note double well potential with minima at \( f = 0 \) and \( f = 1 \)
A convenient reparameterization (for competitive exclusion)

To study the antagonistic interactions in the third quadrant, we rotate our coordinates to \( \sigma = -(\alpha + \beta)/2 \) and \( \delta = \alpha - \beta \):

\[
\partial_t f = D_s \nabla^2 f + f(1-f) \left[ \sigma(2f-1) + \frac{\delta}{2} \right] + \sqrt{D_g f (1-f)} \xi
\]  

(1)

\[\delta = \alpha - \beta = \text{selective advantage}; \quad \sigma = (\alpha + \beta)/2 = \text{"antagonism"}\]

Model \( A' = \text{Model A with nonlinear noise} \ldots\)
Spinodal decomposition for model A’

$$S(k, t) = 4 \sum_{\vec{r}'} \left\langle \left( f(\vec{r}, t) - \frac{1}{2} \right) \left( f(\vec{r} + \vec{r}', t) - \frac{1}{2} \right) \right\rangle e^{i \vec{k} \cdot \vec{r}'}$$

= structure function

\(\delta = \alpha - \beta = \) selective advantage

\(\sigma = - (\alpha + \beta) / 2 = \) "antagonism"

M. Lavrentovich and dnm, PR E 100, 042406 (2019)
Nucleation and growth for $\delta > 0$, $\sigma \geq 0$

Suppose we look at droplets with $0 < \delta \ll \sigma$. The "energy" of such a drop is

$$E[f] = \int \text{d}x \left[ \frac{D_s}{2} (\nabla f)^2 + U(f) \right]$$

We can look for minimal $E[f]$ solutions that look like droplets:

$$\nabla^2 f = -\frac{\sigma}{D_s} f(1 - f)(2f - 1)$$

$$f(r) = \frac{1}{2} - \frac{1}{2} \tanh \left[ \sqrt{\frac{\sigma}{4D_s}} (r - r_0) \right]$$

Droplet energy $E(r_0) = 2\pi \gamma r_0 - \pi c r_0^2$,

line tension $\gamma = (2/3)\sqrt{\sigma/D}$, cond. energy $c = \delta / 3D$

critical droplet size $= R_c = \frac{\gamma}{c} = (2/\delta)\sqrt{D\sigma}$
Green cells must overcome line tension to grow

\[ \sigma = -\left(\alpha + \beta\right)/2, \quad \delta = \alpha - \beta, \quad \delta \ll \sigma \ll 1 \]

Nucleation theory ideas can be used to compute the survival probability in this limit

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Yellow cells enjoy a selective advantage, but must exploit number fluctuations and then overcome a line tension to grow....

“The fate of the false vacuum”
Experimental test of nucleation dynamics for killer yeast (A. Giometto, A. Murray)
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What happens when Model A’ dynamics is subjected to fluid flow?
Nucleation in a flow: Simplified reaction-diffusion model of antagonistic organisms

(Xiaojue Zhu, R. Benzi, drn and F. Toschi)

Go to “board”

**Governing equations**

\[
\begin{align*}
\frac{\partial c_A}{\partial t} + \nabla \cdot (uc_A) &= D \nabla^2 c_A + c_A (1 - c_A - c_B + \epsilon_A c_B) \\
\frac{\partial c_B}{\partial t} + \nabla \cdot (uc_B) &= D \nabla^2 c_B + c_B (1 - c_B - c_A + \epsilon_B c_A)
\end{align*}
\]

**Flow**

\[
\begin{align*}
u_x(x, y) &= F[\alpha \sin(2\pi x/L) + (1 - \alpha) \sin(2\pi y/L)] \\
u_y(x, y) &= F[\alpha \sin(2\pi y/L) + (1 - \alpha) \sin(2\pi x/L)]
\end{align*}
\]

**Parameters**

\[D = 10^{-4}, \quad L = 1, \quad \alpha = 0, \quad \epsilon_A = -0.2, \quad \epsilon_B = -0.3\]
Nucleation in a flow: Simplified reaction-diffusion model of antagonistic organisms

