



Chris Kempes Sante Fe

Georgi Marinov Stanford

Paul Schavemaker ASU, post doc

Boguljub Trickovic ASU, grad student

Mahmoudabadi, G., R. Phillips, M. Lynch, and R. Milo. 2019. Defining the energetic costs of cellular structures. bioRxiv://www.biorxiv.org/content/10.1101/666040v1. Center for Mechanisms of Evolution



#### **Arizona State University**

### **Road Map Tutorial:**

- Mechanisms of evolution: drift and the limits to selection.
- Currency for cellular cost estimates.
- Measuring costs of cellular features.
- Conversion to organismal fitness.

#### **Example applications:**

- Scaling laws in cell biology.
- Origins of genome architecture and the cost of a gene.
- Membranes and cell walls.
- Motility.

Mesmerizing Beauty, Complexity, Diversity, and the Adaptationist Paradigm



"..... from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved." Charles Darwin

The Population-genetic Environment Defines the Limits of Natural Selection



## The Drift-Barrier Hypothesis



The Power of Random Genetic Drift Is Governed by The *Effective* Number of Individuals, N<sub>e</sub>, in the Population

1) Sampling of finite numbers of gametes results in allele-frequency fluctuations.



2) The magnitude of fluctuations scales inversely with population size.



#### Linkage and Selective Interference Causes the Effective Population Size to be <u>Much</u> Smaller Than the Actual Census Size



- a background beneficial mutation lost from the population
- a background deleterious mutation fixed in the population

With free recombination, the outcome would be:





#### Figure 1

A compilation of estimates of the average amount of recombination per unit of physical distance in eukaryotic genomes, derived from 137 meiotic genetic maps. The diagonal lines have slopes of -1.



 $N_e$  = the effective size of a population;

u = base-substitution mutation rate per nucleotide site / generation.

- For a pair of identical nucleotides, 2u = rate at which one or the other mutates to a new state.
- $1/(2N_e)$  = rate of loss of heterozygosity by random genetic drift for a diploid locus.

The equilibrium level of variation is  $\approx 4N_e u$ .

Negative Scaling of Effective Population Size with Organism Size Defines the Range of Mutations Visible to Natural Selection



All mutations with absolute effects  $>10^{-8}$  are available to selection.

All deleterious mutations with effects <10<sup>-4</sup> are free to fix; mutations with advantages <10<sup>-4</sup> are invisible to selection.

# **Distribution of Fitness Effects of Newly Arising Mutations:** large fractions in the domain of effective neutrality.



• Bioenergetics example:

The cost of a 1 base-pair insertion ≈ 100 ATPs.

The life-time energy budget of an *E. coli* sized cell ≈ 10<sup>10</sup> ATPs.

Selective disadvantage of a 1-bp insertion  $\approx 10^{-8}$ .

Evolution of a Growth-rate Phenotype



• Each transition rate is equal to the product of the number of relevant mutations arising per generation and the fixation probability.

• At steady state, the flux rate must be equal in both directions.

• The equilibrium probability of each state is simply proportional to the product of the total set of transition rates towards the state from both directions.



Ideal World of No Linkage

Linked Genomes

## Two Potential Examples of the Influence of the Drift Barrier Across the Tree of Life

• **DNA replication and repair efficiency** – reduction in the mutation rate with decreasing organism size and genome size.

• Maximum biomass production rate – negative scaling of the growth-rate potential with organism size.



Selective disadvantage of a mutator in an asexual population
 = increase in genome-wide deleterious mutation rate



 The mutation rate per nucleotide site scales negatively with the effective population size.

For a given magnitude of random genetic drift, unicellular eukaryotes have lower mutation rates than bacteria because there are more functionally significant genomic sites.

### **Ecological and Physiological Scaling Laws**





Fig. 1. The relationship between  $r_m$  and body weight for 42 species. Inch also the slope  $y = K \cdot x^{-0.249}$  characteristic for the relation between body and metabolic rate per unit weight

Savage et al. (2007, PNAS)

Fenchel (1974, Oecologia)

#### Maximum Growth-rate Scaling Law: the cost of eukaryogenesis and multicellularity.

~1000x decline in maximum growth potential over 20 order-of-magnitude size increase





Mass at Maturity (µg)

- Costs of all cellular features need to be scaled against the total cell budget.
- What is the appropriate currency to use?

