

What causes variation among individuals?

This tutorial will challenge you to design and conduct experiments to determine the cause(s) of variation in a model population. The population you will study is fictitious, but the tutorial is loosely based on a true story. In recent years, in a variety of places in North America, school children, biologists, and ordinary people have discovered populations of frogs with high frequencies of startling deformities, including extra legs. The suspected causes of deformity, some incriminated by stronger evidence than others, include UV radiation, water pollution, and a parasitic trematode worm, called *Ribeiroia*, whose larvae can encyst in the tissues of tadpoles and disrupt their development.

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Your challenge will be to investigate the cause, or causes, of deformity in a frog population that is split between two ponds. More specifically, you will first determine if there is a difference in the frequency of deformities in the two ponds. If there is a difference, you will then conduct experiments to figure out why.

If there is a difference in the frequency of deformity in the two ponds, it could be due to parasitic worms, genetics, a combination of worms and genetics, or random chance. I know, because I wrote these causes into the different scenarios available in the computer simulation you will use.

The FrogPond application

To complete this tutorial, you will need the software application FrogPond. You can download FrogPond from Jon Herron's website at the following URL:

<http://faculty.washington.edu/~herronjc/>

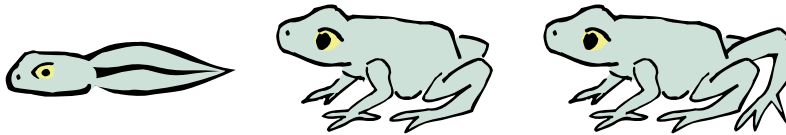
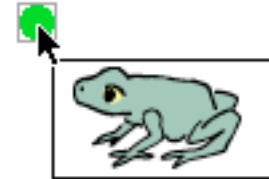
Click on Software, then on FrogPond. There are versions of FrogPond for Windows, Mac OS Classic, and Mac OS X. Download the appropriate version to your computer and launch it.

The first window you will see is an ad for my book. Click on the Proceed button to dismiss it.

Next you'll see a small dialog box asking you to select a scenario from a popup window. Don't worry about what this means. Just pick scenario one, two, or three. (We'll save scenario 4 for later). Then click

the Okay button. Don't be alarmed if it takes a while for the dialog box to go away. Please be patient; the computer is setting up a simulation for you.

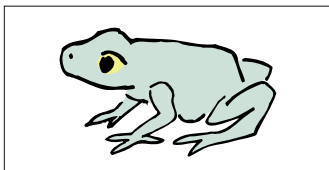
Now you will see FrogPond's main window (see next page). On the left are two large ponds, Top Pond and Bottom Pond. They are full of frogs, represented by the green dots. Click on some of the frogs, and hold the mouse button down, to get a better look at them (right). The smaller dots are tadpoles (below, left). The larger ones are either normal frogs (below, center) or deformed frogs with extra legs (below, right).



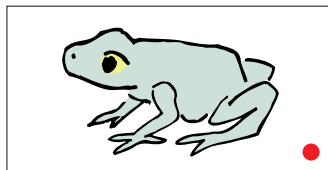
Click the Run button on the lower right. You'll see the frogs move around, mate and make tadpoles (watch closely!), and die. Tadpoles take 50 days to turn into frogs. Frogs live another 150 days after maturing. Click on the Pause button to suspend the simulation.

The red dots in the ponds represent parasitic worms. Notice that when you click on a tadpole or frog to look at it, the box that pops up sometimes has a red dot in the lower right corner. This indicates that the tadpole or frog is infected with the parasite. Infections can only be

Not infected with worms



Infected with worms



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The two large white boxes are the natural ponds you will study: Top Pond and Bottom Pond.

The four small white boxes are experimental ponds where you can conduct your research. To move a frog or worm to an experimental pond, click on it, hold the mouse button down, and drag it to its new location.

The red dots represent parasitic worms.

You can type labels here to keep track of your experiments.

The green dots represent frogs. Large dots are adults, small dots are tadpoles. To see what a tadpole or frog looks like, click on one and hold the mouse button down.

This button will empty the pond of frogs and worms. You can get rid of a particular frog or worm by simply dragging it out of the pond.

This button will run the simulation. Click on it to see the frogs swim around and mate with each other.

Top pond

Bottom pond

100

0

Clear

0

0

Clear

Clear

Day: 0

Run

initiated in tadpoles. A tadpole becomes infected when it bumps into a parasite. Once a tadpole becomes infected, it remains infected for life—even after it turns into a frog.

Note that you can move frogs and worms around, by pointing at them, holding the mouse button down, and dragging them to a new pond. This gives you the ability to design and conduct your own experiments. The questions you need to answer are covered in the following two sections. Note: For each scenario, you must answer Question 1 first. If you skip straight to Question Two, you may waste a lot of time.

Question 1

Is there a higher (or lower) frequency of deformed frogs in Top Pond than in Bottom Pond? How confident are you that your answer is correct?

The frequency of deformed frogs is their proportional representation in the pond. If, for example, six out of ten frogs are deformed, then the frequency of deformed frogs is $6 / 10 = 0.6$, or 0.6.

To answer Question 1, you need to survey the frogs in Top Pond and the frogs in Bottom Pond. It's a good idea to count each frog only once (why?), and to record your data as you go.

Here is a suggestion for a simple way to do your survey. Start with Top Pond. Pick a frog at random, and check to see if it is deformed. If it is, drag it to the upper left experimental pond. If it is normal, drag it to the upper right experimental pond. Note that each experimental pond has a counter next to its lower right corner that keeps track of the num-

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ber of frogs in the pond. Note also that you can type a label above the experimental pond to remind you of what you are putting there. After you have sorted a bunch of frogs into the deformed vs. normal experimental ponds, take the number of deformed frogs and divide it by the total number of deformed + normal frogs. That will give you the frequency of deformed frogs in your survey.

