Fitness competition in caffeine

GOALS

1. Demonstrate that independent evolved strains may differ in their fitness in the evolved environment
2. Demonstrate that independent evolved strains may differ in their fitness between environments (evolved environment vs. original environment)

OVERVIEW

We have been carrying out experimental evolutions with lab strains of *S. cerevisiae* that express vibrant pigments and thus each have a distinct color. Because of these colors, relative abundance of each strain in a mixed culture can be determined by counting colony forming units (CFUs) and calculating the ratio of colors (**Figure 1**). This approach can be used to determine whether yeast with different colors are better adapted to a particular environment.

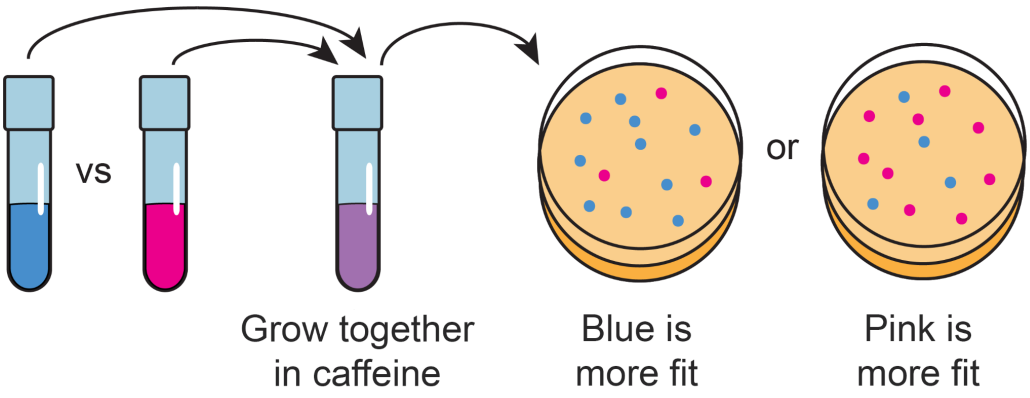

Figure 1: Competition experiment overview. Ratio of CFU colors on agar media indicates the relative abundance of each strain in a mixed culture.

We will use these colors in a competition experiment, which will allow us to determine which yeast from your experiments are best-adapted to caffeine. To do this, you will first grow yeast from the evolution experiment and with different colors in a medium that does not contain caffeine. You will then mix these strains in media containing varying concentrations of caffeine so that they will compete for resources. After the mixed cultures grow, you will plate them onto agar media on plates. After a few days of growth, you can count the ratio of colors on each plate to determine which strain "won" the competition in each concentration (**Figure 2**).

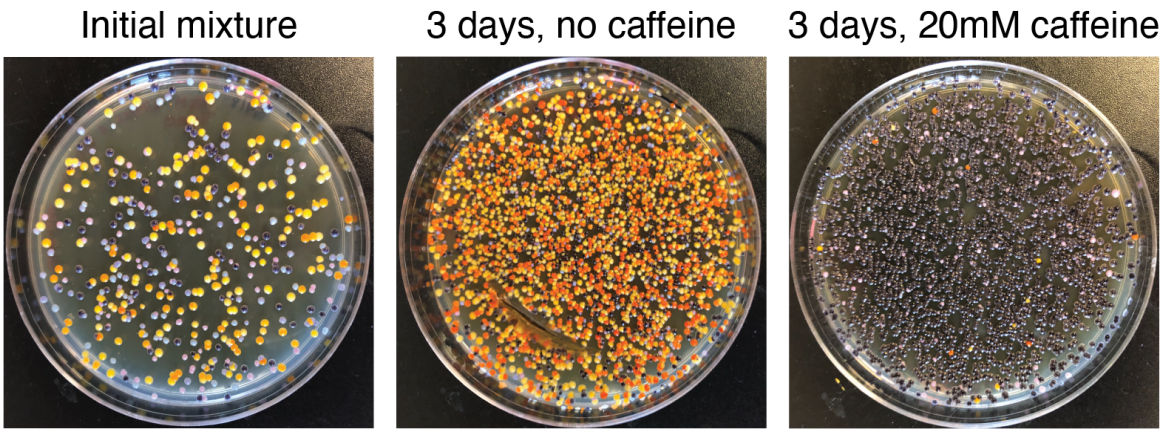

Figure 2: Example of competition experiment outcome. Six strains of different colors evolved in the presence of caffeine were co-cultured in YPD + G418 media containing 0mM or 20mM caffeine for 3 days. A 1:10,000 dilution of each culture was plated onto YPD + G418 agar media, except for 20mM a 1:2,000 dilution . In 20mM caffeine, the black strain outcompetes the others, indicating that it has higher fitness than the ancestral strain in the presence of caffeine, but in the absence of caffeine the yellow and orange strains have higher fitness and outcompete the others.

GLOSSARY

- CFU: Colony Forming Unit; a cell that is capable of growing into a colony of cells when transferred from liquid to solid medium. CFUs are commonly used as a proxy for the number of viable cells in a liquid culture.
- Caffeine: A compound produced by plants found in some products used for foods, including coffee and cocoa beans. Industrial yeast are exposed to caffeine when used for fermenting coffee and chocolate.
- Fitness: A measure of an individual's reproductive success. Note that in this experiment you will not be measuring fitness directly but will instead observe the outcome of competition between individuals with differing fitness in a given environment. Success in this competition is determined by the relative fitness between these individuals.
- G418: Geneticin, an antibiotic commonly used in laboratory experiments. Yeast utilized in this protocol are resistant to G418 due to a plasmid they carry, which also gives them their distinctive color thanks to additional genes on the plasmid that encode pigment production pathways. G418 is necessary for maintenance of the plasmid and additionally helps to prevent contamination by killing other microbes that are not G418-resistant.
- Selection pressure: An environmental condition that favors some genotypes in a population over others.
- YPD: A standard rich yeast medium named for its three ingredients: Yeast extract, Peptone, and Dextrose. Also referred to as YEPD.

PROTOCOL

Day 1: Mix strains in media with different concentrations of caffeine. You will do this as a class!

1. Examine the cultures you saved from the end of your +caffeine (not control!) evolution. If yeast have settled at the bottom of the tube (pelleted), gently shake the tube until they are completely resuspended. The liquid sample of yeast (culture) should be dense enough that you cannot see through it, and the two cultures should be comparable in density.
2. Each group will remove 20ul of their evolved culture and mix them together in a single microcentrifuge tube. Make sure to mix well by vortexing or pipetting up and down several times. For each class, there will be six strains of different colors mixed together, for a total of 120uL.
3. Add 5ul of mixed culture to each of the three test tubes prepared with 0mM, 20mM, and 40mM caffeine. Dispose of all pipette tips as biohazardous waste. Put the tubes in the incubator to grow.
4. Prepare a 1:10,000 dilution of the remaining (105uL) mixed yeast culture from the microcentrifuge via serial dilution. Fill two microcentrifuge tubes with 990ul water and label them “dilution 1” and “dilution 2”. In the tube labeled “dilution 1”, add 10ul of mixed culture and vortex for 5 seconds or invert 10 times to mix. Transfer 10ul from “dilution 1” to “dilution 2” and vortex for 5 seconds to mix.
5. Prepare a 1:50,000 dilution. Label another 1.5mL tube “dilution 3” and fill with 400uL water. Transfer 100ul from “dilution 2” to “dilution 3” and vortex for 5 seconds to mix.
6. Obtain two YPD + G418 agar plates. Label one “Before competition, 1:10,000”, and the other “Before competition, 1:50,000.” Label with your classroom number to tell your competitions apart from other classes!
7. Use a sterile plate spreader to spread 150ul from “dilution 2” onto the 1:10,000 dilution plate, and 150uL “dilution 3” onto the 1:50,000 plate. Put the plates in the incubator to grow.

Day 4: Plate mixed cultures onto YPD + G418 agar media.

1. Examine the cultures you inoculated on Day 1. If yeast have settled at the bottom of the tube (pelleted), gently shake the tube until they are completely resuspended. The liquid sample of yeast (culture) should be dense enough that you cannot see through it, though the culture grown in the highest concentration of caffeine may appear less dense than the others.
2. Prepare a 1:10,000 dilution of each mixed yeast culture (0mM, 20mM, 40mM caffeine) via serial dilution. For instance, for the YPD + G418 media condition, fill two microcentrifuge tubes with 990ul water and label them “YPD + G418 dilution 1” and “YPD + G418 dilution 2”. In the tube labeled “YPD + G418 dilution 1”, add 10ul of mixed culture, and vortex for 5 seconds or invert 10 times to mix. Transfer 10ul from “YPD + G418 dilution 1” to “YPD + G418 dilution 2” and vortex for 5 seconds to mix.

You should have 3 tubes of "dilution 2", one for each concentration of caffeine (0mM, 10mM, 20mM)

3. Prepare a 1:50,000 dilution of each mixed yeast culture (0mM, 10mM, 20mM caffeine) via serial dilution. Label a tube "dilution 3" and the concentration of caffeine, fill it with 400uL water, add 100uL of "dilution 2", and vortex for 5 seconds or invert 10 times to mix. You should have 3 tubes of "dilution 3", one for each concentration of caffeine (0mM, 10mM, 20mM).
4. Obtain six YPD + G418 agar plates. Label one "Competition in 0mM caffeine, 1:10,000", and another "Competition in 0mM caffeine, 1:50,000." Repeat this for 20mM and 40mM caffeine for a total of 6 plates, 2 per concentration. Label with your classroom number to tell your competitions apart from other classes!
5. Use a sterile plate spreader or glass beads to spread 150ul from "dilution 2" onto the 1:10,000 dilution plate for the corresponding concentration of caffeine, and 150uL "dilution 3" onto the 1:50,000 plate. Repeat for each concentration. Put the plates in the incubator to grow.

Day 7: Calculate ratio of colors among CFUs as a proxy for relative fitness.

1. For each condition (before competition, competition in 0mM, competition in 20mM, competition in 40mM), your instructor will help you determine which of the two dilutions (1:10,000 or 1:50,000) is easier to count.
2. Count the number of colonies on each selected plate. Remember to count from the initial mixture that you plated on Day 2, as well as the competitions you plated on Day 3. Use Table 1 to record your colony counts, per directions in Question 1.
3. Follow directions in Questions 1 and 2 to complete the tables.
4. Answer the questions and extension questions.

TABLE 1: Number of colonies

Color						
Name of strain						
Initial mixture						
0mM caffeine competition						
20mM caffeine competition						
40mM caffeine competition						

TABLE 2: Relative abundance of colors

Color						
Name of strain						
Initial mixture						
0mM caffeine competition						
20mM caffeine competition						
40mM caffeine competition						

QUESTIONS

1. Complete Table 1:
    a. Write the color and strain info (Your group or another group's?) for each strain that you mixed for your competition.
    b. In rows 3-6, record the number of colonies you counted for each strain/color on each plate: the initial mixture you plated on Day 2, and the three competitions you plated on Day 4.
2. Complete Table 2:
    a. Write the color and strain info as in Table 1
    b. In rows 3-6, calculate the relative abundance of each strain for each plate. This is calculated as:

        number of colonies of color A / total colonies

        So if on your initial plate you counted 57 blue (color A) colonies, and 109 total colonies (including blue), the relative abundance of blue colonies is:

        57 blue / 109 = 0.52

        The relative abundances of each color should add to approximately 1 for each plate.
3. Do you see an equivalent number of colonies of each color on the plate you made on Day 2? What does this tell you about your initial mixture of the yeast strains?
4. Does the relative abundance of each color strain differ across the three media conditions you plated from on Day 3?
5. Which strain had the greatest increase in relative frequency from the before competition plate (Table 2 row 3) to the after competition in 0mM caffeine plate (Table 2 row 4)? What does this tell you about the fitness in the absence of caffeine, when the selection pressure has been removed?
6. Which strain had the greatest increase in relative frequency from the before competition plate (Table 2 row 3) to the after competition in 40mM caffeine plate (Table 2 row 6)? What does this tell you about the fitness in the presence of caffeine, when the selection pressure is maintained? (If no strains grew in the 40mM competition, answer this for the 20mM competition, Table 2 row 5!)

EXTENSION QUESTIONS

1. How does your evolved yeast strain compare to those of other groups? Did it do better or worse than other groups'? How do you know?
2. Do yeast with a high fitness in one environment (presence of caffeine) always have a high fitness in other environments (absence of caffeine)? Use data from the competitions to support your answer.
3. Do all yeast adapted to the same environment (evolved in the same dose of caffeine) have the same fitness in that environment? Use data from the competitions to support your answer.