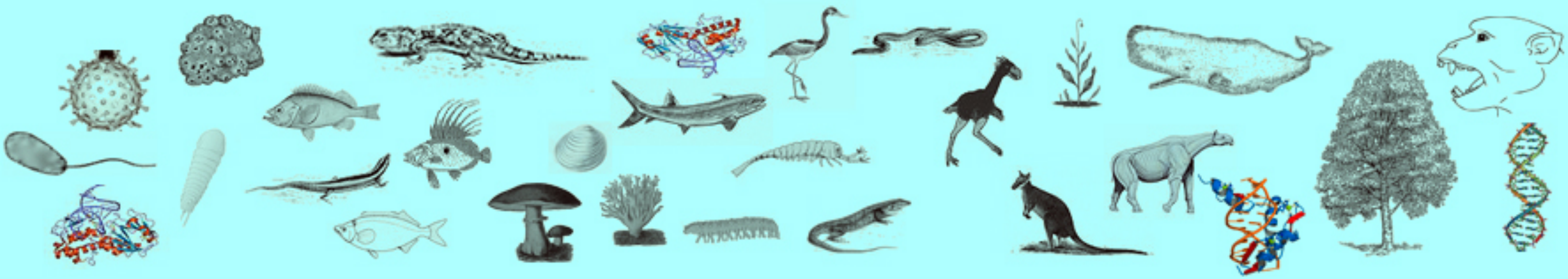


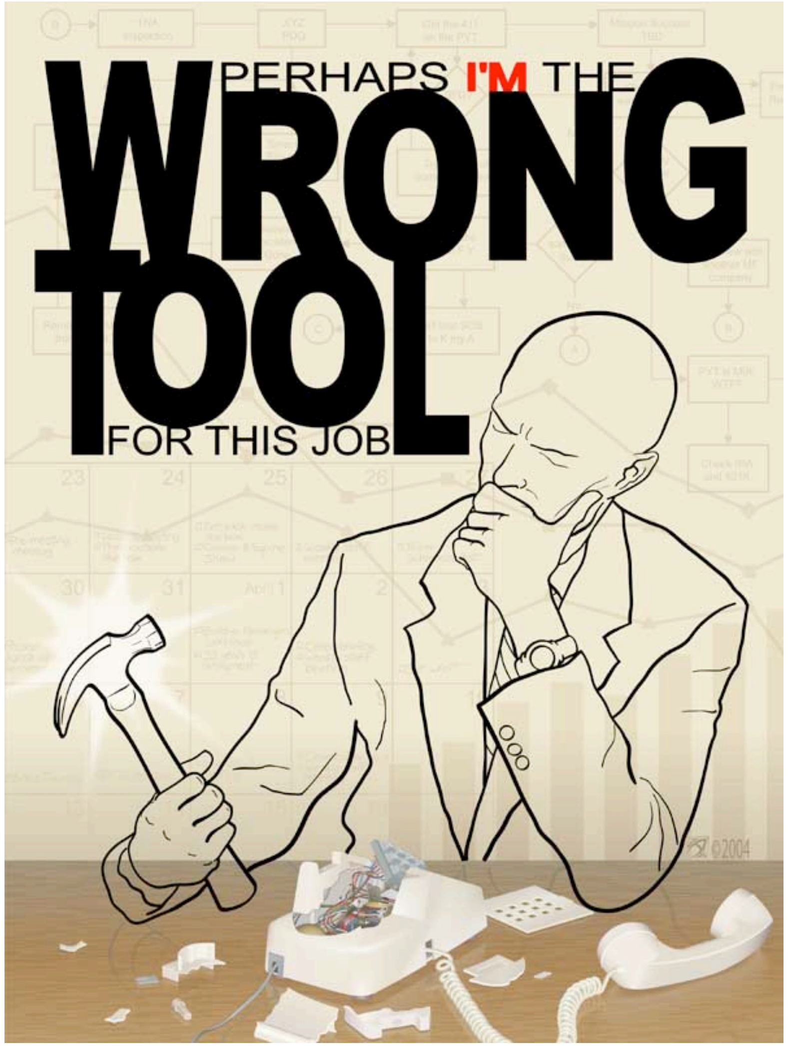
EVOLUTIONARY CONSIDERATIONS IN SYNTHETIC BIOLOGY

Orkun S Soyer
University of Exeter

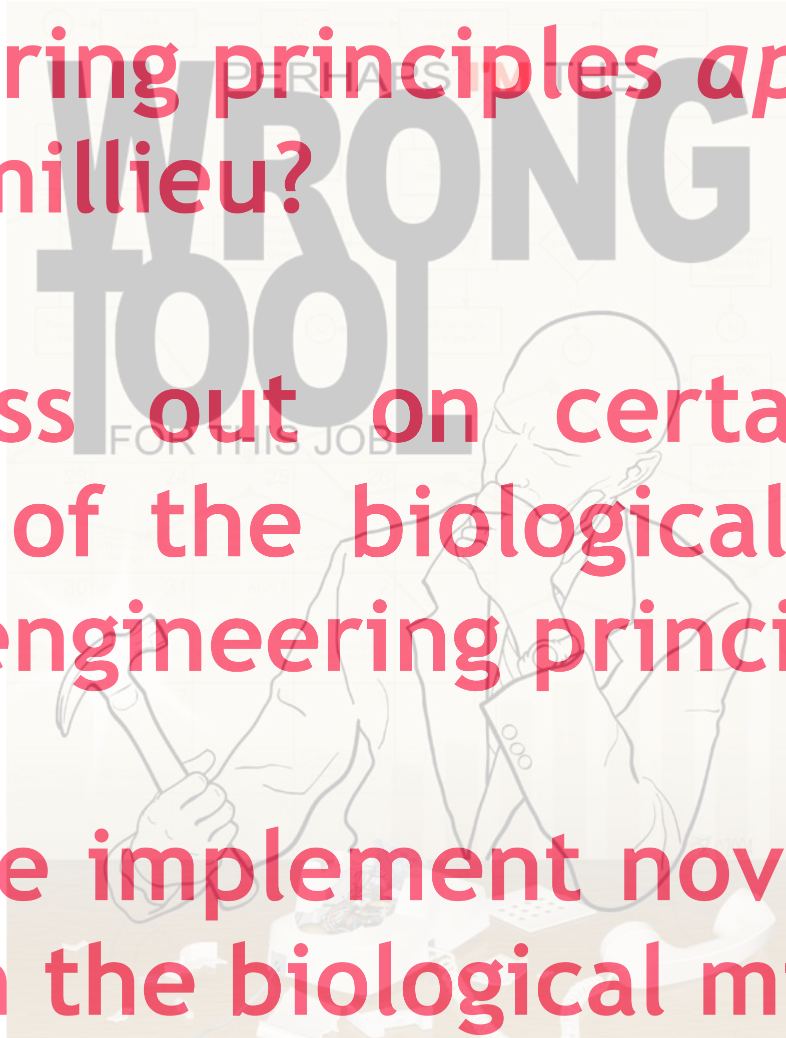
Applying *engineering principles* to (re)design biological systems

SYNTHETIC	BIOLOGY
Standards	Plasticity
Modularity	Evolvability
Simplicity	Complexity
Reliability	Stochasticity

Applying *engineering principles* to (re)design biological systems



Applying *engineering principles* to (re)design biological systems

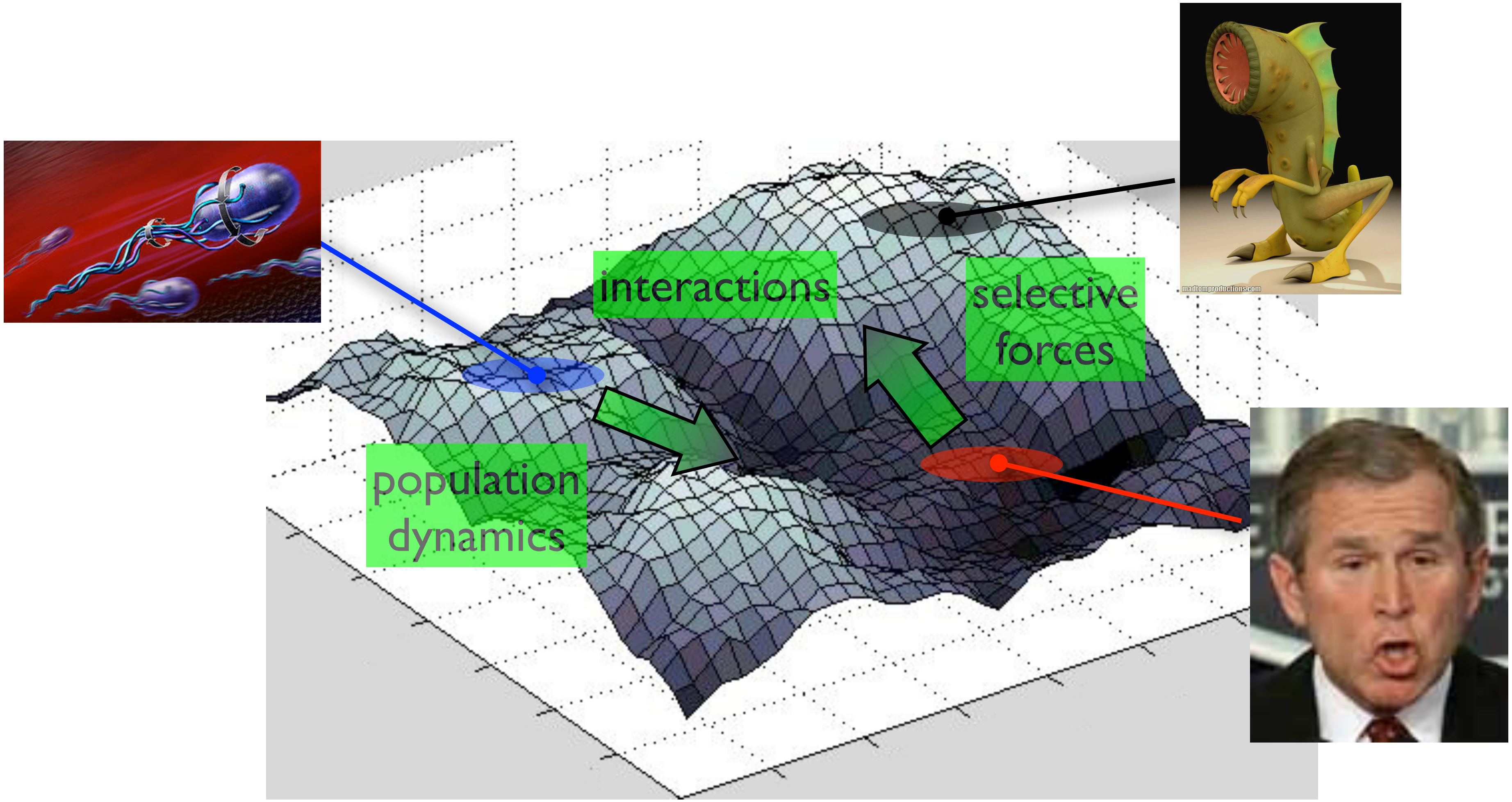


Do engineering principles *apply* for the biological millieu?

Do we miss out on certain, unique properties of the biological millieu by *enforcing* engineering principles on it?

How can we implement novelties, thus *innovate* in the biological millieu?

EVOLUTIONARY PROCESSES AS DESIGN PRINCIPLES



Can we *learn* from evolution how to *engineer* biological systems?

