Constraints on communities and characters

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“Molecular biology has been a great leveller and has made thinking unnecessary in many areas of modern biology. With the disappearance of theory has also come the decline of experimentation.”

Sydney Brenner (1997)
A path to undoing the “great leveling”?

The Totalitarian Principle:
“Everything that is not forbidden is mandatory”
--Gell-Mann

Translation to Biology:

(1) What is not possible in biological systems and why not?

(2) “Numbers matter!” -- Daniel Fisher (2017)
What constraints operate in evolving, interacting, replicating (microbial) populations and communities?
Constraints at two levels
Part I: Communities

Zak Frentz
Doeke Hekstra
Stanislas Leibler

Rockefeller University &
Simons Center for Systems Biology
Institute for Advanced Study

Frentz*, Kuehn*, Leibler.
For microbial communities:

1. Are abundance dynamics repeatable across identically maintained ecosystems?

2. Is there any simple statistical structure that emerges from measurements on ensembles of ecosystems? What are the appropriate variables?
Closed ecosystems: controlled, complex systems

**A**

Algae: *Chlamydomonas reinhardtii*

**B**

Bacteria: *Escherichia coli*

**C**

Ciliates: *Tetrahymena thermophila*

Persist for months/years when hermetically sealed and supplied with light.

Dynamics for many replicates

Systematic error in temperature: 0.1C
illumination levels: 30%
Variation across replicates increases in time

\[ \sigma_i(t) = \sigma(\log(N_i(t))) \]

Hekstra & Leibler, Cell (2012)
Variation is structured along ‘ecomodes’

\[
C_{ij}^R(t) = \frac{\text{Cov}_R(\log N_i, \log N_j)}{\sigma(\log N_i)_R \times \sigma(\log N_j)_R}
\]
Variation is structured along ‘ecomodes’
Variance accumulates
In time.....

...in a well defined way.
(primarily [1,1,1] direction)

What drives this variation?
Intrinsic? Extrinsic?
Replicate populations in controlled conditions: custom holographic microscopes

<table>
<thead>
<tr>
<th></th>
<th>Holography</th>
<th>Previous work</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>±0.02 °C</td>
<td>±0.1 °C</td>
</tr>
<tr>
<td>Illumination</td>
<td>±2.5%</td>
<td>~±15%</td>
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</table>
A new instrument: holographic microscopes

Resolution ~ 2µm in x,y; ~40µm in z over ~5µL volume
Measurement every 6.6 minutes.

Frentz*, Kuehn*, Hekstra & Leibler. RSI (2010)
Population dynamics – single replicate

20 000 measurements over 92 days.
Short timescale fluctuations are Poisson.
1200 lux illumination (High light)
Strongly deterministic dynamics
No accumulation of variance

Hekstra & Leibler (2012)

30% variation in light levels

PRX 2015

2% variation in light levels
Are the dynamics always deterministic?
Dynamics are repeatable in phase space

Low Light (25%)

High Light (100%)
Morphological variation in *T. thermophila* at 25%
Morphological changes predict abundance changes
Dynamics under different light levels

Structure of variation across replicates.

Illumination constant in time
Constraints on abundance dynamics

20 to 100% all systems

Components
Variation in light `pushes' system along ecomode

@ 12 days
Questions:

- How do modes emerge from ecology (interactions, metabolism) and evolution (if they do at all?)?
- Spatial structure? Gas exchange? Interactions?
- Are ecomodes observed in more complex communities? (Mikhail Tikhonov, Pankaj Mehta)
- Are there common ecomodes and environmental variables across microbial communities in diverse settings?
“...the whole organization [of the organism] is so tied together during its growth and development, that when slight variations in any one part occur....other parts become modified.”

Darwin, 1859
Trade-offs constrain phenotypes
Why do we observe specific phenotypes?

Selected for high Trait #2?  
Selected for low Trait #1?  
Selected for both?
A model system: motility in *E. coli*

microns, seconds
Selecting for swimming and growing

Adler (1966)
Wolfe & Berg (1989)
Evolution of migration

LB medium

$T_d = 30\text{ min (liquid)}$

12 hr/ round

$\sim 12$ generations/round

David Fraebel
What drives faster migration?

\[
\frac{\partial \rho}{\partial t} = D_b \nabla^2 \rho - \nabla \cdot (k(c)\rho \nabla c) + g(\rho, c)
\]

\[
\frac{\partial c}{\partial t} = D_c \nabla^2 c - f(\rho, c)
\]

Keller & Segel (1978)
Tindall et al. (2008) Review
Croze et al. (2011)
Prediction: for fastest migration increase both speed and growth.
Growth rate declines with selection

Growth measured in well-mixed liquid media
Swimming speed increases with selection

High-throughput single cell tracking

Track ~100 cells/strain, 5 minutes each
~20,000 run events/strain

Harry Mickalide

Jordan*, Kuehn*, Ketifori, Leibler PNAS (2013)
Trade-off constrains evolution of faster migration
Fast parallel genetic evolution of faster migration

De novo mutations relative to founder.

