Flux Manuscript Outline

BACKGROUND / INTRODUCTION

* Basic intro material and background…
  + Microbial systems are used to understand ecology and evolution
  + Traditionally, evolutionary studies focus on understanding adaptation to constant environments
    - Aside: chemostat/turbidostat/etc vs. serial passage
* The gap…
  + Relatively limited understanding of adaptation in (to?) fluctuating conditions.
    - Either a very simplified view
      * See: studies with low, high, fluctuator treatments (I know there is at least one, probably a few)
    - Or focused on more “ecological” questions
      * evolution studies with degrading environments and slow vs. fast transitions to degraded state
  + Valuable to understand how organisms adapt to stressful (non-ancestral state) conditions that are variable vs. constant for stress.
* We do…
  + We evolve yeast for 500 generations in 11 different treatments variable for stress amount (from “no” chemical stress to moderate, but sublethal, chemical stress) and dynamics (constant vs fluctuating with variable range) to assess the following research questions:
    - (1) Does evolution in (a) stressful and/or (b) fluctuating environments lead to increased stress tolerance?
    - (2a) Is elevated stress tolerance associated with a fitness cost in low/no stress conditions? (2b) if so, is this cost lower for fluctuating histories?
    - (3a) Do the evolutionary paths (genotypic change) to stress tolerance differ between fluctuating and constant stress histories? – drop this question if we choose not to include the genetic / genomic results.

METHODS

* Strains, media and culture methods
* Experimental design
  + Evolutionary dynamics
  + Endpoint assays of relative fitness
* Library construction and sequencing
* Sequence data processing & calculations
  + Sequence datasets
  + Barcode cross-contamination rate
  + Fitness calculations
* Statistical analysis
  + Analysis & visualization tools
  + Reads
  + Power Analysis
  + Fitness change in 250 generations of experimental evolution
    - Evolutionary treatment
    - Individual barcode inc/dec
  + Evolutionary dynamics

RESULTS

* Initial fitness
  + *FIGURE: histogram of initial fitness in seven environments*
  + Initial fitness is hump-shaped and has little variation among strains.
  + Average strain is more fit than the reference, and this difference increases with increasing assay environment stress
  + Very high stress environments (COPR 120) limit ability to accurately measure fitness (see spread in points here).
* Contamination rate & Power
  + Contamination
    - *FIGURE: none*
    - No outside contamination – no growth in growth blanks
    - Cross-Contamination rate was very low – present results from single well controls
  + Power
    - *FIGURE: power surface, 2-panel*
    - RMSE for deltafitness: 2.54
    - Power to detect treatment differences in fitness (high): 99.9% power to detect 2.54% fitness difference, 87% power to detect 1% fitness difference.
    - Power to detect fitness change for individual BCs (low): 90% power to detect 5% fitness change, 40% power to detect a 2.5% fitness change, 10% power to detect a 1% fitness change
* Fitness Assays
  + Single Environment / treatment effect model results
    - *FIGURE: violin plots with heatmaps*
    - In CM (No added stress):
      * EH0 treatment more fit than all others.
      * Followed by EH0\_40 and EH40, the two other lowest stress treatments assayed.
      * Higher stress treatments (EH20\_60, EH0\_80, EH40\_80, EH80) all equal in fitness to one another and lower fitness than previously mentioned treatments.
      * Treatments with higher stress exposure are likely to have negative relative fitness change. – COST.
      * Trend: higher mean stress AND max stress associated with lower fitness in “no stress” environment. Trend significant at low stress, but not higher stress.
    - In SALT80 (high amount of added stress):
      * EH80 treatment more fit than all others.
      * Followed by EH40\_80, then EH0\_80 and EH20\_60
      * Trend: higher mean stress AND max stress associated with higher fitness in “high stress” environment. Trend significant except at very low stress.
    - In SALT40 (low amount of added stress):
      * Pattern intermediate between CM and SALT80 environments.
      * Some indication that exposure to moderate stress results in elevated fitness in this environment (EH40\_80)
    - In SALT 120 (very high amount of added stress)
      * Pattern identical-to, but weaker than results seen in SALT 80.
  + Multi-environment / individual barcode-level results
    - *FIGURE: colorful stacked bars for fitness increase by t-test OR more data rich “X” plots for fitness in two environments*
* Evolutionary dynamics
  + *FIGURE: heatmaps*