Predator escape: an ecologically realistic scenario for the evolutionary origins of multicellularity

Student handout

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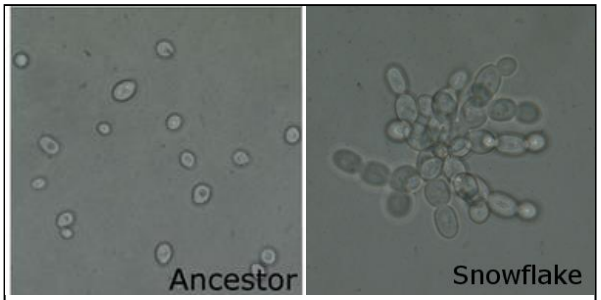
Introduction. The evolution of multicellularity was one of a few events in the history of life that allowed for increases in biological complexity. The first step in this transition is the evolution of multicellular clusters. Once clusters have evolved, there is a shift in the level of natural selection- from single-cells to whole clusters. Over many generations, cluster-level adaptation results in the evolution of increased multicellular complexity (e.g., cellular division of labor, the evolution of developmental programs, etc). This process is described in the movie entitled 'Video overview of the yeast experiment'.

Here we will examine the very first step of this process- the evolution of cellular clusters. In the lab, scientists have shown that simply selecting for fast settling through liquid media can result in the evolution of cluster-forming 'snowflake' yeast. Gravity (imposed by Ratcliff et al (2012)[1] with a centrifuge) is a simple way to select for cluster formation, because clusters of cells fall through liquid media faster than single cells. As a result, if a random mutation arises that results in cluster formation, these will have a *huge* competitive advantage over the ancestral unicellular yeast.

While these experiments are easy to do and give researchers a lot of experimental control, they aren't a very good model for types of selection that unicellular organisms face in nature. After all, there aren't any centrifuges in nature. In this lab, students will use unicellular and snowflake yeast to test a key hypothesis about this transition[2]: that predation by small-mouthed organisms can select for cluster formation.

Goals. Give rotifers (small animals that prey on single-celled organisms) unicellular and multicellular yeast. Observe rotifer predation, and then calculate the relative survival of uni and multicellular yeast during predation. Perform a statistical analysis on this result.

The actors



The prey. Unicellular yeast (strain Y55) on the left. Multicellular yeast (strain C1W3) on the right.


The predator. This rotifer's mouth is at the left, and the stomach is visible through the transparent body. It has been eating red yeast.

Snowflake yeast (above) evolved from single-celled ancestors after three weeks of 'settling selection', or artificial selection for faster settling through liquid media. Genetically, this resulted from a single

mutation that knocked out a gene required for mother-daughter cell separation after mitosis. This experiment was important because it showed that simple multicellularity can evolve rapidly, but it does not use a very ecologically-realistic selective agent.

Rotifers are microscopic animals that prey upon single-celled organisms like algae and bacteria. For a long time, scientists have hypothesized that predation could provide a similar selective environment to settling through liquid- namely that predators would be capable of eating (and killing) small, single-celled organisms, resulting in selection for multicellular clusters too large to be eaten. Rotifers live in aquatic environments, like ponds, marshes, and wet moss. They eat food by creating a vortex with the cilia on their head, which funnels microbes into their mouth. Their bodies are largely transparent and they move slowly, which makes them ideal for this lab. We will give hungry rotifers uni and multicellular yeast, then examine their ability to eat each growth form.

Task 1: Observing rotifer predation.

This experiment utilizes two yeast strains: strain Y55 was isolated from a vineyard in France, and is a regular, unicellular yeast. Multicellular strain C1W3 was derived from Y55 after three weeks of selecting for rapid settling through liquid media. We have labeled the unicellular yeast (strain Y55) red, and the multicellular yeast (strain C1W3) blue. Ask your instructor if you are interested in how this was done. Here you will mount rotifers on the microscope, and observe their predation by the rotifers.

Mounting live rotifers for microscopic examination.

Materials

·Yeast (both strains Y55 and C1W3) fixed and stained with Congo red and methylene blue (supplied in kit). **Be sure to wear gloves and protective eye glasses. These stains are toxic.**
· (2) Glass depression slide (alternative: plastic depression slide)
· (2) 22mm x 22mm coverslips
·Micropipette capable of pipetting 100 µL of liquid
·Micropipette capable of pipetting 1 mL of liquid (alternative: plastic pipettes)
·Corresponding micropipette tips
·Rotifers

Procedure

1. Add 100 µL of predator to depression slide.
   Hint: Get rotifers from the bottom of the container
2. Add 5 µL of blue stained C1W3 multicellular yeast
3. Add 5 µL of red stained Y55 unicellular yeast
4. Add coverslip and immediately view on microscope

Observations

You must observe at least 25 rotifers (a larger sample size is encouraged if time permits) and make a determination on which yeast is the predominant yeast in the stomach of a given rotifer. Note the behaviors of the rotifers. How do they eat? Can you observe any yeast being consumed? How long does it take them to fill their stomach? Record this information in a table.