Sorting the *entire* population in top pond would be pretty tedious, so you will probably want to stop early. How many frogs do you think you need to survey to get a fairly accurate estimate of the true frequency of deformities in Top Pond?

After you have an estimate you are comfortable with for top pond, survey Bottom Pond. Then compare the frequencies for the two ponds. Depending on whether you are doing Scenario 1, 2, or 3, it is possible that there is really no difference in the frequency of deformity in Top versus Bottom Pond, and that any apparent difference is just due to random chance. That is, it was just the luck-of-the-draw in the frogs you happened to survey that is making it look like there are more deformed frogs in one pond or the other. Do you think this is the case? Or do you think there really is a difference between the frequency of deformity in Top versus Bottom Pond?

When you are finished with your survey, you can return the frogs from the experimental ponds to Top and Bottom ponds. An efficient way to do this is by clicking the clear button under each experimental pond. This will present a dialog box letting you tell the computer what to do with the frogs.

If you think any apparent difference in the rate of deformity is just due to chance, skip the section on Question 2 and read on. If you think

there really is a substantial difference between Top versus Bottom Pond, proceed to Question 2.

Question 2

If there is a difference in the frequency of deformed frogs in Top Pond versus Bottom Pond, then what is its cause?

Possible answers are:

1. The frogs in one pond are, on average, genetically more prone to deformity than the frogs in the other pond (the worms are irrelevant);
or
2. The deformities are caused by the worms, and one pond has more worms than the other (genetics is irrelevant).

Your challenge now is to design and conduct experiments that will allow you to determine which answer is correct. Here are some things you might do in the course of your experiments:

- Examine the frogs, and classify and count them.
- Record your data in tables or graphs.
- Move frogs (and, if you like, worms) from the big ponds to the smaller experimental pools. Run the simulation for a while (an entire generation takes 200 days), then examine the frogs in the pool. Note that you can type labels into the rectangles above the pools to keep track of what you've put there.

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- Mate particular frogs with each other. Move two frogs to a pool. Encourage them to mate by making sure they are touching each other. Run the simulation for a short time. You will see the parents produce one or two tadpoles. Let the tadpoles grow up, then examine them.
- Get rid of frogs or worms by dragging them out of a pool or pond, or by using the clear button.
- If you need to reset your simulation, you can do so with the Reset command under the File menu. (Note, however, that if you do this you will have to repeat your surveys of Top Pond and Bottom Pond. In different runs of the same scenario, the pond with the higher rate of deformities may change.

You will have noticed that I have only told you pieces of what you might do during your experiments. I haven't told exactly what experiments to set up. That is up to you. I will, however, offer three pieces of general advice. First, an effective way to proceed is to pick one of the two possible answers as your working hypothesis. It doesn't matter which one. Then do something with the frogs. Predict what will happen if your hypothesis is right, and what will happen if it is wrong. Then collect data to see what actually happened. Second, you may have to repeat an experiment several times before a clear pattern emerges in the data. Third, if your experiment does not produce a clear outcome, then try a different experiment.

When you are confident you know what is going on with the frogs in your ponds, proceed to the next section.

Learning the Truth

Because FrogPond is a simulation, it allows you to do something you cannot do in the real world. You can ask the application to tell you the Truth about the simulation you are running. Under the Tools menu, select Tell the truth. Enter the password 'Kermit'. Then click Now tell me.

Were you right? If not, try to figure out why your experiments failed to produce a clear answer, or why they led you astray. Try to design a new experiment that will give you the right answer. Then run your new experiment and see if it works.

Try another scenario

Under the File menu, select Reset. Pick a different scenario. Then repeat your investigations of Question 1 and Question 2. When you think you know what's going on, ask FrogPond to tell you the Truth.

If you get tired of the laborious surveys that you have to do to answer Question 1, here is a shortcut. Under the Tools menu, select Sample. This will give you a small dialog box. Select the pond you want to survey, and the number of frogs you want to check. Then click Sample. FrogPond will do the survey for you and tell you the result.

FrogPond's method of surveying is a little bit different than yours. It picks a frog at random, then throws it back in the pond. Then it picks another frog at random. This procedure has a serious flaw: It may count the same frog twice. But in the context of this simulation, it also offers an advantage. Even though there are only 100 frogs in the pond, you can ask FrogPond to sample 1000 frogs. Yes, this counts every frog

several times. But it also gives you a pretty accurate estimate of the true frequency. (If you don't believe me, check for yourself.)

If, in the Sample dialog box, you ask FrogPond to sample both ponds, it will report the *relative risk* of deformity. This is the frequency of deformity in Top Pond divided by the frequency in Bottom Pond. If the relative risk is close to 1, then the frequency of deformity is about the same in both ponds. If the relative risk is a lot smaller than one, it means that the frequency of deformity is higher in Bottom Pond. If the relative risk is a lot bigger than one, it means that the frequency of deformity is a lot higher in Top Pond.

If you are still having fun, and if you feel confident in your ability to design experiments and interpret the results, try to figure out what's going on in Scenario Four.

Bibliography

The following papers investigate the causes of deformity in real populations of frogs:

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- Burkhart, J. G., J. C. Helgen, et al. 1998. Induction of mortality and malformation in *Xenopus laevis* embryos by water sources associated with field frog deformities. *Environmental Health Perspectives* 106: 841-848.

- Gardiner, D. M., and D. M. Hoppe. 1999. Environmentally induced limb malformations in mink frogs (*Rana septentrionalis*). *Journal of Experimental Zoology* 284: 207-216.
- Gilliland, M. G., and P. M. Muzzall. 2002. