Module 2: Evolution

Description: This module explores the history of life on Earth and various mechanisms of evolution through mathematical modeling and simulation as well as hands-on laboratory experiments. Key concepts include Earth geohistory, emergence, and the evolution of multicellularity.

Protocols:

Evolution of multicellularity in snowflake yeast

Introduction

Transitions from unicellularity to multicellularity, throughout history, led to greater biological complexity and cellular differentiation. Although single cells must sacrifice their autonomy, the development of multicellularity allows organisms to behave collectively and perform more complex tasks. The first step in this evolutionary transition is the formation of cellular clusters, which can be studied experimentally by using gravity to select for cells that fall at different rates through liquid media.

This method of experimental evolution examines the two-step process that produces multicellular yeast from single-celled ancestors. First, cells must evolve cluster formation. Since clusters of cells will fall through liquid media more quickly, multicellular and unicellular yeast can be easily separated with more multicellular clusters being found at the bottom of the cell column. Second, clusters must be able to satisfy the three requirements for Darwinian evolution: (1) variation, i.e. different types of clusters must form, (2) heritability, i.e. cluster variations must pass from one generation to the next, and (3) variations must affect cell fitness, i.e. their ability to settle quickly. When these conditions are met, competition arises between yeast cells with different cluster phenotypes resulting in natural selection of clusters with more beneficial mutations. By studying this first step in the evolution of multicellularity we gain deeper insight into how the vast biological diversity surrounding us was developed.

Goal: Examine the evolutionary transition to multicellularity by selecting for the evolution of cluster formation in unicellular yeast.

Materials

Note: Each group member (2 per group) will complete the experiment individually, but some equipment will be shared.

Shared materials:

- vortex mixer
- shaking incubator
- micropipettes (100-1000uL & 5-50uL)
- stir plate
- 2L YPD media (recipe below)
- small propane torch
- ethanol (70% in squeeze bottle)

- aluminum foil
- tape (for labeling)
- autoclave tape
- markers
- loop (for picking yeast spores)
- sterile glass beads (dia. = 3.0mm) in test tubes
- microscope slides & coverslips

Materials per workstation:

- waste beaker
- micropipette tips (100-1000uL & 1-10uL)
- pipette pump
- parafilm (at least 2 pieces/day)
- gloves
- dry wipes
- protective eyeglasses
- sterile, capped 25mm X 150mm test tubes with 10mL YPD media (2/day/person, after inoculation)
- test tube racks
- ruler
- timer/stopwatch (on smartphone)
- test tube stand (optional: 3D printed holder)
- 1.5mL microcentrifuge tubes
- serological 5mL pipettes (at least 2/day/person, after DAY 0)
- DI water (approximately 20mL)
- YPD media plates (stored in fridge)
- C1W1/ACE2 plated yeast spores
- C1W3 plated yeast spores

Media preparation

Objectives: [1] Make 2L of YPD media and **[2]** Prepare test tubes of YPD media for evolution experiment

[1]

1. To make one liter (1L) of YPD media, dissolve the following in 1L of DI water (adjust accordingly to make 500mL of YPD in each bottle):
   - 20g dextrose
   - 20g peptone
   - 10g yeast extract
2. Seal the bottles halfway, apply autoclave tape, and label with name, date, and material (YPD media)
3. Autoclave one 500mL bottle for 30 minutes, set the second aside to use for pouring plates

[2]

1. Using a 25mL serological pipette and pump, aliquot 10mL of YPD media into 20 test tubes (two tubes are needed for each day of transfers, so each group will need at least 30 tubes of YPD media total, but extras are a good idea)
2. Cap all test tubes and apply a strip of autoclave tape to cover the tops

3. Label test tube rack with name, date, and material (YPD media)
4. Autoclave for 30 minutes

Pouring plates

Objectives: [1] Add agar to YPD media (before autoclaving) and **[2]** Pour sterile YPD into petri dishes

[1]

1. Add 15g/L of agar to YPD media bottle (adjust amount based on volume of YPD)
2. Autoclave for 30 minutes

[2]

1. Set up and maintain a sterile workstation
2. After autoclaving, pour sterile YPD media (just enough to cover the bottom of the plate) into petri dishes
3. Cover immediately (tops should be kept face down at all times) and leave to polymerize
4. Seal plates in a bag and label with name, date, and material (YPD media)