On a blank sheet of paper, draw a picture of a rotifer eating yeast. Use arrows to indicate the movement of water around the rotifer head.

Task 2: Quantifying rotifer predation.

In this experiment, you will quantify the number of each type of yeast cell in rotifer stomachs. In comparison to the previous exercise, you will actually quantify predatory selection, and will analyze your results statistically. This approach is more rigorous- it will not only allow us to calculate the relative fitness of multi:unicellular yeast, but it will also allow us to determine if this result is statistically robust.

Imaging flattened rotifers. If the school has a microscope with a digital camera: students can take images of flattened rotifers (photos at right) for counting the number of red and blue yeast inside their stomachs. To do this, follow the protocol above, but let the yeast and rotifer mix stand for ~3 minutes prior to pipetting onto a microscope slide. Rather than using the concavity slide, transfer 10 µL of the yeast-rotifer mixture onto a standard slide and flatten by placing a coverslip on top. Otherwise, use the images provided with the lab. You should see images like those to the right.

If the lab does not have a microscope camera, your instructor will provide you with electronic or printed images of rotifers that we imaged using the above protocol.

In either case, each student will obtain an image of a flattened rotifer. Each student will record the number of yeast of each color in their rotifer and then the number in the rotifers of their group members. Each circle in the stomach of a rotifer is one yeast cell (lower right).

Both red and blue yeast are visible in the stomach.

Each of the dark circles above is a yeast cell in the stomach of a rotifer. These are all red unis.

Data Collection

In the table below, count the number of red unicellular and blue multicellular yeast found in your rotifer stomach. Include the number of each yeast strain your group-mates find in their rotifers. Finally, sum the total number of uni and multicellular yeast your group found across all of your rotifers, and put this in the 'total' box

	Rotifer 1	Rotifer 2	Rotifer 3	Rotifer 4	Rotifer 5	Total
Number of red unicellular yeast						
Number of blue multicellular yeast						

Relative survival during predation

Now we will calculate the relative survival of multi to unicellular yeast during rotifer predation. This is a key element in their Darwinian fitness, because yeast that are eaten by predators are killed and cannot pass their genes on to future generations. First, calculate the proportion of killed yeast that are multicellular:

$$\text{Proportion multicellular consumed} = \frac{\#\ blue\ multicellular\ yeast}{\#\ blue\ multicellular\ yeast + \ \#\ red\ unicellular\ yeast}$$

Statistical analysis

To determine if the above difference is significant, we will perform a statistical analysis. In essence, this analysis determines the probability that the difference in predation between uni and multicellular yeast would have been observed by chance. For example, if you flip a coin 100 times and you get 53 heads and 47 tails, this difference isn't large enough that we're could say with much confidence that the coin was biased towards heads. As the results get more divergent from our expectation of 50:50, the chance that the coin really is fair goes down. We're going to use the same principles here to determine if the differences we see in yeast death by rotifers is significant.

We will use a chi-square test, which compares the observed frequencies of uni and multi cells to expected frequencies. To generate the expected frequency of red vs blue cells, assume that both uni and multicellular yeast stock solutions were at the same cell density (cells / mL). Assuming there was no rotifer preference for either yeast strain, we expect that half the total number of yeast counted should be multicellular, and half should be unicellular. Therefore, to calculate the 'expected' number of multis and unis (for use below), divide the total number of counted cells by two.

The chi squared statistic (denoted χ^2 because χ is the Greek letter 'chi') is calculated by summing the squared difference between the observed and expected number of multicellular yeast in the rotifer stomachs, and the unicellular yeast in rotifer stomachs.

$$\chi^2 = \sum \frac{(\#\ Obs - \#\ Exp)^2}{\#\ Exp}$$

For example, say I counted 200 yeast cells in total, so I expect there to be 100 multi and 100 uni cells in the rotifer stomach. But, when we counted them, I found there were 50 multi cells and 150 uni cells. The χ^2 statistic is calculated as:

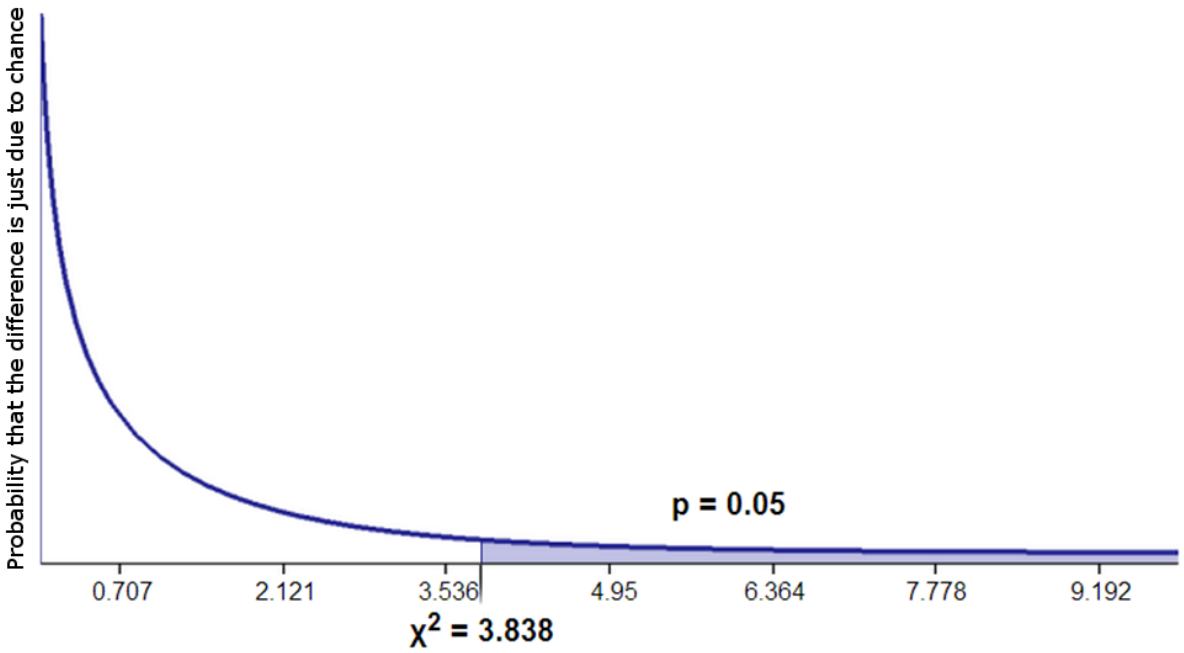
$$\chi^2 = \frac{(50-100)^2}{100}\ [this\ is\ the\ multi\ expectation] + \frac{(150-100)^2}{100}\ [the\ uni\ expectation] = 50$$

Fill out the following table with the information necessary to conduct a chi-square analysis.

Number of observed multis consumed (# Obs)	Number of expected multis consumed (# Exp)	$\frac{(\#\ Obs - \#\ Exp)^2}{\#\ Exp}$ for multis	Number of observed unis consumed (# Obs)	Number of expected unis consumed (# Exp)	$\frac{(\#\ Obs - \#\ Exp)^2}{\#\ Exp}$ for unis

What is your chi squared statistic? Make sure to show your work (either here or in the boxes above).

Finally, we need to use the chi squared statistic to determine the probability that we got the difference between uni and multi predation simply by chance if rotifers really have no preference. As you can see on the distribution below, if your χ^2 statistic is greater than 3.9, then there is a less than 5% chance that your results were caused by chance alone. At that point, we're pretty confident that the rotifers really do have a preference. If your χ^2 statistic is greater than 3.9, the difference in predation you observed is statistically significant at a level generally accepted by scientists to be robust. If this was your result- congratulations, most scientists will now believe that your result is real!



Chi squared distribution. When the chi squared test statistic is above 3.84, then the chance that the difference in rotifer predation was due just to chance is below 5%. At that threshold, we are pretty sure the rotifers have a preference!

Discussion

Depending on instructor preference, students will answer discussion questions in their lab notebooks or discuss these questions as a class. At the culmination of this lab, you will be asked to incorporate your thoughts and write up a full lab report.

References

1. Ratcliff, William C., R. Ford Denison, Mark Borrello, and Michael Travisano. "Experimental evolution of multicellularity." Proceedings of the National Academy of Sciences 109, no. 5 (2012): 1595-1600.

2. Grosberg, Richard K., and Richard R. Strathmann. "The evolution of multicellularity: a minor major transition?" Annu. Rev. Ecol. Evol. Syst. 38 (2007): 621-654.