(Xaiojue Zhu, R. Benzi, drn and F. Toschi)

$$\varepsilon_A \leftrightarrow \alpha; \quad \varepsilon_B \leftrightarrow \beta$$

$$\frac{\partial c_A}{\partial t} + \nabla \cdot (uc_A) = D \nabla^2 c_A + c_A(1 - c_A - c_B + \varepsilon_A c_B)$$

$$\frac{\partial c_B}{\partial t} + \nabla \cdot (uc_B) = D \nabla^2 c_B + c_B(1 - c_B - c_A + \varepsilon_B c_A)$$

$$u_x(x, y) = F[\alpha \sin(2\pi x/L) + (1 - \alpha) \sin(2\pi y/L)]$$

$$u_y(x, y) = F[\alpha \sin(2\pi y/L) + (1 - \alpha) \sin(2\pi x/L)]$$

$$\sigma = -(\varepsilon_A + \varepsilon_B) / 2 = \text{antagonism}$$

$$\delta = \varepsilon_A - \varepsilon_B = \text{selective advantage}$$

$$\alpha = 0$$
Dynamics of Totals & A-Fraction $\xi$

Scalar
1. \[ \frac{\partial c_A}{\partial t} + \nabla \cdot (uc_A) = D\nabla^2 c_A + c_A(1 - c_A - c_B + e_{ABC}) \]
2. \[ \frac{\partial c_B}{\partial t} + \nabla \cdot (uc_B) = D\nabla^2 c_B + c_B(1 - c_B - c_A + e_{BCA}) \]

Flow
- \[ u_x(x, y) = F[\alpha \sin(2\pi x/L) + (1 - \alpha) \sin(2\pi y/L)] \]
- \[ u_y(x, y) = F[\alpha \sin(2\pi y/L) + (1 - \alpha) \sin(2\pi x/L)] \]

Parameters
- \[ D = 10^{-4}, L = 1, \alpha = 0, e_A = -0.2, e_B = -0.3 \]

\( c_A, c_B < 0 \)

Change of variables, let
- \( c_T = \frac{c_A + c_B}{2} \)
- \( f = \frac{c_A}{c_A + c_B} = \frac{c_A}{c_T} \)

\[ \frac{\partial}{\partial t} (c_T) + \nabla \cdot (uc_T) = D\nabla^2 c_T + c_T(1 - c_T) \]

So, even when \( c_T \approx 1 \), \( \frac{\partial c_T}{\partial t} \approx -2\sigma f(1-f) \)

So, the antagonism parameter \( \sigma \) decreases the total population at A/B interfaces, consistent with the well-mixed dynamics associated with Eqs (1) & (2) (see Prigoloff et al.)

Hyperbolic fixed point at \( (c_A^*, c_B^*) = (\frac{c_A + c_B}{2}, 1) \)

but only if \( e_A + e_B \ll e_A e_B \)

\( \Rightarrow \) at the fixed point \( c_A^* + c_B^* = 1 + \frac{e_A e_B}{(e_A + e_B) - e_A e_B} = 0 \)
What is the dynamics of $f(x,t)$?

First we study the well-mixed case...

$$\frac{df}{dt} = \frac{1}{c_T} \frac{dc_A}{dt} - \frac{c_A}{c_T} \frac{dc_T}{dt} (1-f) c_T$$

$$= (1-c_A-c_B + e_A e_B f) f - \frac{f}{c_T} \left[ \frac{e_A}{e_A + e_B} c_T + (e_A + e_B) c_T^2 f (1-f) \right]$$

Everything simplifies provided $e_A$, $e_B << 1 \iff c_T \approx 1$

$$\frac{df}{dt} = e_A f (1-f) - [e_A + e_B] f f (1-f)$$

or

$$\frac{df}{dt} = f (1-f) \left[ e_A - (e_A + e_B) f \right]$$

$$\Rightarrow e_A - (e_A + e_B) f = \left( \frac{\Delta}{2} - \delta' \right) + 2 \sigma f$$

$$= \frac{\Delta}{2} + \sigma (2 \Delta - 1) \Delta$$

$$\frac{df}{dt} = f (1-f) \left[ \frac{\Delta}{2} + \sigma (2 \Delta - 1) \right]$$

Same equation as La Ventovich/... draft paper.

