adding metabolism: Virtual microbes 

*evolution predictable???
focus on metabolism

(1) de novo evolution in artificial metabolic universe (possible reactions cf KEGG, but much smaller)

\[ \text{what will happen when the tape is played } N \text{ times?} \]

(a) from identical initial conditions in a constant environment

(b) from identical initial conditions in a strongly variable environment and brought in lab conditions (LTEE)

(2) What should we expect to evolve after WGD of YEAST using its metabolic network as starting condition

\[ \text{what will happen when the tape is played } N \text{ times?} \]

Compare to what happened in the unique case of YEAST on earth

(3) "causal drift": what changes in metabolic rates cause diabetes?
“Virtual Microbes”
a paradigm system for bottom up modeling of multiple modes of adaptation in biological-like complex complex adaptive systems

Thomas Cuypers and Bram van Dijk

Cell with
Genome with
genes (TFs, pumps, enzymes) with parameters (Vmax, K, binding)
metabolism
grow and divide
Mutate
(duplication/deletions, HGT, par. changes)

In ’universe’
potential metabolic reactions
Resource influx space

NO PREDEFINED FITNESS

Grid world

Importers/Exporters
Enzymes
Transcription factors
Protein expression
Transporting/catalysis
Growth of cell volume
Metabolic universe

Table 1 Metabolic universe: Using VirtualMicrobes, we generated an artificial biochemistry comprised of 9 metabolites, 8 importers, 8 exporters and 43 conversion reactions, with two non-substitutable building blocks and a single energy molecule.

From: Contingent evolution of alternative metabolic network topologies determines whether cross-feeding evolves

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Class</th>
<th>Diffusion rate</th>
<th>Degradation rate</th>
<th>Toxicity</th>
<th>Mass</th>
<th>Influx rate</th>
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<tbody>
<tr>
<td>R</td>
<td>Resource</td>
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<td>0.0003</td>
<td>0.077</td>
<td>9</td>
<td>0.002</td>
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<td>B1</td>
<td>Building block</td>
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<td>0.0100</td>
<td>0.058</td>
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<td>0</td>
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<tr>
<td>B2</td>
<td>Building block</td>
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<td>0.0100</td>
<td>0.103</td>
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<td>0</td>
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<tr>
<td>E</td>
<td>Energy carrier</td>
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<td>0.1000</td>
<td>0.065</td>
<td>1</td>
<td>0</td>
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<tr>
<td>M1</td>
<td>--</td>
<td>0.014</td>
<td>0.0006</td>
<td>0.105</td>
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<tr>
<td>M2</td>
<td>--</td>
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<td>0.0003</td>
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<tr>
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<tr>
<td>M4</td>
<td>--</td>
<td>0.014</td>
<td>0.0014</td>
<td>0.158</td>
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<tr>
<td>M5</td>
<td>--</td>
<td>0.016</td>
<td>0.0008</td>
<td>0.047</td>
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</tbody>
</table>

Reactions

- R → B2 + E
- R → B1 + E
- R → M1 + 3 E
- R → M2 + 5 E
- R → M4 + 3 E
- R → M5 + 4 E
- R → M3 + 2 E
- B1 → M1 + 2 E
- B1 + M4 → R

Importers: one for each non-energy metabolite (8 total)

Exporters: one for each non-energy metabolite (8 total)
De Novo evolution in a constant environment (1 resource)

Meijer, v. Dijk & H. 2020
2 types of Evolved metabolism generate predictable ecosystems

“core, shell and cloud”

U-like-shape of pangenome
Fig. 3: Metabolic dependencies in cross-feeding communities.
Cross-feeding evolves in 1 of 2 types of metabolism in space. Self-sufficiency regained when mixed (switching).
conclusion

- Crossfeeding can evolve in space without explicit costs/tradeoffs or environmental variability.
- Selfsufficiency can also evolve in exactly the same "universe".
- Crossfeeding and selfsufficiency contingent outcomes from their LUCA.
- Crossfeeding and selfsufficiency are predictable outcomes from evolved metabolism.
- Selfsufficient mutants exist in crossfeeding ecosystem but do not take over.
- Switching spatial system (biofilm) to wellmixed lead to switching between crossfeeding and selfsufficiency.
De Novo Evolution in variable environment

"WHAT" has evolved?, How to observe?

WT 1

WT 2

WT 3

WT n

LCA of evolved population

Identical for all replicates

Harsh, fluctuating environment (2 resources)

van Dijk et al 2019
De Novo Evolution in variable environments

“What” has evolved?, How to observe?

Common metabolic cycle

LCA of evolved population

Harsh, fluctuating environment

Identical for all replicates
De Novo Evolution in variable environments

"WHAT" has evolved?, How to observe?

WT 1

WT 2

WT 3

WT n

LCA of evolved population

Similar "fitness"

Harsh, fluctuating environment

Identical for all replicates
De Novo Evolution in variable environments

“WHAT” has evolved?, How to observe?

Dissimilar “fitness”

LCA of evolved population

Harsh, fluctuating environment
Identical for all replicates
BUT: very diverse GRN (or none) and metabolic reaction to alternative environments
Experimental evolution:
starting with pre-evolved “wildtypes”

Well known example of experimental evolution:

Long term evolutionary experiment (LTEE) (Lensky 1991)
One strain of E.Coli is evolving in lab-conditions since 1988
(>70000 generations) in 12 replicates
in a serial transfer protocol (diluted in new medium very 24 hours)
still adapting (getting “better”)
Continued new ways of observing & new insights

This case study:

In silico evolution of the above pre-evolved “wildtypes” (WT 1-16)
in similar serial transfer protocol

study “generic” features of such an evolutionary process
To WHAT does the population adapt?
HOW does it adapt?
Multiple observables
Similarities/differences to E. coli?
In serial transfer protocol they all evolve to “Trust the hand that feeds them” (anticipate 24 hr cycle)

Minimize Lag-phase
Exhaust all food after 24 hours remaining JUST alive
OR
remaining JUST ready to divide
Maximizing growth rate OR Yield evolved trade-off and distinct strategies
By individual based regulation OR collective tuning
By individual based regulation OR collective tuning

A) No coexistence

B) Quasi-stable coexistence

C) Balanced polymorphism

D) All replicates

WT01
WT02
WT03
WT04
WT05
WT06
WT07
WT08
WT09
WT10
WT11
WT12
WT13
WT14
WT15
WT16
Diversified evolved wildtypes all evolve anticipation of 24 hr cycle *un-predicted predictability*

BUT in unpredictable ways

Some WT’s adapt in a predictable way, others in very different ways

*predictibility is an unpredicable outcome of evolution*
Conclusions/Observations

- What is fitness / what has evolved not obvious
- Evolutionary attractors can be characterized as a combinatorial set of a limited set of alternatives
- Autonomous and Collective “problem solving” (metabolism) “easy” alternatives
- Non-autonomy not because of lack of genes...
- Spatial embedding, also without spatial patterns important
- Trade-off’s not innate but evolved properties
- GRN very variable (presence and shape)
- Predictability, even in well defined environments depends on prior evolution
  
  *Predictability is an unpredictable outcome of (prior) evolution*