LEARNING FROM EVOLUTIONARY PROCESSES

Plasticity

Innovation

Functional continuity with structural change

Robustness

Evolvability

Single two-state protein motifs as plastic building blocks of response dynamics

Interface focus

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Published online

Adaptive dynamics with a single two-state protein

Attila Csikász-Nagy* and Orkun S. Soyer

the FEBS Journal

Regulating the total level of a signaling protein can vary its dynamics in a range from switch like ultrasensitivity to adaptive responses

Orkun S. Soyer, Hiroyuki Kuwahara and Attila Csikász-Nagy

Microsoft Research – University of Trento Centre for Computational and Systems Biology, Italy

Features rendering biological systems robust are byproducts of evolution under fluctuating (and co-evolving) environments

Molecular Systems Biology 4; Article number 202; doi:10.1038/msb.2008.44
Citation: *Molecular Systems Biology* 4:202

www.molecularsystemsbiology.com

molecular systems biology

Parasites lead to evolution of robustness against gene loss in host signaling networks

Marcel Salathé[1,3] and Orkun S Soyer[2,*]

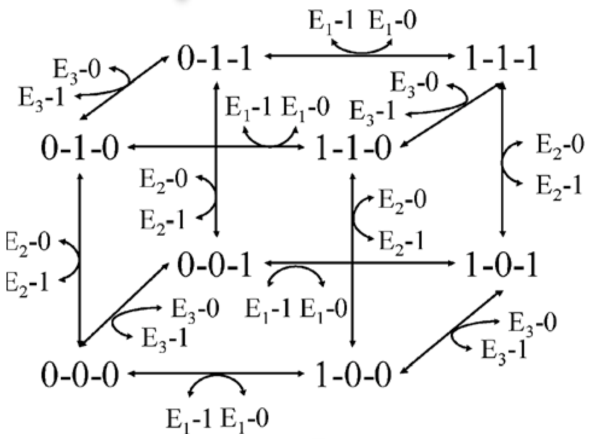
OPEN ACCESS Freely available online

PLoS COMPUTATIONAL BIOLOGY

Evolution under Fluctuating Environments Explains Observed Robustness in Metabolic Networks

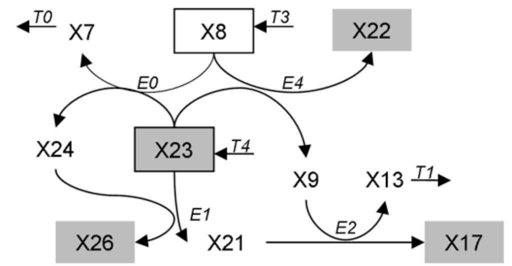
Orkun S. Soyer[1]*, Thomas Pfeiffer[2]*

Media M1
(0-1-1)



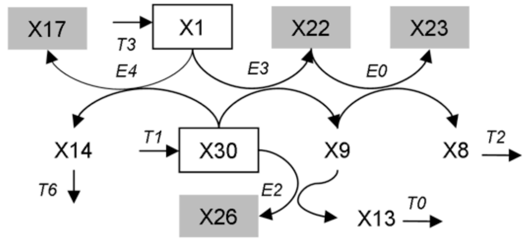
Biomass
(1-1-1)

Network final/V0 - Run ID 227



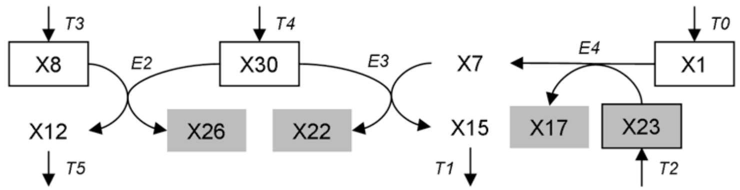
M1 – network evolved in minimal medium 1. Robustness is determined in medium 1

	E_0	E_1	E_2	E_4	T_0	T_1	T_3	T_4
W_{KO}	0	0	0	0	0.02	0.3	0	0
dos	12	11	17	20	8	3	16	9



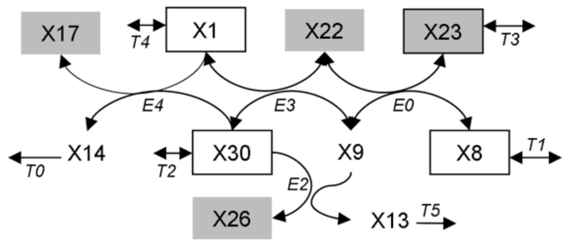
M2 – network evolved in minimal medium 2. Robustness is determined in medium 2

	E_0	E_1	E_2	E_3	T_0	T_1	T_2	T_3	T_6
W_{KO}	0	0	0	0	0.1	0	0.07	0	0.03
dos	14	21	17	15	4	15	5	12	5



R – network evolved in rich medium which contains the metabolites present in minimal medium 1 and 2. Robustness is determined in rich medium

	E_2	E_3	E_4	T_0	T_1	T_2	T_3	T_4	T_5
W_{KO}	0	0	0	0	0.08	0	0	0	0.05
dos	20	13	16	4	5	7	4	8	5



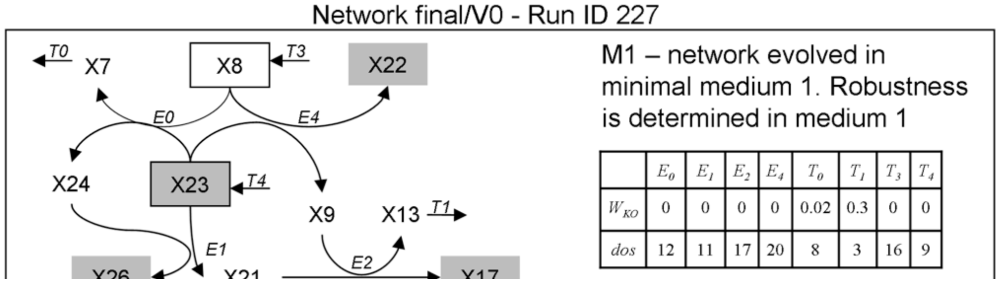
Legend

X0 Metabolite required for biomass formation

X0 Metabolite present in the environment

V – network evolved in the fluctuating environment, changing between minimal medium 1, minimal medium 2, and rich medium. Robustness is determined for each media individually, and over all media

	E_0	E_2	E_3	E_4	T_0	T_1	T_2	T_3	T_4	T_5
$W_{KO}(M1)$	0	0	0	0	0	0	0.4	0	0.04	0.1
$W_{KO}(M2)$	0	0	0	0	0.09	0.3	0	1.4	0	0.1
$W_{KO}(R)$	0.8	0	1.0	0	0.06	0.8	0.3	0.5	0.5	0.2
$W_{KO}(V)$	0	0	0	0	0	0	0	0	0	0.1
dos	8	12	8	9	3	3	3	2	2	2

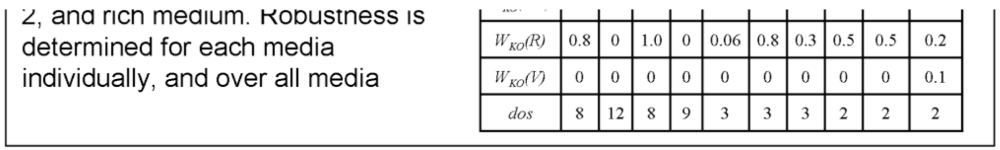


	E_0	E_1	E_2	E_4	T_0	T_1	T_3	T_4
W_{KO}	0	0	0	0	0.02	0.3	0	0
dos	12	11	17	20	8	3	16	9

Fluctuating environments result in the evolution of metabolic networks with more redundant paths and promiscuous enzymes.

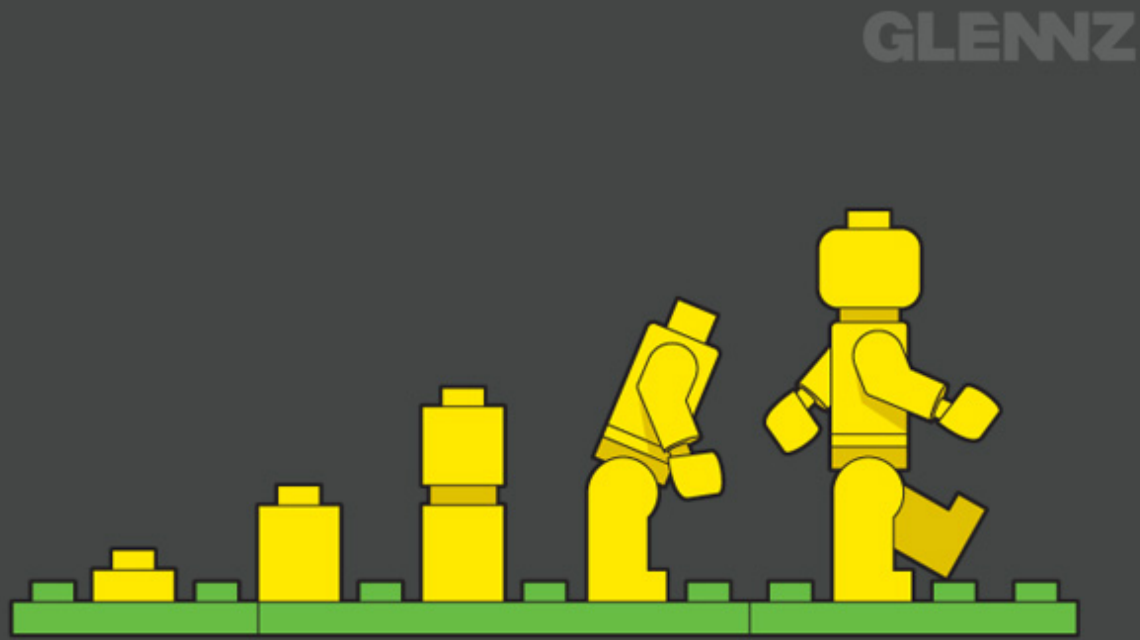
Both features result in robustness against knockouts.

Robustness is lost upon subsequent evolution under stable environments



$W_{KO}(R)$	0.8	0	1.0	0	0.06	0.8	0.3	0.5	0.5	0.2
$W_{KO}(V)$	0	0	0	0	0	0	0	0	0	0.1
dos	8	12	8	9	3	3	3	2	2	2

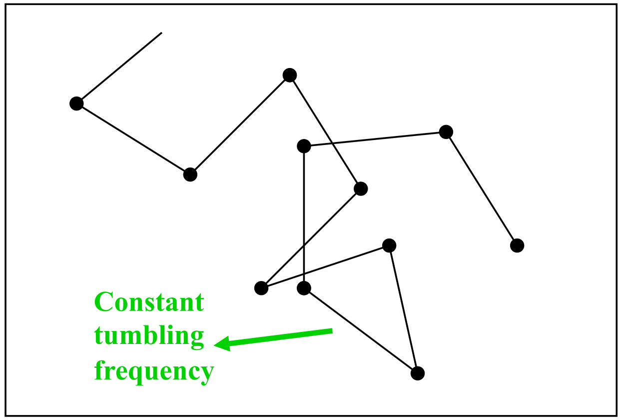
Evolution,
Innovation & Evolvability



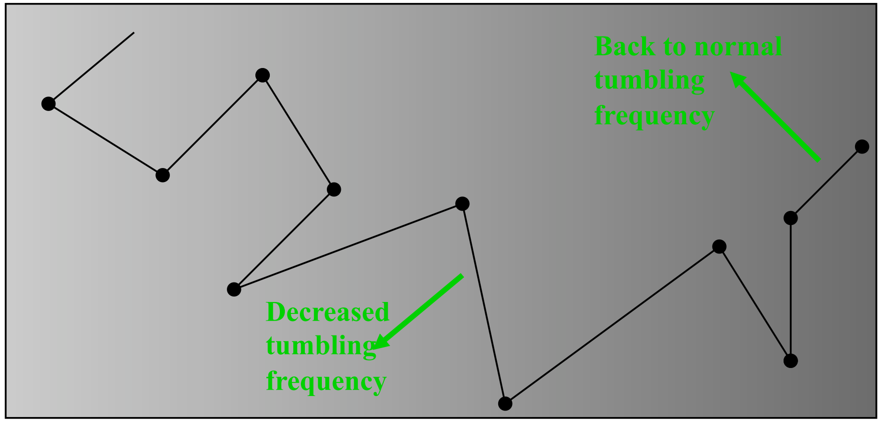
Chemotaxis in *Escherichia coli*

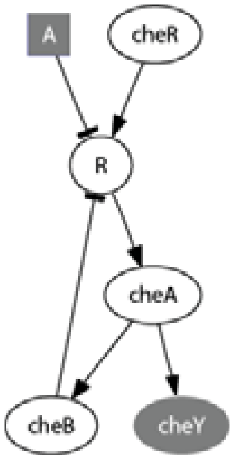
Chemotaxis in *E. coli* is based on temporal comparison of signal levels (i.e. it requires memory and adaptation)