Initial inoculation

Objectives: [1] Set-up and maintain a sterile workstation, **[2]** Inoculate tubes of YPD media with C1W1/ACE2 yeast spores and allow colonies to grow for 24 hours, and **[3]** Plate C1W3 spores on YPD media and incubate for three days

[1]

- Wear appropriate safety equipment: gloves, glasses, etc.
- Tape aluminum foil over work area and gather necessary materials
- Light burner and work close to the flame to reduce contamination risks (but don't burn yourself)
- Avoid touching pipette tips to the sides of the test tube and/or waste bag
- Flame all tubes and caps before pipetting AND before sealing
- Avoid touching skin or other surfaces with gloved hands
- Clean hands with 70% ethanol (away from the flame!) if contamination is suspected

[2]

- Set up sterile workstation (see directions above)
- Get one plate of C1W1/ACE2 yeast spores, one tube of YPD media, and loop
- Label YPD test tube with strain (C1W1), date, and initials
- Sterilize loop, then pick yeast colonies from C1W1/ACE2 plate
- Inoculate tube of YPD media with picked colonies (on loop)
- Flame rim and cap before sealing test tube, flame loop to sterilize
- Place YPD+C1W1/ACE2 test tube into incubator (30 deg C) and set to shake vigorously for 24 hours

[3]

- Using sterile forceps, grab the C1W3 filter paper and place it on a YPD media culture plate

- Add ~50uL of DI water to the paper and then streak it around the agar plate with the forceps (leave filter paper on edge of plate)
- Incubate at 30 deg C for 3-4 days to see colony growth

Culture transfers

Objectives: [1] Measure cell column height after allowing yeast cultures to settle for ~30 minutes, **[2]** Perform settling selection (fast v. slow) by transferring yeast cultures to fresh YPD media tubes, and **[3]** Take samples (1:10 dilution) and image yeast cells with phonescope

[1]

1. Set up sterile workstation
2. Retrieve YPD+C1W1/ACE2_SLOW and YPD+C1W1/ACE2_FAST tubes from shaking incubator and place in test tube stand
3. Allow yeast cultures to settle for 15 minutes, then measure the height of each cell column with a ruler, take a picture, and determine settling speeds

[2]

1. Get fresh 10mL YPD media test tubes (2), 5mL serological pipettes (2) & pipette pump, parafilm, and dry wipes
2. Label fresh YPD media tubes with strain (C1W1), date, initials, and settling selection (fast or slow)
3. Mix cells in test tube with vortex mixer before using pipette pump to collect 5mL of culture in 5mL serological pipette
4. Carefully seal bottom of pipette with parafilm and set to stand, undisturbed, in a test tube rack for 10 minutes, then carefully remove parafilm and follow protocol for either fast or slow settling
5. Wait 3-5 minutes, then repeat steps 2 & 3 with second 5mL serological pipette; make sure to leave ~100uL in YPD+C1W1/ACE2 test tube for microscopy sample
6. While waiting for gravity selection, complete steps 1-3 of [3] and then continue with settling selection
    a. Fast settling:
        i. transfer bottom 1mL from serological pipette to a fresh YPD media tube
        ii. discard remaining yeast culture (top 4mL) in waste bag
        iii. flame mouth of test tube and cap before sealing
    b. Slow settling:
        i. discard bottom 4mL from serological pipette in waste bag
        ii. transfer remaining yeast culture (1mL) to fresh YPD media tube
        iii. flame mouth of test tube and cap before sealing
7. Place both tubes (YPD+C1W1/ACE2_SLOW and YPD+C1W1/ACE2_FAST) into incubator (30 deg C) and set to shake vigorously for 24 hours

[3]

1. Using 100-1000uL micropipette, pipette 900uL of DI water into two 1.5mL microcentrifuge tubes
2. Label each tube with strain (C1W1), # of transfers (none, 1st, 2nd, etc.), and settling selection (fast or slow)
3. Using 1-100uL micropipette, pipette 100uL of each yeast culture from either YPD+C1W1/ACE2_SLOW or YPD+C1W1/ACE2_FAST tube into a 1.5mL microcentrifuge tube with 900uL of DI water

