Better than Before and Better Together

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Cooperation as a Function of Complexity

- Fitness is the capacity to survive and reproduce; fitness can be measured as the capacity of a variant type to displace another type in competition for available resources.
- Symbioses, such as mutualism, are pervasive features of the natural world, thus collaboration may be just as important as competition in driving biological innovation.
- Although collaborative interactions are pervasive in Nature, do they actually increase the fitness of collaborating partners?
- Are collaborative systems that are more complex, consisting of multiple variants, more productive than simpler systems consisting of one or few variants?

Previous Work Shows:

<table>
<thead>
<tr>
<th>Strains</th>
<th>Relevant characteristic</th>
<th>Growth rate for x μM</th>
<th>Glucose uptake (μM)</th>
<th>Steady state (胁迫)</th>
<th>Glycerol production (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JA122</td>
<td>Derivative of RJ253, F. P. not wild type; used here to amplify his potential to grow.</td>
<td>0.46 ± 0.01</td>
<td>1.18 ± 0.08</td>
<td>1.06 ± 0.08</td>
<td>196 ± 20</td>
</tr>
<tr>
<td>CV101</td>
<td>Derivative of JA122 selected after 775 generations, tests small phenotype on Kenyon 12, Kanm.</td>
<td>0.40 ± 0.01</td>
<td>2.60 ± 0.18</td>
<td>3.07 ± 0.08</td>
<td>362 ± 17</td>
</tr>
<tr>
<td>CV116</td>
<td>Derivative of JA122 selected after 775 generations, tests large phenotype on Kanm.</td>
<td>0.30 ± 0.02</td>
<td>1.85 ± 0.06</td>
<td>0.68 ± 0.06</td>
<td>± 0</td>
</tr>
<tr>
<td>CV103</td>
<td>Derivative of JA122 selected after 775 generations, tests large phenotype on Kanm.</td>
<td>0.40 ± 0.01</td>
<td>1.81 ± 0.07</td>
<td>0.74 ± 0.06</td>
<td>40 ± 0</td>
</tr>
</tbody>
</table>

In 1987, Heffin et al. evolved in the laboratory a community of E. coli strains starting with a single common ancestor. The population was culled for 775 generations with glucose as a limiting resource.

Questions

- Are E. coli that evolved into a community more fit than their common ancestor, which was a single clone?
- Does collaboration among evolved E. coli boost fitness? If collaborating variants are more fit, are they also, as a group, more productive?

Results

Part 1: The Reconstruction

![Figure 1 A, B Reconstruction of the consortium with glucose scavenger, strain E3 (CV103) and either of two waste consuming clones E1 and E6 (CV101 & CV116).](image)

E3 is always most abundant; cross-feeding is inferred from the fact frequencies are constant after ~15 generations. When E strains are grown, individually or collectively, in the presence of A, their common ancestor, A is eliminated after ~20 generations.

Part 2: Fitness Comparison

![Figure 2 Differences in fitness between individuals and groups, relative to their common ancestor.](image)

Fitness coefficients were calculated as the slope of the linear regression (experiment reference), as a function of elapsed generations. Cell generations elapsed equals (time * dilution rate)/2.

Part 3: Productivity Comparison

![Figure 3 Yield differs among evolved strains, consortia and their common ancestor.](image)

Co-evolved consortia produce more cells, biomass, and total protein.

Methods

Label Bacteria with Green Fluorescent Protein

- One-On-One Competitions
- Teams of Two versus Ancestor
- Team of Three versus Ancestor
- Cell Counting with Flow Cytometry
- Quantify Biomass
- Quantify Protein Content

Results

![Figure 4 Insert Green Fluorescent Protein gene into the E. coli chromosome.](image)

![Figure 5 Plate GFP-labeled colonies and unlabeled colonies, archive labeled colonies in 20% glycerol at -40°C.](image)

![Figure 6 Culture E coli were competed in "chowestas" fed continuously with a simple medium of salts-glucose.](image)

![Figure 7 Competition. At 0 h GFP cells 1:1 ratio with unlabeled cells. At 48 h free, of green cells has increased, and at 72 h green cells have outcompeted unlabeled cells.](image)

![Figure 8 Strain frequency by flow cytometry.](image)

![Figure 9 Biomass 250 μl of culture was filtered, dried overnight at 65°C, then weighed to 1μg.](image)

![Figure 10 Total protein was quantified on cell extracts by standardizing with a protein-specific dye](image)

Conclusions

- Co-evolved communities can be reconstructed in lab.
- Evolved clones are all more fit than their common ancestor, but not more fit than each other.
- Community fitness is greater than individuals’ fitness, but fitness is not additive.
- Community fitness and productivity increases as a function of its genetic complexity.

Acknowledgements

DDY, AA and FR were funded by NNX12AB87G-EXO from NASA, AC and AR by University of Toronto Cancer Center.