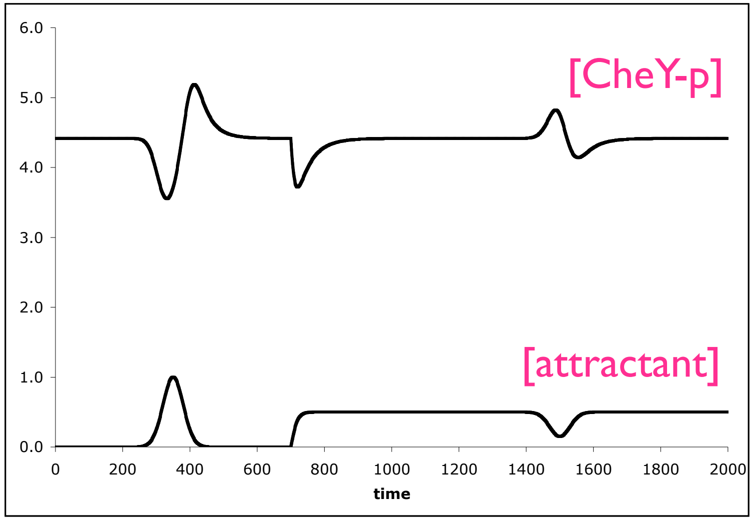
No food gradient: Random walk



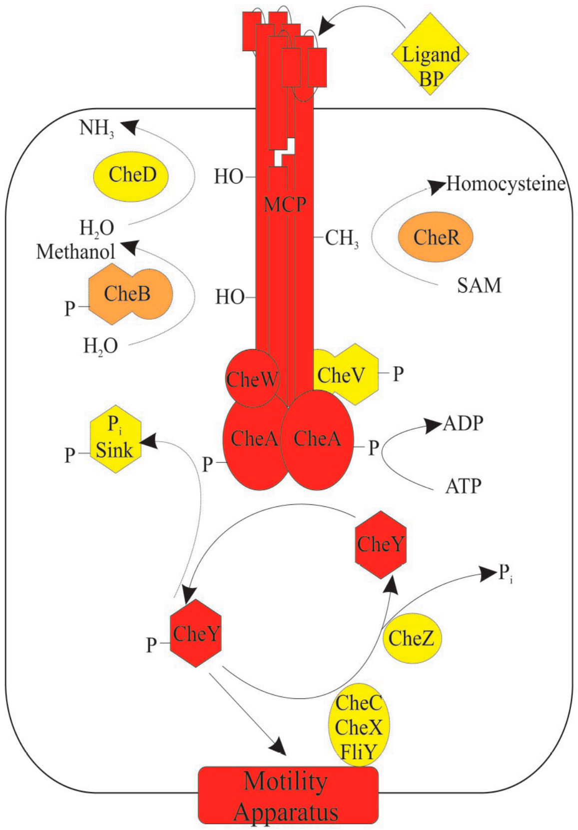
Increasing food gradient: Biased random walk







Evolution of Bacterial Chemotaxis



All bacteria seem to have the adaptation mechanism implemented

Szurmant H and Ordal GW (2004), *Microbiol. Mol. Biol. Rev.*

Adaptation seem to be the best chemotaxis strategy

Clark DA and Grant LC (2005), *PNAS*
Celani A and Vergassola M (2010), *PNAS*

There seem to be no other chemotaxis strategy possible!

Schnitzer MJ (1993), *Phys Rev E*

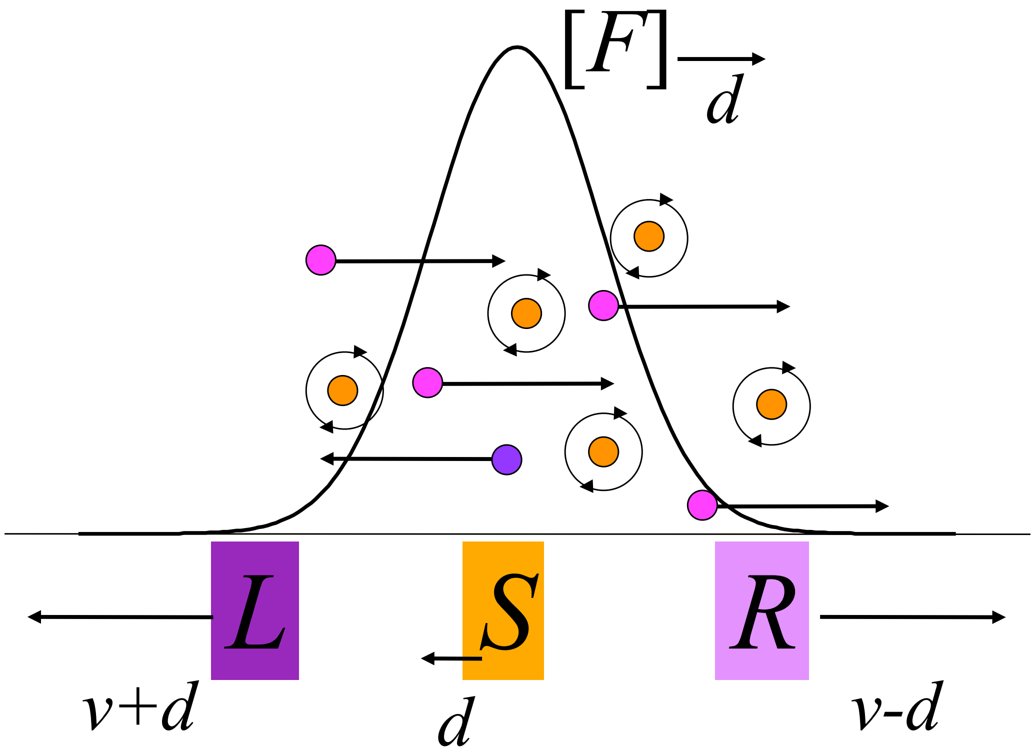
Simplifying chemotaxis behaviour

$$L \underset{\beta/2}{\overset{\alpha_L}{\rightleftharpoons}} S \underset{\beta/2}{\overset{\alpha_R}{\leftrightharpoons}} R$$

$$\frac{\partial L}{\partial t} = (v+d)\frac{\partial L}{\partial x} - \alpha_L L + \frac{\beta}{2} S$$

$$\frac{\partial R}{\partial t} = -(v-d)\frac{\partial R}{\partial x} - \alpha_R R + \frac{\beta}{2} S$$

$$\frac{\partial S}{\partial t} = d\frac{\partial S}{\partial x} + \alpha_R R + \alpha_L L - \beta S$$



Considering chemotaxis strategies (responses)

$$L \underset{\beta/2}{\overset{\alpha_L}{\rightleftarrows}} S \underset{\beta/2}{\overset{\alpha_R}{\leftrightarrows}} R$$

linear

Simplest response possible

$$\alpha_L = \alpha_0 + \lambda\left[F\right]$$
$$\alpha_R = \alpha_0 + \lambda\left[F\right]$$

adaptive

Mimicking the response seen in *E. coli*

$$\alpha_L = \alpha_0 + \lambda(v + d)\left[F\right]'$$
$$\alpha_R = \alpha_0 - \lambda(v - d)\left[F\right]'$$

Optimal chemotaxis strategies

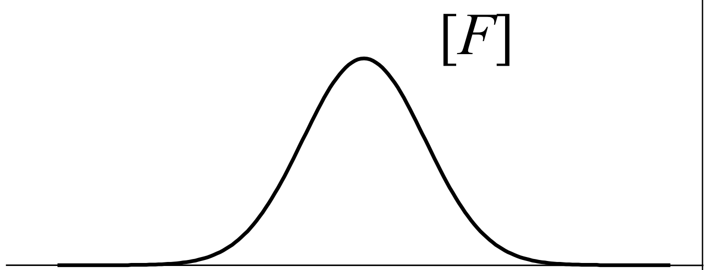
α_0, β, λ

Food found in a given time period

$$\frac{\partial L}{\partial t} = (v+d)\frac{\partial L}{\partial x} - \alpha_L L + \frac{\beta}{2} S$$

$$\frac{\partial R}{\partial t} = -(v-d)\frac{\partial R}{\partial x} - \alpha_R R + \frac{\beta}{2} S$$

$$\frac{\partial S}{\partial t} = d\frac{\partial S}{\partial x} + \alpha_R R + \alpha_L L - \beta S$$

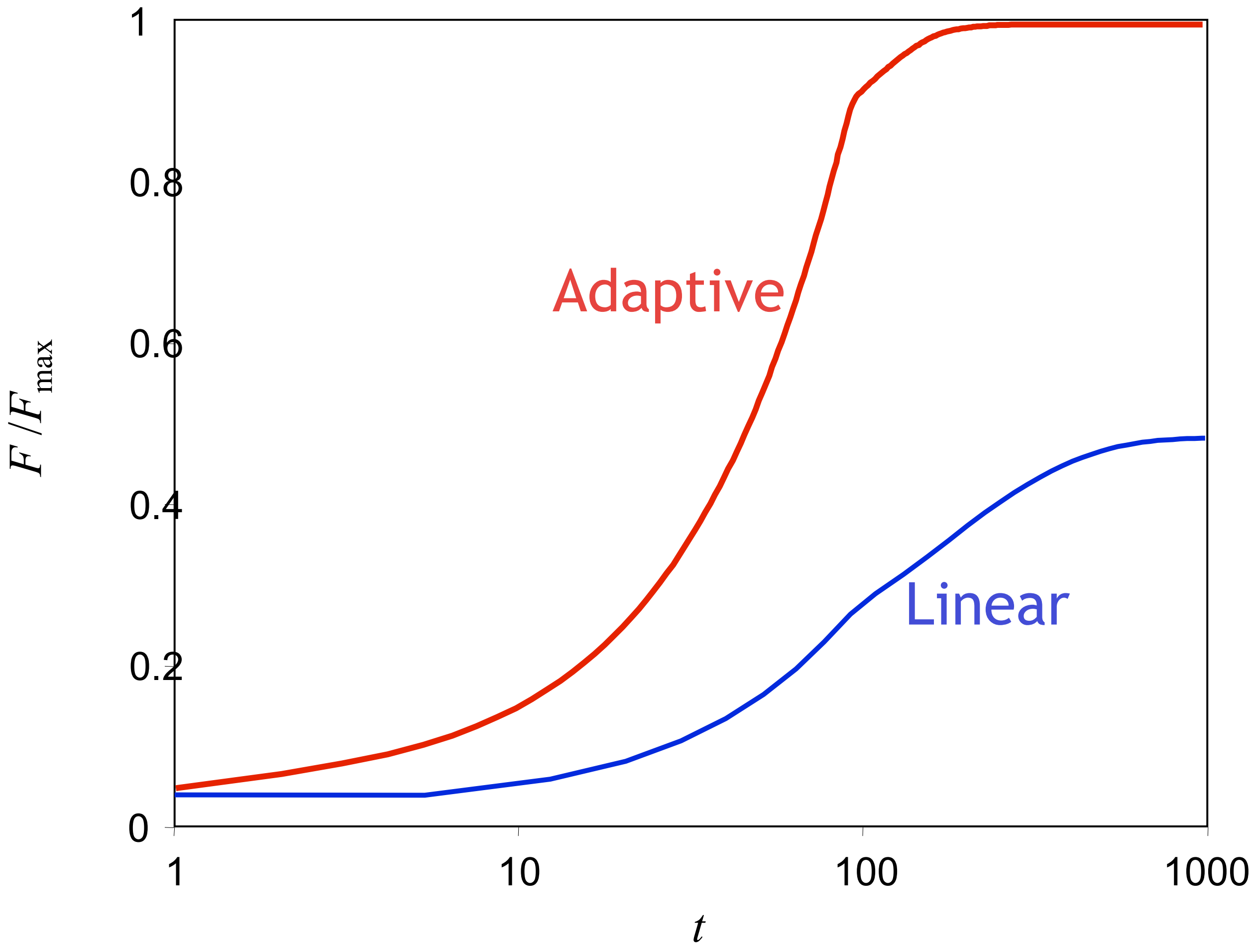


$$\alpha_L = \alpha_0 + \lambda[F]$$

$$\alpha_R = \alpha_0 + \lambda[F]$$

$$\beta$$

Both strategies work!
But, adaptive bugs are smarter and faster...