#### Ecoli ATP requirement

#### Contents [hide]

- 1 ATP requirement for the creation of an E. coli cell
  - 1.1 Empirical versus theoretical estimates of the ATP cost per cell
  - 1.2 Experimental determination of the ATP cost under anaerobic conditions
  - 1.3 Experimental determination of the ATP cost under aerobic conditions
  - 1.4 Theoretical ATP requirement per cell under aerobic and anaerobic conditions
  - 1.5 Decomposing experimental costs into biosynthetic and maintenance costs
  - 1.6 References

#### ATP requirement for the creation of an E. coli cell

compiled and written by Phillip Mongiovi and Ron Milo ₫

(with glucose as carbon and energy source, cell of 0.28 pg dry mass, ~40 minutes division time. Theore deduct maintenance costs that are constant per unit time)

Experimental: 12 billion-20 billion

Theoretical: 6 billion-11 billion

Cell yield per consumed carbon increases with the caloric content of the resource



Species	Size	$\mathbf{C}$	Ν	Р	S	Κ	Na	Mg	$\mathbf{C}\mathbf{a}$	Cl
Prochlorococcus sp.	0.16	15323	1682	87	82	49	410	371	25	173
Synechococcus sp.	1.00	14906	1755	122	72	78	248	104	49	120
Vibrio natriegen	3.50	8333	1837	157	116	320	400	73	8	1320
Escherichia coli	3.80	7675	1880	263	74	62	210	61	10	104
Pycnococcus provasoli	10	14000	1900	72	77	89		19	4	
Nannochloris atomus	14	14000	2000	81	29	78		19	$^{2}$	
Means		11503	1494	115	124	167	255	106	232	429
Seawater		2.25	0.03	0.002	28	10.2	469	52.7	10.3	546
Cellular enrichment		5,100	50,000	57557	4.4	16.4	0.5	2.0	22.6	0.8

	$\mathbf{Sr}$	Fe	Mn	Zn	$\mathbf{Cu}$	$\mathbf{Co}$
Pycnococcus provasoli	8	910	150	66	38	7
Nannochloris atomus	4	1100	93	140	19	7
Saccharomyces cerevisiae		354	31	642	46	
Nitzschia brevirostris	330	790	590	69	46	14
Emiliania huxlevi	44000	460	940	50	9	39
Means	796	680	395	129	62	24
Seawater	89	0.00054	0.00036	0.0054	0.0024	0.000020
Cellular enrichment	8.9	1.260.000	1.086.000	24.000	26,000	1.182.000

A)

#### Measuring Cell Maintenance and Growth Requirements With a Chemostat





• From known metabolism, convert resource consumption to generated ATPs.

### Lifetime Energy Requirements of Cells



• Total ATP consumption / cell division:  $C_T = C_G + tC_M$ , where t = cell division time (hours).

- The four components of cellular cost calculations.
- Direct vs. opportunity costs.
- Conversion to a selection coefficient.

### The Four Primary Cost Components



### The Cost of Biosynthesis of Elementary Building Blocks



#### Energetic and evolutionary consequences of a genomic modification:

Total baseline energetic cost:

 $S_{cost} = S_{DNA} + S_{RNA} + S_{PRO}$ 

Net selective advantage of expressed features:  $s_{net} = s_{direct} - s_{cost}$ 

All scaled relative to the total cost of building a cell.



Selective disadvantage = reduction in population-level growth rate =  $s_c$ 

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Fitness prior to trait modification = 1
Fitness after investment in the trait = 1 - s_c
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Ancestral cell-division time proportional to C. Division time after trait modification proportional to C + c.

Assuming c << C,

 $s_c = In(R) \cdot (c / C),$ 

=  $ln(2) \cdot (c / C)$  for binary fission.

 $s_c$  = energetic fitness cost of the trait.

C = total energy budget of ancestral cell.

c = added energy cost of the trait.

R = offspring number / generation.

Ilker and Hinczewski (2019, Phys. Rev. Letts.)

### Origin of Genome Complexity by Nonadaptive Mechanisms

- All embellishments to gene structure impose weak mutational and bioenergetic disadvantages.
- Efficiently removed by selection in prokaryotes with large effective population sizes, but can accumulate in an effectively neutral fashion in eukaryotes.



### Three Levels for the Cost of a Gene:

1) Chromosome: synthesis of nucleotides for replication, and amino acids for nucleosomes in eukaryotes; helix unwinding; etc.

**2) Transcription**: synthesis of ribonucleotides for steady-state number of transcripts; turnover rates; mRNA capping; intron splicing; polyadenylation; etc.

**3) Protein**: synthesis of amino acids for steady-state number; chain elongation; turnover rates; post-translational modification; etc.

• All measured relative to the total energy budget of the cell in units of ATP hydrolyses.

Frequency Distribution of the Costs for All Genes



 Multicellular eukaryotes – absolute costs are ~10 to 100x those in bacteria, but the relative costs are smaller, and often too small to be perceived by selection.



Fig. 4. Fractional costs of average genes in bacteria and unicellular eukaryotes (relative to total cellular energy budgets), subdivided into components at the level of replication, transcription, and translation.

## A Singular Event: the Origin of the Mitochondrion

Did this give rise to a Lane/Martin bioenergetic revolution that led to the evolution of:

- Novel protein folds
- Expansion in gene number and genome size
- Introns
- Internal complexity of cells
- Multicellularity
- Development
- Sex
- Etc.



c Euglena



Figure 2 | The cellular power struggle.