4. Mix samples with vortex mixer before placing ~10uL onto a microscope slide and covering with a coverslip
5. Take images of yeast cells with phonescope and make note of cell type (unicellular v. multicellular), characteristics (cluster formation, cluster variation, etc.), and cell size; use different slides for each sample (YPD+C1W1/ACE2_SLOW or YPD+C1W1/ACE2_FAST)

Additional step on the third day of culture transfers:

[4a] Transfer C1W3 spores from plate to liquid YPD media and leave to grow overnight (control in case of culture contamination)

1. Get one plate of C1W3 yeast spores, one tube of YPD media, and loop
2. Label YPD test tube with strain (C1W3), date, and initials
3. Sterilize loop, then pick yeast colonies from C1W1/ACE2 plate
4. Inoculate tube of YPD media with picked colonies (on loop)
5. Flame rim and cap before sealing test tube, flame loop to sterilize
6. Place YPD+C1W3 test tube into incubator (30 deg C) and set to shake vigorously overnight

Additional step for fourth and subsequent days of culture transfers:

[4b] Begin settling selection (fast only) with C1W3 cells and transfer yeast cultures to a fresh YPD media tube and take samples for microscopy

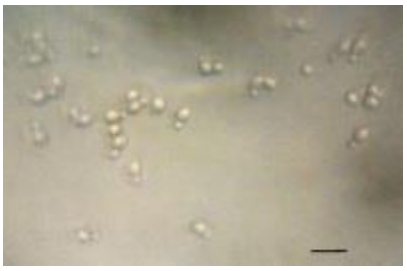
1. Get a fresh 10mL YPD media test tube, a 5mL serological pipettes & pipette pump, parafilm, and dry wipes
2. Label fresh YPD media tubes with strain (C1W3), date, initials, and settling selection (fast)
3. Mix cells in YPD+C1W3 tube with vortex mixer before using pipette pump to collect 5mL of culture in 5mL serological pipette
4. Carefully seal bottom of pipette with parafilm and set to stand, undisturbed, in a test tube rack for 10 minutes, then carefully remove parafilm and follow protocol for either fast or slow settling
5. While waiting for gravity selection, complete steps 7-__ and then continue with fast settling selection
    - Fast settling:
        1. transfer bottom 1mL from serological pipette to a fresh YPD media tube
        2. discard remaining yeast culture (top 4mL) in waste bag
        3. flame mouth of test tube and cap before sealing
6. Place both tube (YPD+C1W3_FAST) into incubator (30 deg C) and set to shake vigorously for 24 hours
7. Using 100-1000uL micropipette, pipette 900uL of DI water into a 1.5mL microcentrifuge tube
8. Label tube with strain (C1W3), # of transfers (none, 1st, 2nd, etc.), and settling selection (fast)
9. Using 1-100uL micropipette, pipette 100uL of yeast culture from YPD+C1W3 tube into 1.5mL microcentrifuge tube with 900uL of DI water
10. Mix sample with vortex mixer before placing ~10uL onto a microscope slide and covering with a coverslip
11. Take images of yeast cells with phonescope and make note of cell type (unicellular v. multicellular), characteristics (cluster formation, cluster variation, etc.), and cell size in relation to other yeast strains and selection protocols (C1W1 v. C1W3 and fast v. slow)

NOTE: Repeating **[1]** – **[3]** and **[4b]** as needed, extends the experiment (35 generations of yeast after 7 days).

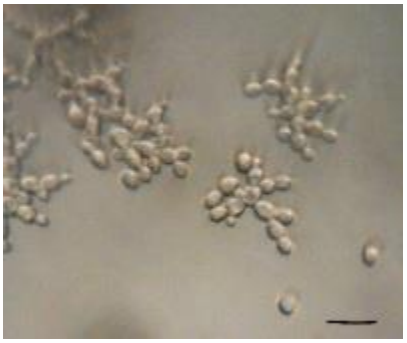
Results

C1W1/ACE2 (muticellular yeast with unicellular gene re-introduced) imaged with smartphone microscope

no transfers:



result of fast selection after 6 days:



result of slow selection after 6 days:

Note: Scale bar is ~ 30um.