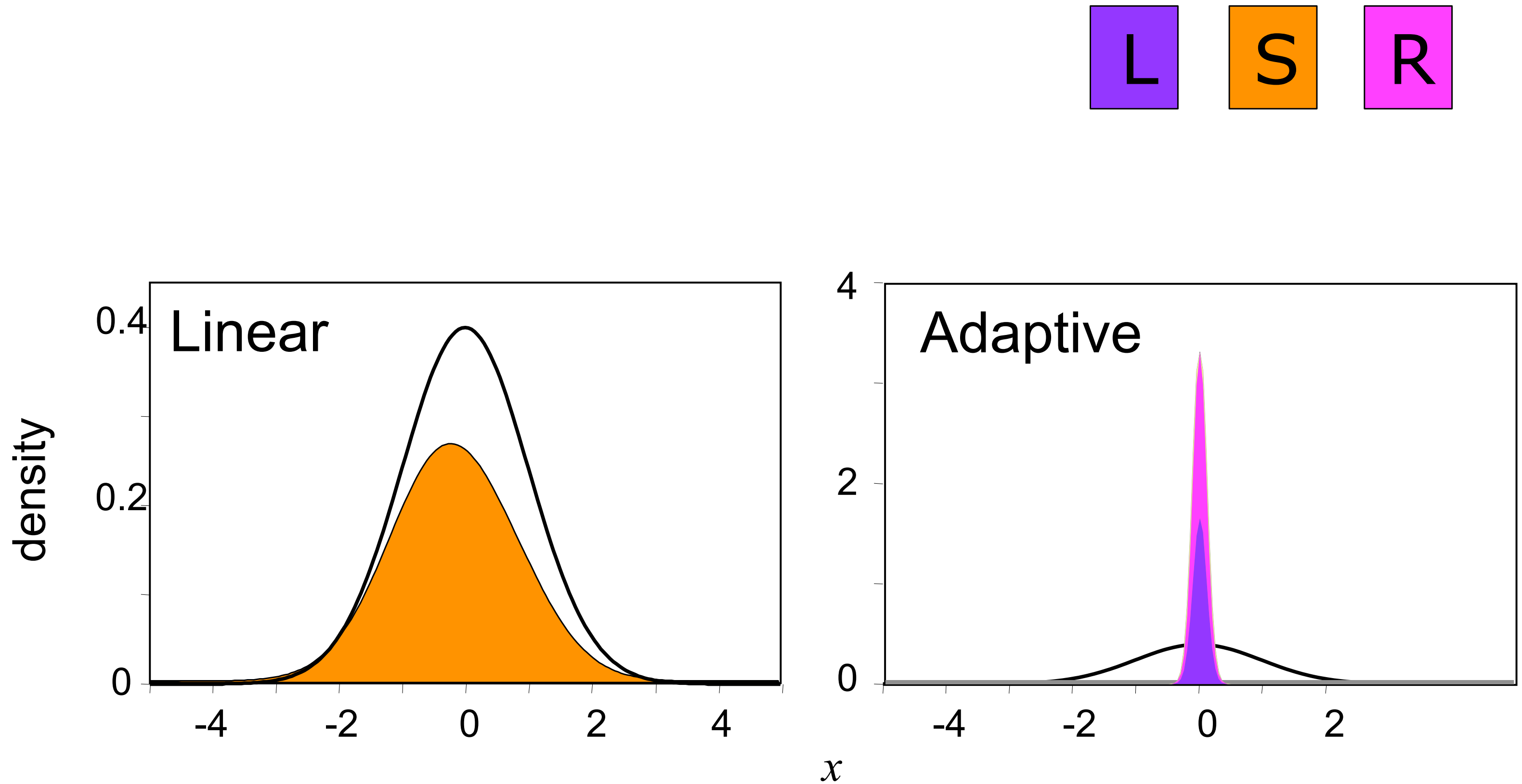


d=0

Optimal parameter values

α_0	0.01	0.01
β	100	0.02
λ	100	3.6

How do these strategies work?

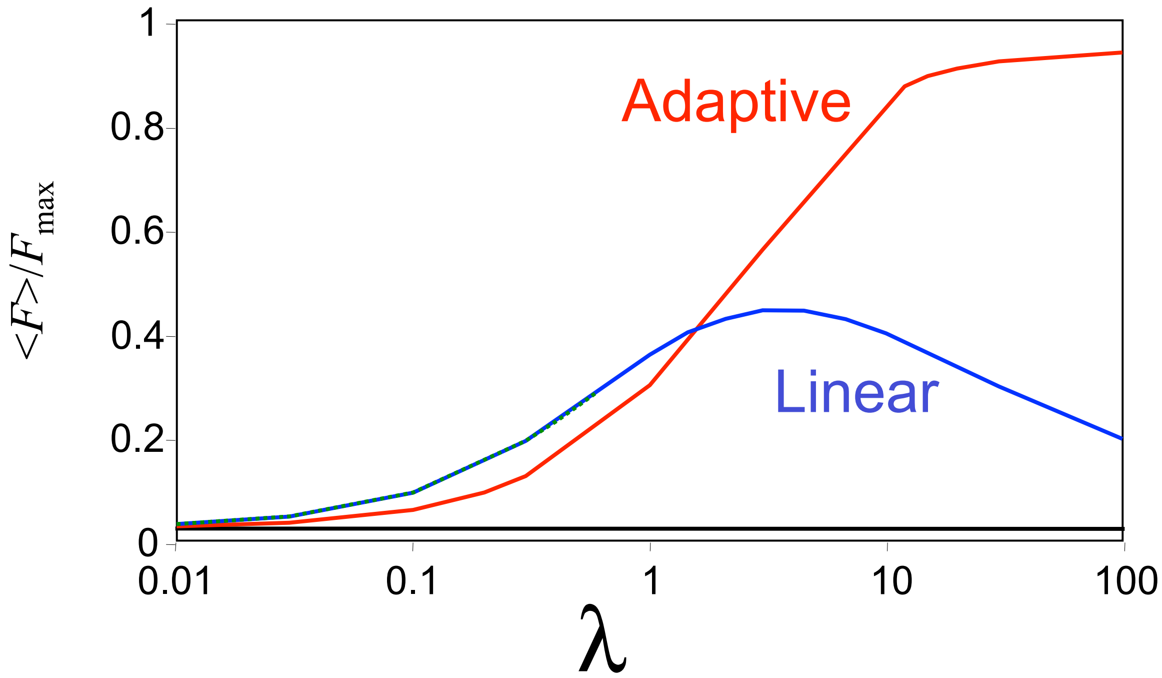


Stumbling on food and staying with it

Gradient climbing

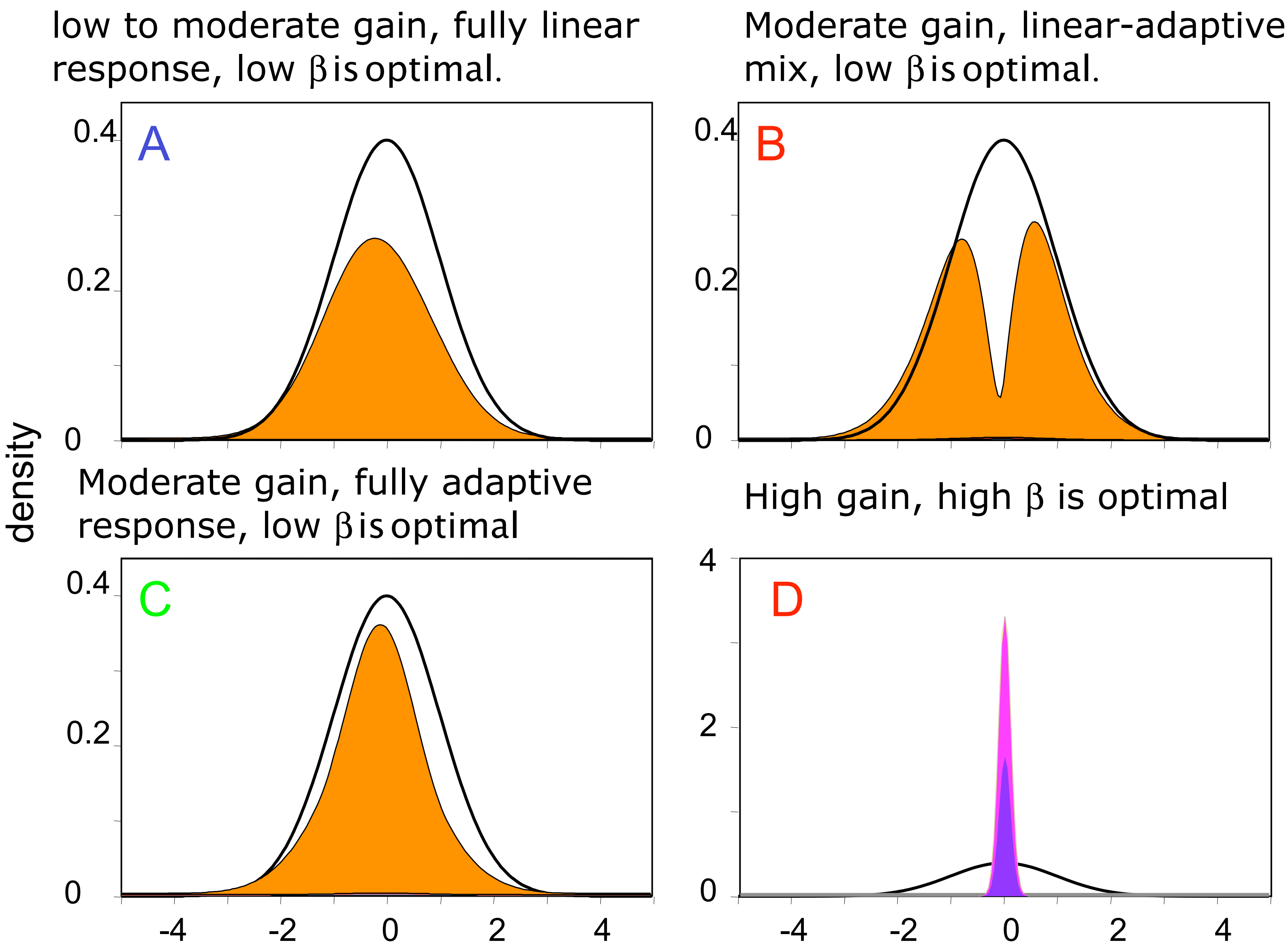
What if sensitivity is the driving selective pressure?

$$\alpha = \alpha_0 + \lambda\left[F\right] \qquad \alpha = \alpha_0 + \lambda(v+d)\left[F\right]'$$