Additional terms when there are spatial gradients...

$$\frac{df}{dt} = -\frac{1}{c_T} \nabla \cdot (\vec{u} c_A) + \frac{\sigma}{c_T} \nabla^2 c_A - \frac{c_A}{c_T} \left[ -\nabla \cdot (\vec{u} c_T) + D \nabla^2 c_T \right] + \ldots$$

$$\approx -\nabla \cdot (\vec{u} c_A) + D \nabla^2 c_A + c_A (\nabla \cdot \vec{u}) + \ldots$$, if $c_T \approx 1$

$$= -c_A \nabla \cdot \vec{u} + D \nabla^2 f + f (1-f) \left[ \frac{\Delta}{2} + \sigma (2 \Delta - 1) \right]$$

Deterministic generalization of Model A to include flow.
The dynamics of the droplet radius $R(t)$ is given by

$$\frac{dR(t)}{dt} = -\frac{D}{R(t)} + \frac{\delta}{2} \sqrt{\frac{D}{\sigma \tau_g}}$$

(require $R(t) \gg w = \text{interface width}$)

→ critical droplet radius $R_c = \gamma / c = (2 / \delta) \sqrt{D \sigma}$

→ dying droplets should vanish with a square root singularity,

$$R(t) = \sqrt{R_0^2 - 2D(t-t_0)},$$

where $R_0$ is the radius of a dying droplet has well below the maximum $R_c$ at time $t_0$

→ Once the droplet is above the maximum, we should eventually have a circular, expanding pushed wave with

$$R(t) \approx vt, \quad v = (\delta / 2) \sqrt{D / \sigma \tau_g}$$

simulations: selective advantage $= \delta = \varepsilon_A - \varepsilon_B = 0.1$

antagonism $= \sigma = -(\varepsilon_A + \varepsilon_B) / 2 = 0.25$
1. Initial radius=0.11 without flow

\[ c_A = 1, c_B = 0 \]

\[ c_A = 0, c_B = 1 \]

\[ R < R_c \]
2. Initial radius=0.12 without flow

\[ c_A = 1, \; c_B = 0 \]
\[ c_A = 0, \; c_B = 1 \]

\[ R > R_c \]
The effect of a saddle flow on a (slightly) subcritical droplet of a selectively favored species.

Initial radius=0.11, F=0.0025

- $R(t=0) < R_c$ without flow.
- The saddle flow elongates the droplet, and resulting flat regions are relatively free from the confining effects of line tension.
- Although there is a selective advantage, the inward flows due to the saddle are larger than the outward pushed wave velocity due to the selective advantage.
- The net effect is to produce a shorter extinction time.
Initial radius=0.11, F=0.025

(Fluid driving force $F$ at the saddles is now a factor of 10 bigger.)

Red droplet of the selectively favored phase dies even more rapidly...
Time series for initial radius=0.11, increasing flow strength F at the saddle

\[ c_A(t) = \pi R^2(t); \quad R(t) = \sqrt{R_0^2 - 2D(t-t_0)} \]

The predicted linear vanishing of \( c_A(t) \) is rounded into a foot, due to the smoothing effect of diffusion?

The selectively favored droplet dies even more rapidly when born on a saddle point

Extinction time \( T_E \approx T_0 - AF^2; \quad T_E(F) \) must be an even function of \( F \).
Hence, \( T_E(F) \approx T_E(0) - AF^2 \) for small \( F \)....
Larger droplets can be strongly influenced by periodic boundary conditions!!

Red variable species is initially at the saddle point. $<c_A>$ grows non-monotonically. Also as time goes by, $A$ splits up and reconnects.
A stable radius is possible when droplet with a selective advantage is born on a sink.
Effects of spatially modulation with line sink and a selective advantage

A layer of Species A is put in the middle of domain with width 0.07. A perturbation of $0.01 \times \sin(2\pi \times 4 \times x)$ is added to the interface.
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- Spatial population genetics of antagonistic organisms
- Fluid mechanics
- Active dynamics & antagonism: spinodal decomposition and nucleation in Model A'

Thank you!


http://streetanatomy.com