Analysis with breseq (Barrick lab, UT)
clpX: “breaking a repressor”

Reconstructed clpX E185* mutation in ancestral background

clpXE185*: Stop codon in middle of open reading frame

flhDC

Motility/chemotaxis operons

David Fraebel, Tom Kuhlman
Pleiotropy governs trade-off

Growth rate

Swimming speed

Predicted front migration rate [cm h\(^{-1}\)]

clpX mutant migration rate \(\sim30\%\) faster than founder.
Select for growth and swimming in minimal medium.

M63 Galactose (0.18%) Minimal Medium

\[ T_d = 5.5 \text{ hrs (liquid)} \]

48 hr/round

Diane Schnitkey
Opposite phenotypic evolution:
Fast growth, slow swimming

Growth rate

Probability

|v_r| [μm s^{-1}]

0 10 20 30 40

0 0.05 0.1 0.15

founder
red round 5
white round 10

Growth rate

F 5 10

0 0.1 0.2 0.3 0.4 0.5
Evolution in opposing directions

Rich medium

Minimal medium

Evolu-on in opposing direc-ons
Minimal medium: parallel genomic evolution

(Leucine -> Arginine @ position 22)
**galS: “Breaking a repressor” (again!)**

Mutation L22R in helix-turn-helix DNA binding domain likely disrupts repression.

Reconstructed *galSL22R* mutation in ancestral background
Pleiotropy governs trade-off

\[
\frac{|v_r|}{|v_r(\text{founder})|} \quad \frac{k_g}{k_g(\text{founder})}
\]

Speed

Growth

galS mutant migration rate \sim 100\% faster than founder.
Conclusion: phenotypes evolve by breaking repressors
Genetic covariance determines phenotypic evolution

Direction of phenotypic evolution described by:

\[ \Delta \phi = G \beta \]

\[ G = \begin{bmatrix} \sigma_{v_r}^2 & \rho \sigma_{v_r} \sigma_{k_g} \\ \rho \sigma_{v_r} \sigma_{k_g} & \sigma_{k_g}^2 \end{bmatrix} \]

\[ \beta = \left( \frac{\partial \log(s)}{\partial |v_r|}, \frac{\partial \log(s)}{\partial |v_r|} \right) \]

Measured from experiment

Inferred from reaction-diffusion simulation for each medium

Lande (1979)
Gene-covariance determines phenotypic evolution

Direction of phenotypic evolution described by:

\[
\Delta \vec{\phi} = \mathbf{G} \vec{\beta}
\]

\[
G = \begin{bmatrix}
\sigma_{v_r}^2 & \rho \sigma_{v_r} \sigma_{k_g} \\
\rho \sigma_{v_r} \sigma_{k_g} & \sigma_{k_g}^2
\end{bmatrix}
\]

\[
\vec{\beta} = \left( \frac{\partial \log(s)}{\partial |v_r|}, \frac{\partial \log(s)}{\partial |v_r|} \right)
\]

Measured from experiment

Inferred from reaction-diffusion simulation for each medium

What about G?

- Trade-off implies $\rho < 0$
- Adjust $\sigma_{v_r}/\sigma_{k_g}$ until $\Delta \phi \downarrow \text{predicted}$ aligns with $\Delta \phi \downarrow \text{observed}$
- For $\rho < -0.1$, find: $\sigma_{v_r}/\sigma_{k_g} \geq 1$ (Rich Medium) and $\sigma_{v_r}/\sigma_{k_g} \leq 0.3$ give good agreement

Lande (1979)
Genetic covariance determines phenotypic evolution

\[ \rho = -0.75 \]

\[ \sigma_{\downarrow|\downarrow r} / \sigma_{\downarrow|\downarrow r} | \] 

Genetic variance couples with selection pressure to ultimately determine direction of evolution

\[ p(\phi) = \frac{1}{2\pi \sqrt{|G|}} \exp(\phi - \langle \phi \rangle)^T G^{-1} (\phi - \langle \phi \rangle) \]
Why do we observe specific phenotypes?

Because it’s genetically “easy” to evolve them

Schluter “Genetic lines of least resistance” (1996)
Lind, Farr & Rainey. eLife (2015) – extragenic negative regulators most common mutations
Michael Savageau. PNAS (1997)
Conclusions

• Selection reproducibly leads to faster migration through soft agar in rich and minimal media

• Evolution of faster migration is constrained by trade-off between run speed and growth rate

• Trade-off comes from antagonistic pleiotropy of mutations that produce faster migration

• Genetic variance of each trait depends on environment, alters direction of evolution compared to selection pressure alone

Fraebel, Mickalide, Schnitkey, Merritt, Kuhlman, Kuehn. eLife (2017)