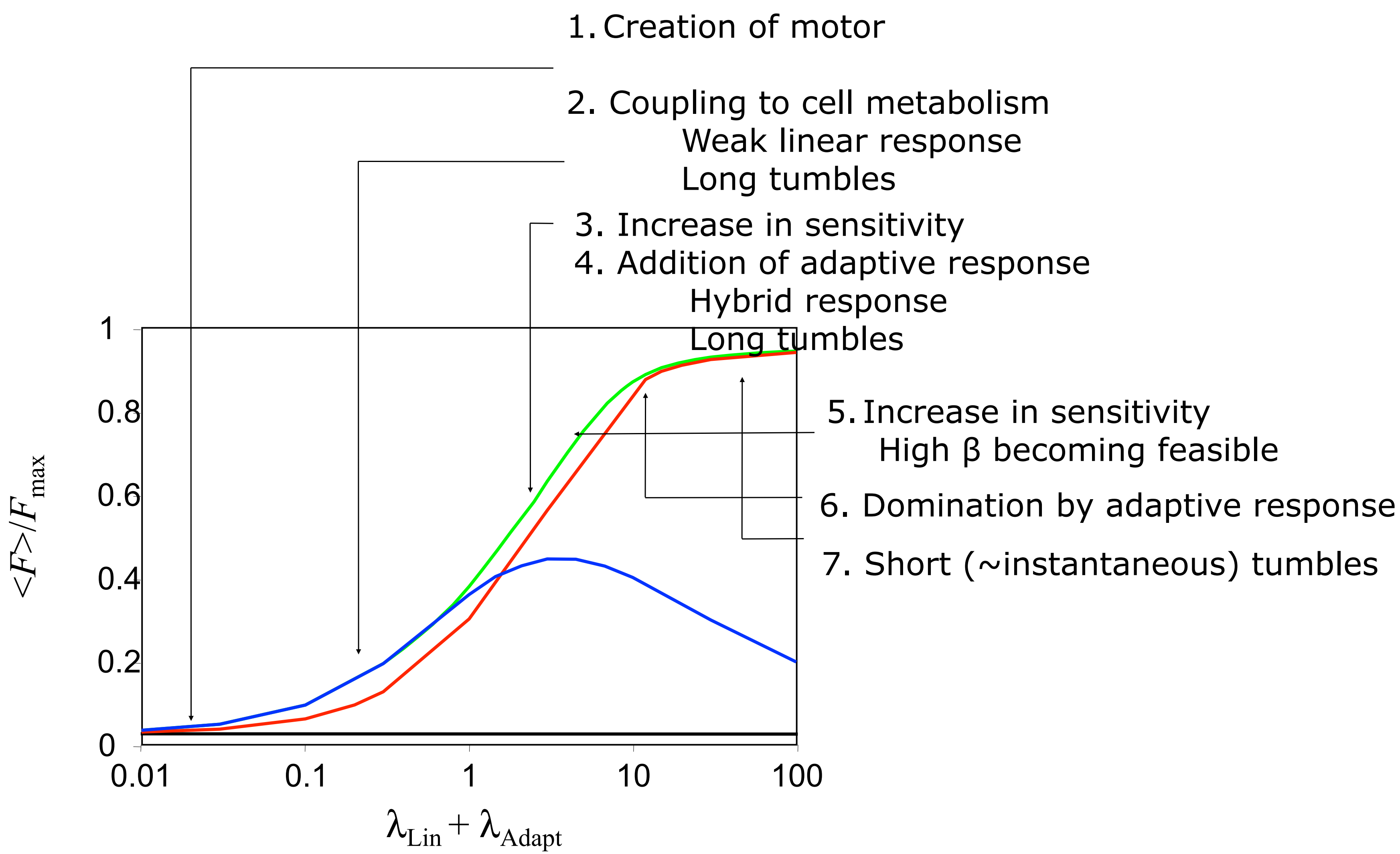


d=0
t=1000

Distinct chemotaxis strategies



Evolution of chemotaxis!



Evolution of chemotaxis: Thoughts for synthetic biology

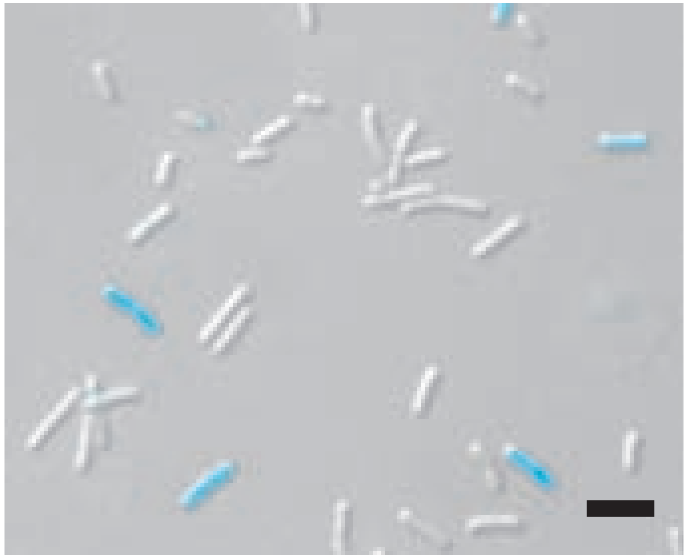
A *simpler to implement* alternative design for chemotaxis (with mediocre performance that is good enough for co-localisation with signal)

Where initial designs might *evolve to* or where final designs might *evolve from* is not trivial!
Be aware of the *principle* of;
Functional continuity with structural change

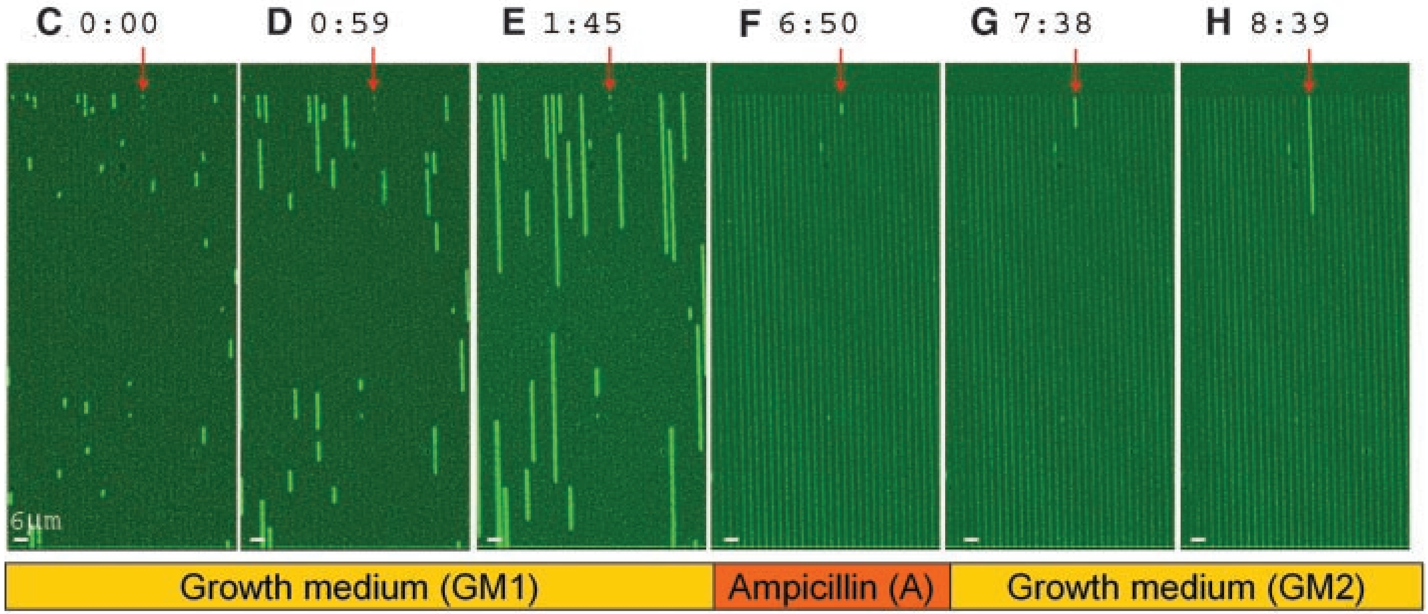
Stochasticity
Nonlinearity
Evolvability

The observation...

Several bacteria display distinct phenotypes in an otherwise clonal population

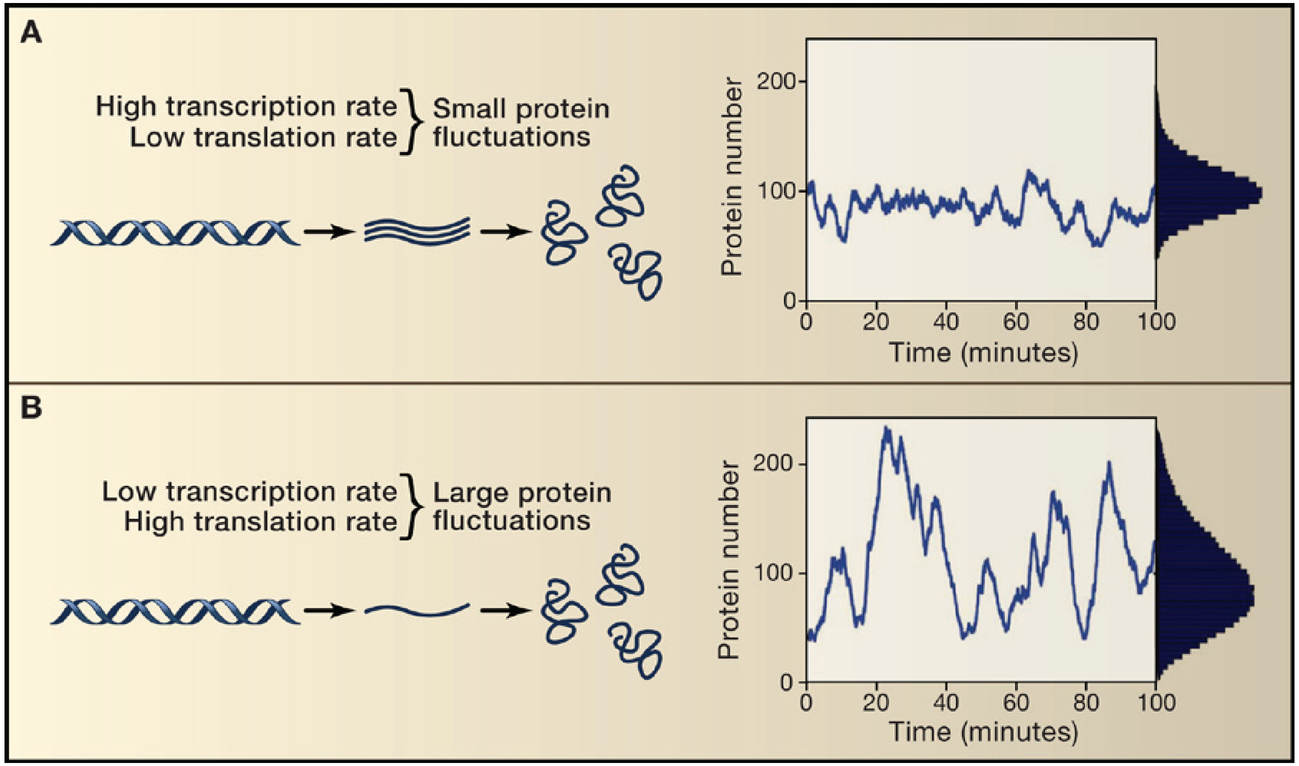


Maamar, H, et al. Noise in gene expression determines cell fate in Bacillus subtilis. Science 317, 526-529 (2007).

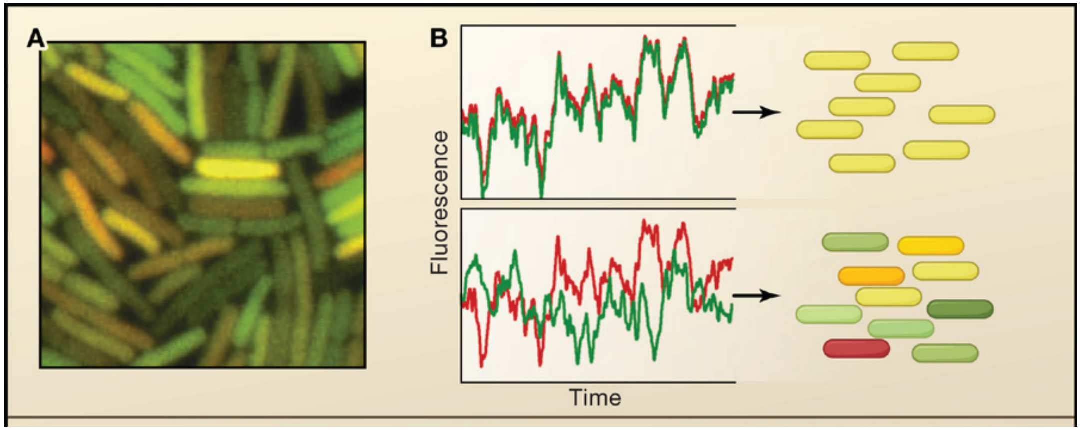


Balaban, NQ et al. Bacterial persistence as a phenotypic switch. Science 305, 1622 (2004).