#### Membrane scaling and prokaryote-eukaryote divide:

- 10 to 20% of a eukaryotic cell's total energy budget is associated with membranes, which is comparable to the ~20% composition in bacterial species.
- The cost of synthesizing mitochondrial membranes is ~5% of a eukaryotic cell's energy budget.
- The total membrane area of mitochondria is not much different than that of the cell surface area.
- The number of ATP synthase complexes and ribosomes in eukaryotic cells is approximately the same as expected for a bacterial cell of comparable volume.

### Surface Area of Mitochondria vs. Plasma Membrane

#### Paramecium mitochondria



ATP synthase is restricted to the tips of cristae



### The Price of Mitochondrial Membranes

Total cost of membranes =

(no. of lipid molecules / surface area)x (cost / lipid molecule) x surface area

$$C_L \simeq (3.08 \times 10^6) \cdot \overline{c}_L \cdot A,$$

Relative to total cellular ATP requirements, cost of mitochondrial membranes

=  $0.05 V^{0.04} \approx 5\%$  of cell's energy budget



Size-dependent Scaling: Numbers of ATP Synthase Complexes and Ribosomes / Cell

• Continuity of scaling across bacteria and eukaryotes.



### Cell walls:

• How much do the added embellishments in bacteria, fungi, and plants cost?

• Why have most eukaryotes lost cell walls?

#### Structure of Gram-negative cell wall



Auer and Weibel 2017

#### Lipopolysaccharide Structure



Auer and Weibel 2017

#### *Escherichia coli* (Gram negative)

Bacillus subtilis (Gram positive)



- Cost of cell wall is less than that of the cell membrane, but still 5 to 10% of total budget.
- In both cases, the total cost of cell exterior is ~30% of the cell's energy budget.

#### **Cost of Swimming at Low Reynold's Numbers:**

is this trivially small, as suggested by Purcell and others?

 Efficiency of conversion of chemical energy into mechanical swimming with flagella is uniformly low, ~2%, owing to Brownian motion, rotational diffusion, flagellar flexibility, helical motion, etc.

• The cost of swimming is much less than the cost of building flagella.

### Flagellar motility – Escherichia coli





Turner, et al. (2000) Real-Time Imaging of Fluorescent Flagellar Filaments Jarrell and McBride (2008) The surprisingly diverse ways that prokaryotes move

### Variation in flagellum base structure in bacteria



Beeby, et al. (2016) Diverse high-torque bacterial flagellar motors assemble wider stator rings using a conserved protein scaffold

### Variation in flagellar filament thickness



Wang, et al. (2017) A structural model of flagellar filament switching across multiple bacterial species Thomson, et al. (2017) Bacterial Flagellins: Does Size Matter?

### Internal flagella – Leptospira interrogans



Malmstrom, et al. (2009) Proteome-wide cellular protein concentrations of the human pathogen Leptospira interrogans

### Many flagella – Ovobacter propellens





TEM, scale bar:  $1 \, \mu m$ 

~ 400 flagella

Scale bar: 1 µm

Fenchel and Thar (2004) "Candidatus Ovobacter propellens": a large conspicuous prokaryote with an unusual motility behaviour

### Eukaryotic flagellum – Chlamydomonas reinhardtii





Axoneme (9 + 2)

Silflow and Lefebvre (2001) Assembly and Motility of Eukaryotic Cilia and Flagella Lessons from Chlamydomonas reinhardtii Mitchell (2000) Chlamydomonas flagella



How much energy does such activity demand? Applying Stoke's Law to the low Reynolds number situations in which microbes live, for a sphere with radius r, the power required for swimming,

$$P = 6\pi r \eta v^2,\tag{4}$$

scales with the product of the viscosity of the medium  $(\eta)$  and the squared swimming velocity  $(v^2)$ , and to account for the inefficient conversion from cellular chemical energy to motion, this must further be divided by ~ 0.02 (noted above).

Power requirement for swimming is nearly isometric with cell volume:

- $\approx$  (6 x 10<sup>-18</sup>) V joules/sec, where V is cell volume in um<sup>3</sup>
- $\approx$  (3 x 10<sup>5</sup>) V ATP hydrolyses / hour

 $\approx$  0.1% of basal metabolic rate

• In *E. coli*, the lifetime cost of swimming is ~1000x less than the cost of building the flagella, which constitutes ~8% of the total cellular energy budget.

#### KEY EVENTS IN THE EMERGENCE OF EUKARYOTIC CELLULAR COMPLEXITY



Increased cell size —— Increased random genetic drift —— Shift in mutational features and rates

Moderate increase in gene number

Massive increase in noncoding DNA

Mobile-element proliferation

### **Center for Mechanisms of Evolution**

- Cell biology, biophysics, biochemistry, population genetics.
- Empirical, computational, theoretical.
- Hiring: faculty, research scientists, postdocs, students.



Arizona State University





Kerry Jeremy Geiler-Samerotte Wideman