How? The Molecular Basis...
1. Noise



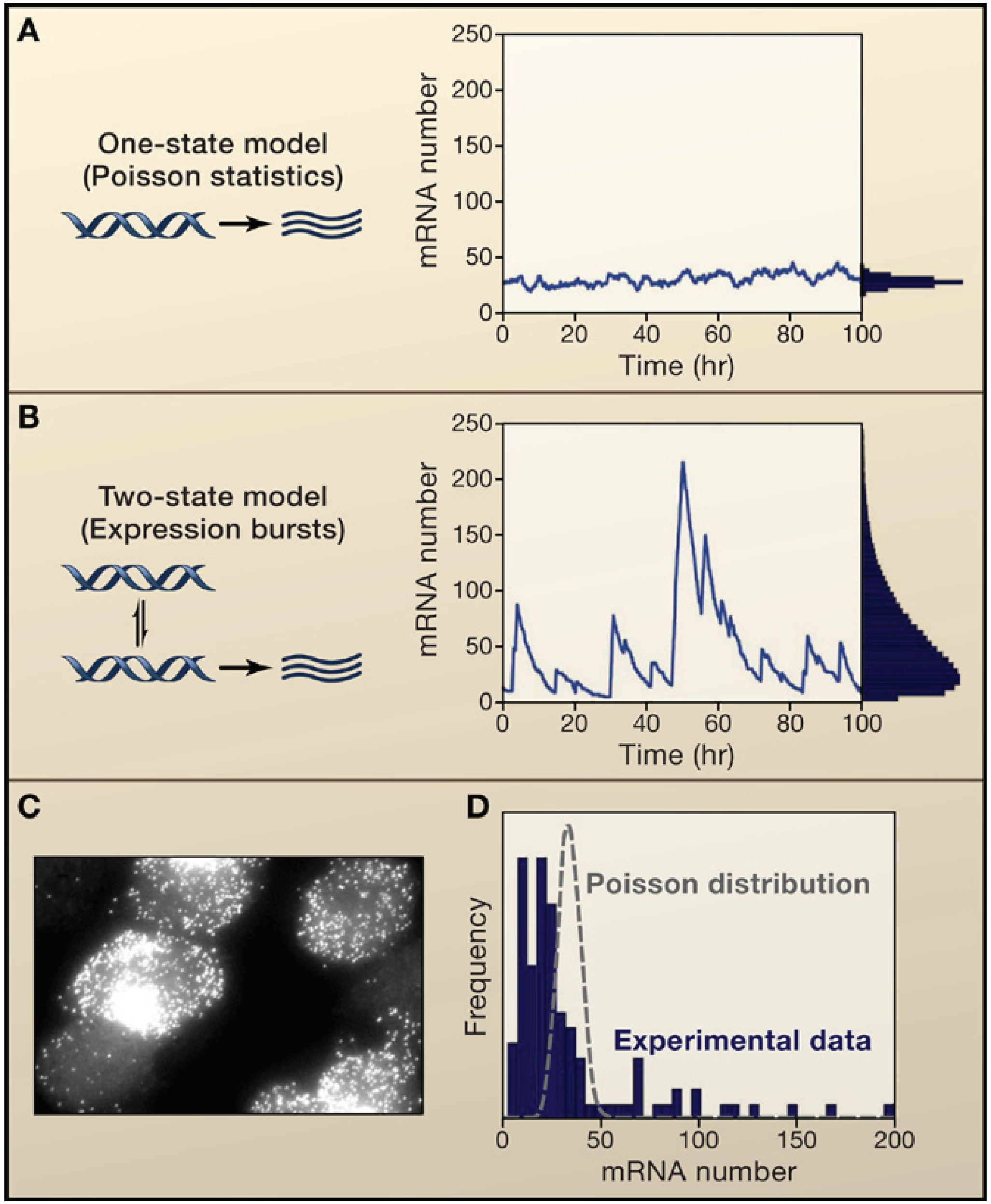
Noise is inherent in gene regulatory networks.



Raj, A. & v Oudenaarden, A. Nature, nurture, or chance: stochastic gene expression and its consequences. Cell 135,(2008).

How? The Molecular Basis...
1. Noise

Noise is inherent in gene regulatory networks.



Raj, A. & v Oudenaarden, A. Nature, nurture, or chance: stochastic gene expression and its consequences. Cell 135,(2008).

The Molecular Basis...
2. Bistability

A bistable gene regulatory network gives rise to stochastic switching at population level.

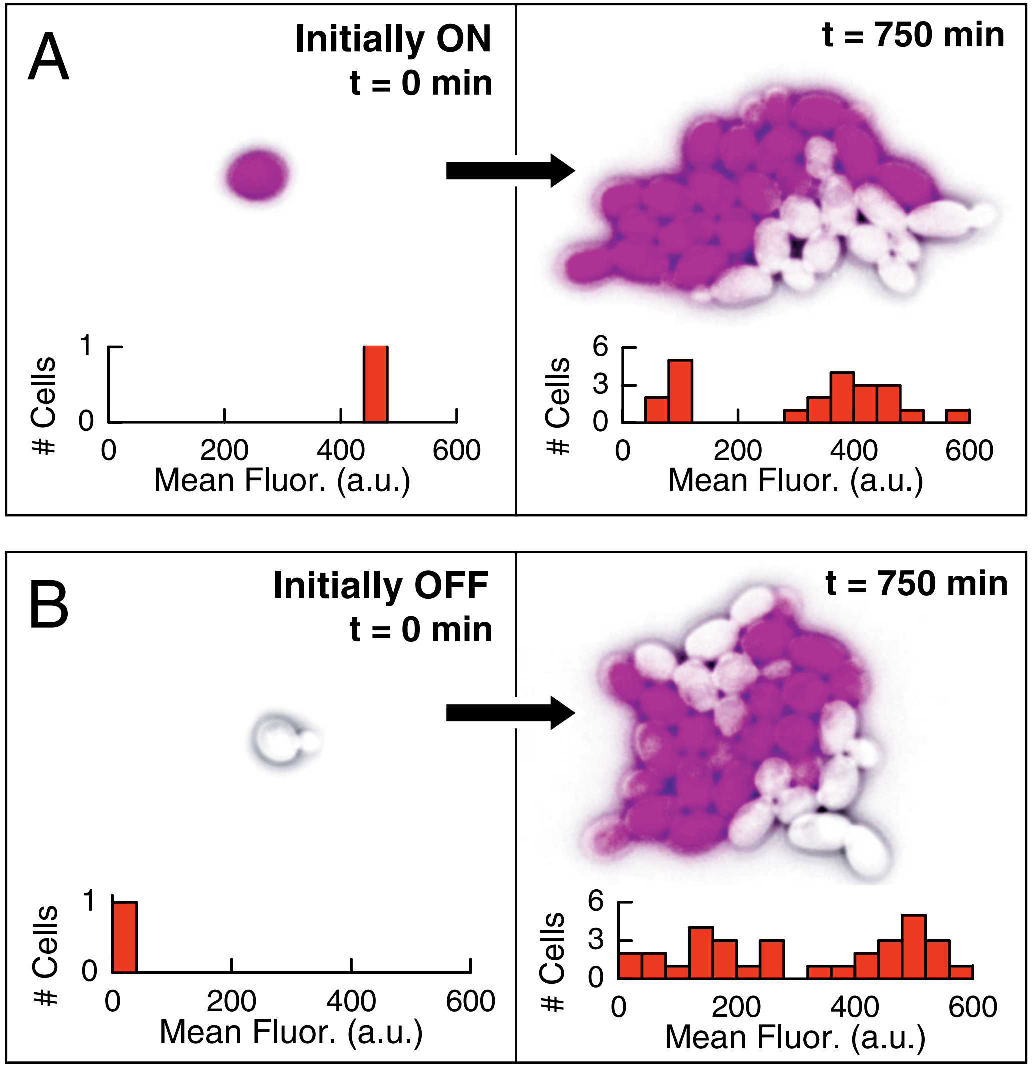
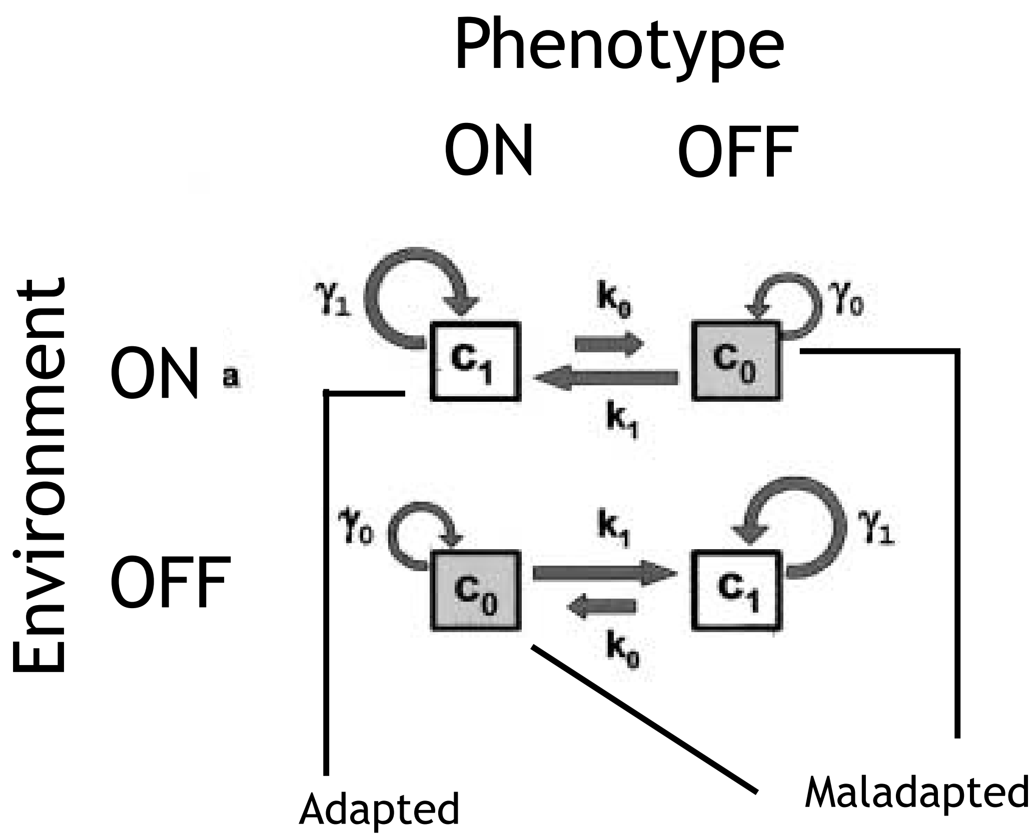


Figure 1. Cells Switch between Expressing and Nonexpressing States

van Oudenaarden, A. et al. Heritable stochastic switching revealed by single-cell genealogy. PLoS Biol 5, e239 (2007).

The Explanation...

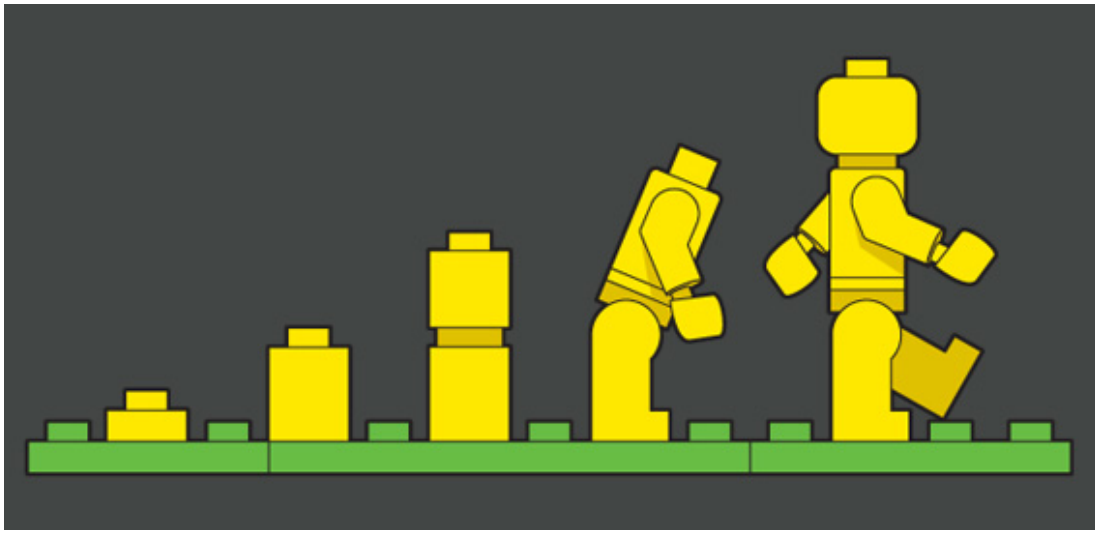
Beneficial heterogeneity:
Under fluctuating environments stochastic switching can provide an advantage to the population.



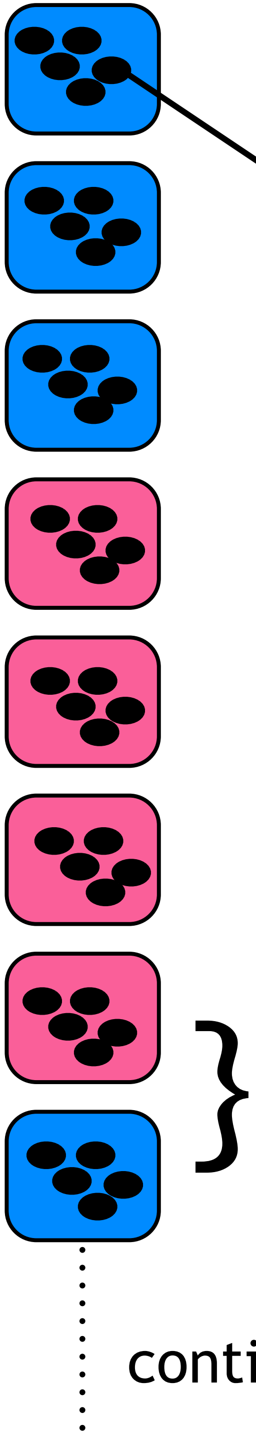
Thattai, M. & van Oudenaarden, A. Stochastic gene expression in fluctuating environments. Genetics 167, 523-530 (2004).

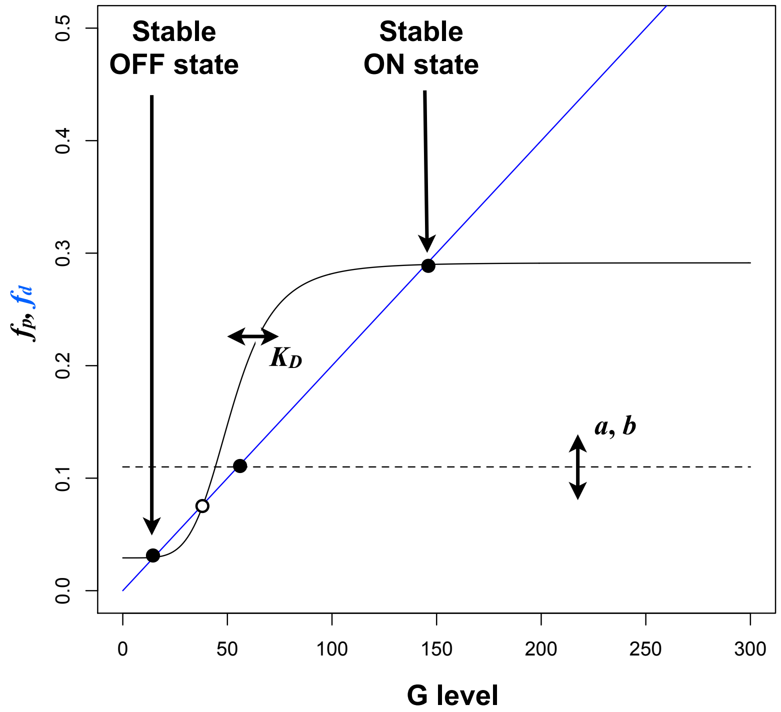
Correlation does not imply causation

Is fluctuating selection sufficient for the evolution of bistable and noisy gene regulation in individuals?



In silico evolution under fluctuating selection





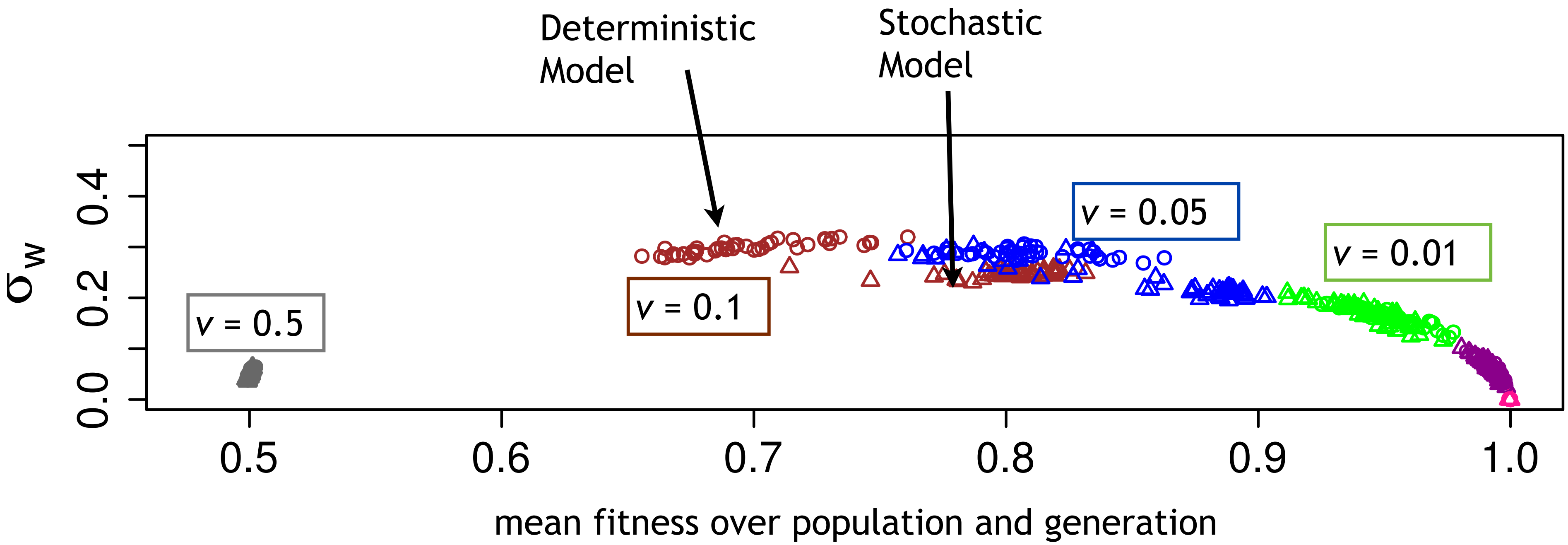
Evolving parameters;
a, b, N, and K_D

Starting from;
a=b=1, N=0, and K_D=50

Environmental switching probability per generation *v* = 0.5, 0.1, 0.05, 0.01 ...

continue *ad infinitum*

Cells adapt to fluctuating environments



Evolving parameters;
a, b, N, and K_D

Starting with a linear system;
a=b=1, N=0, and K_D=50

Cells adapt to fluctuating environments

- Under all rates of environmental switching (ES) analysed, cells showed some level of adaptation

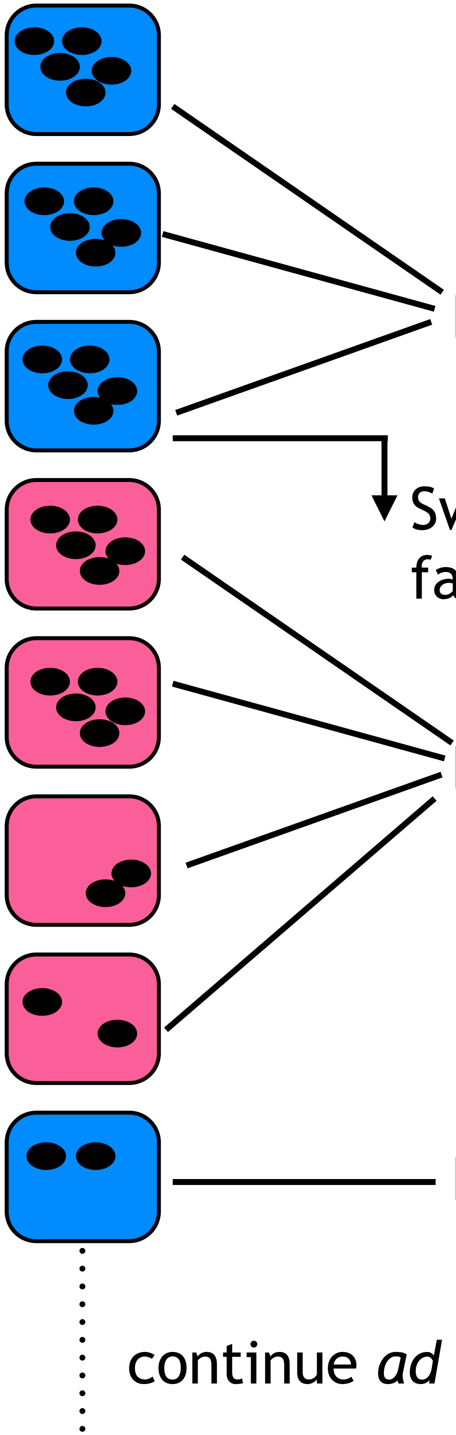
- Stochasticity in gene regulation improved adaptation only under intermediary rates of ES

- Higher nonlinearity and bistability in gene regulation evolved only in the stochastic phenotype model and only under those rates of ES where stochasticity was found to be beneficial

How can we understand these results?

Why did nonlinearity and bistability evolve in these simulations? and why did it evolve only under a certain range of environmental fluctuations?

In silico evolution under fluctuating selection



Produce lots of protein

Switch phenotype as fast as possible → via mutations, OR ?

Produce very little protein

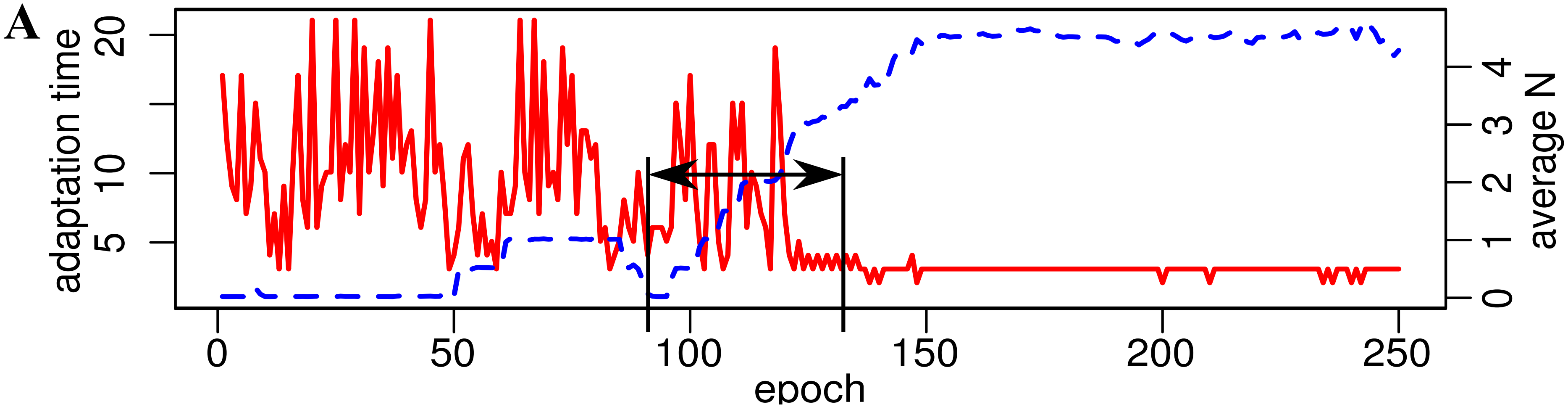
Produce lots of protein

continue *ad infinitum*

Selection for increased *evolvability*

Switch phenotype as fast as possible

Cells evolved increased evolvability



A model with deterministic environment; Epochs of 10 generations.

Adaptation time is defined as “number of generations for mean population fitness to reach above 0.7”.

Stochastic Switching As A Byproduct Of Evolution of Evolvability

- Fluctuating environments can select for the evolution of higher evolvability

- Specific nonlinear gene regulatory dynamics underpin higher evolvability at molecular level

- In the presence of noise, increasing nonlinearity further enhances diversity and gives rise to bistability

- Bistability and noise can give rise to stochastic switching, which can immensely enhance adaptation time

LEARNING FROM EVOLUTIONARY PROCESSES

Plasticity

Degradation as a tool to regulate response dynamics

Innovation

Use of evolutionary simulations as design tools: Functional continuity with structural change

Robustness

Fluctuations as driver and maintainer of structural features underlying robustness to deleterious mutations

Evolvability

Nonlinearity and noise as potential sources of faster adaptation

University of Exeter

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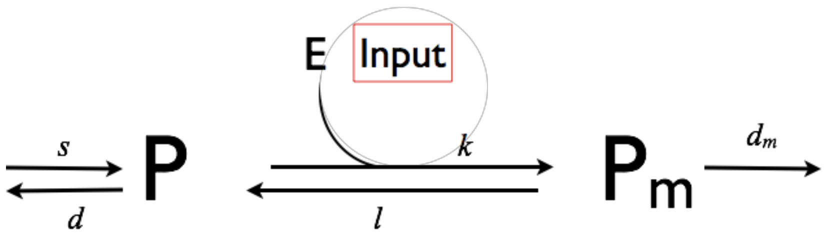
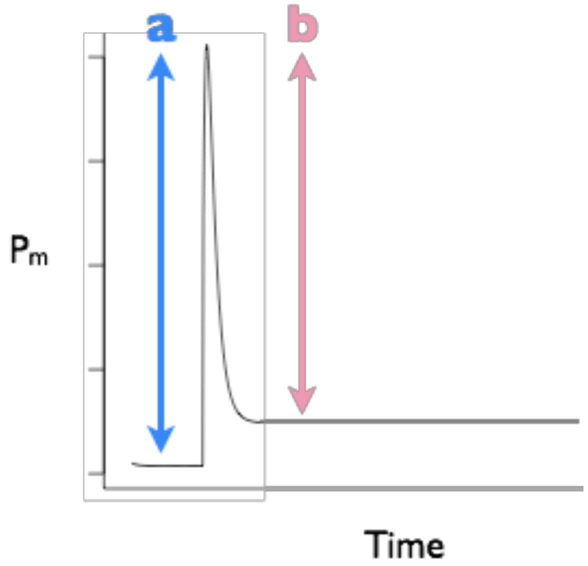
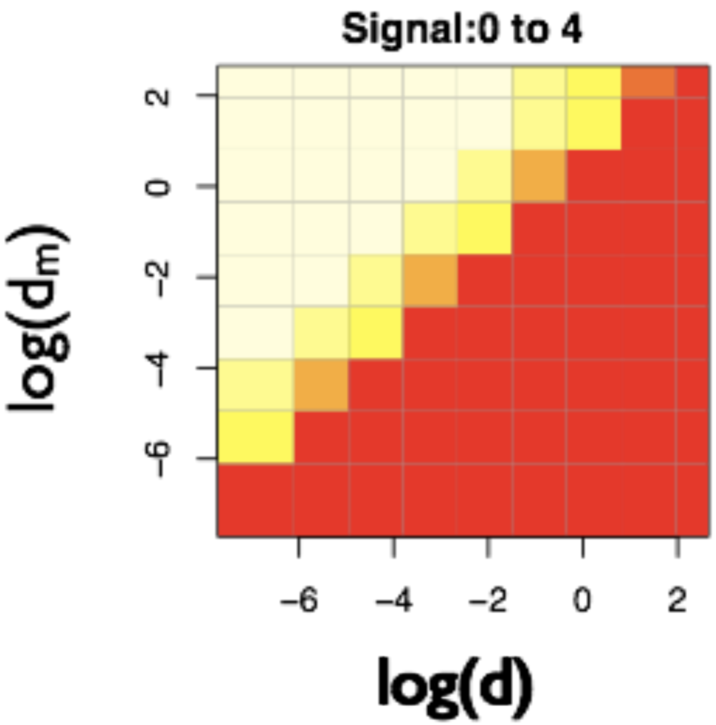
Richard Goldstein
MRC, Mill Hill, London

Single two-state protein motifs as building blocks of response dynamics



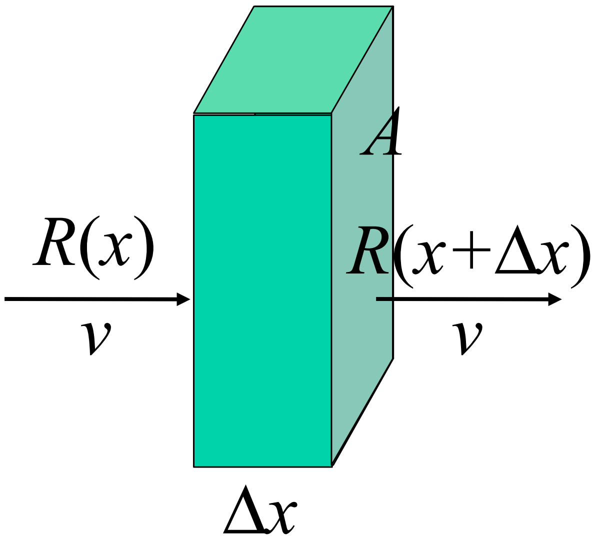




b/a = 0 step response
b/a = 1 perfect adaptation

Mathematics to the rescue

$$\frac{\partial R}{\partial t} = \boxed{-v\frac{\partial R}{\partial x}} - \alpha_R R + \frac{\beta}{2}S$$



$$V = A\,\Delta x$$

Flow in left:

$$\Delta n_+ = R(x)\,Av\,\Delta t$$

Flow out left:

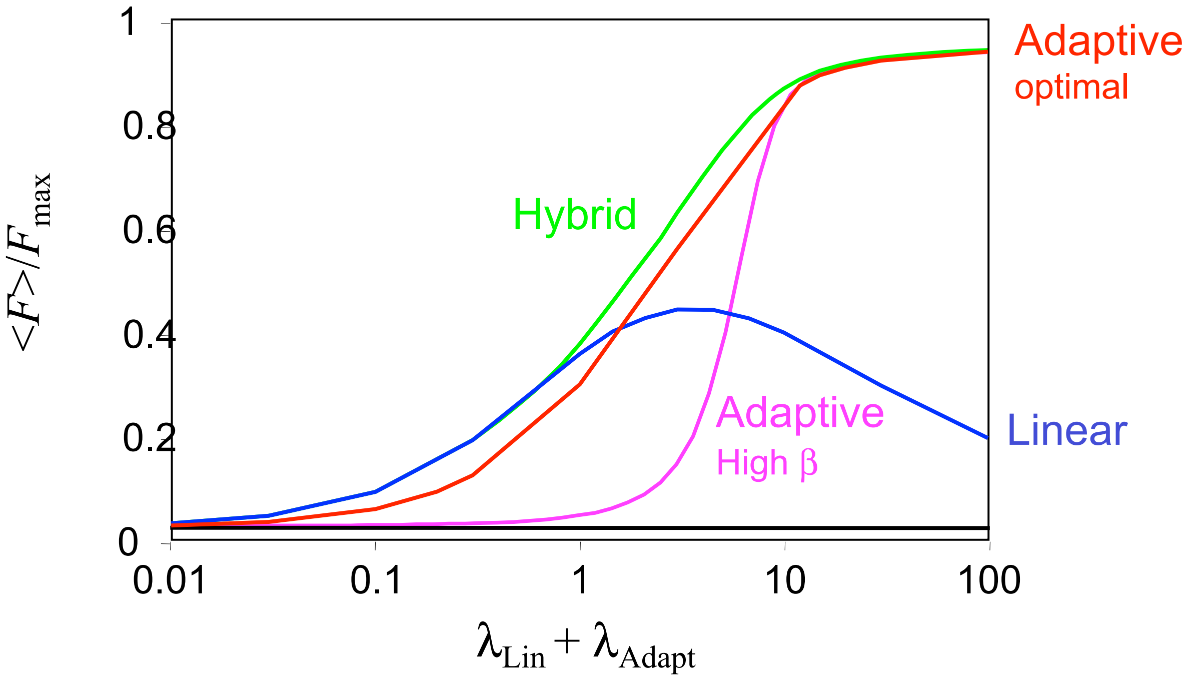
$$\Delta n_- = -R(x+\Delta x)\,Av\,\Delta t$$

$$\Delta n = v\,\Delta t\,A\big(R(x) - R(x+\Delta x)\big)$$

$$\Delta R = v\,\Delta t\,A\big(R(x) - R(x+\Delta x\big) / A\,\Delta x$$

$$\Delta R / \Delta t = -v\frac{\big(R(x+\Delta x) - R(x)\big)}{\Delta x}$$

Sensitivity as exaptation for adaptation!



What previous works have missed

Tumbling is not instantaneous, or it was not always!

Schnitzer MJ (1993), *Phys Rev E*

$$\frac{\partial L}{\partial t} = (v+d)\frac{\partial L}{\partial x} - \alpha_L L + \frac{\beta}{2} S$$

$$\frac{\partial R}{\partial t} = -(v-d)\frac{\partial R}{\partial x} - \alpha_R R + \frac{\beta}{2} S$$

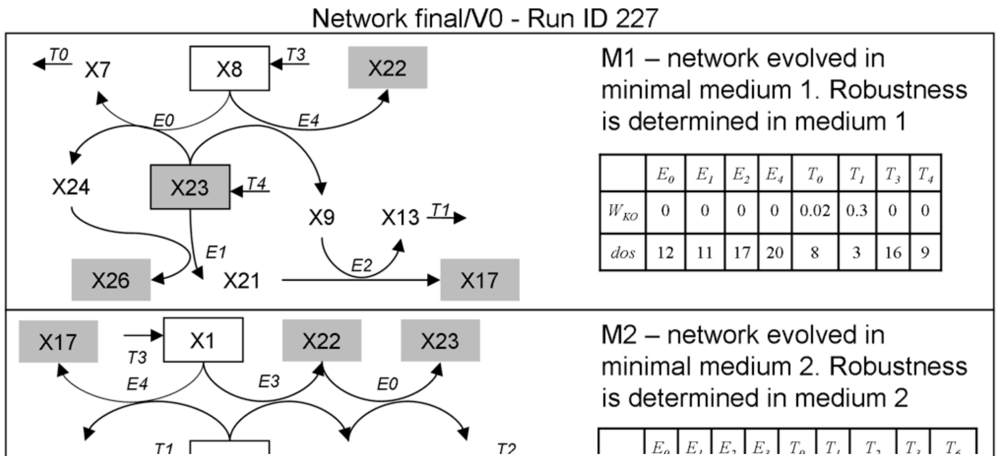
$$\frac{\partial S}{\partial t} = d\frac{\partial S}{\partial x} + \alpha_R R + \alpha_L L - \beta S$$

vs

$$\frac{\partial L}{\partial t} = (v+d)\frac{\partial L}{\partial x} - \frac{\alpha_L}{2} L + \frac{\alpha_R}{2} R$$

$$\frac{\partial R}{\partial t} = -(v-d)\frac{\partial R}{\partial x} - \frac{\alpha_L}{2} L + \frac{\alpha_R}{2} R$$

$$\beta \gg \alpha_L, \alpha_R$$

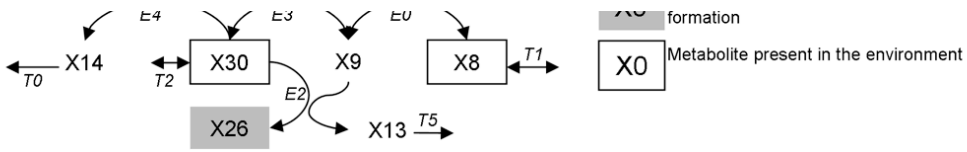


	E_0	E_1	E_2	E_4	T_0	T_1	T_3	T_4
W_{KO}	0	0	0	0	0.02	0.3	0	0
dos	12	11	17	20	8	3	16	9

Replaying the tape of evolution.
DO EVOLUTIONARY PROCESSES LEAVE FINGERPRINTS?

Redundancy in metabolic networks is an evolved response to fluctuating environments

=> bugs from stable environments should be less versatile



V – network evolved in the fluctuating environment, changing between minimal medium 1, minimal medium 2, and rich medium. Robustness is determined for each media individually, and over all media

	E_0	E_2	E_3	E_4	T_0	T_1	T_2	T_3	T_4	T_5
$W_{KO}(M1)$	0	0	0	0	0	0	0.4	0	0.04	0.1
$W_{KO}(M2)$	0	0	0	0	0.09	0.3	0	1.4	0	0.1
$W_{KO}(R)$	0.8	0	1.0	0	0.06	0.8	0.3	0.5	0.5	0.2
$W_{KO}(V)$	0	0	0	0	0	0	0	0	0	0.1
dos	8	12	8	9	3	3	3	2	2	2

METABOLIC NETWORKS: WHY HUB MOLECULES? WHY SCALE-FREE? WHY ROBUST?

- Toy model of enzymes and metabolites, with enzyme trade-off for specificity/rate.
- Evolve under selection for biomass production (fixed/fluctuating selection)
- Networks evolved under fixed selection display hub molecules and scale-free connectivity
- Networks evolved under fluctuating selection display increased robustness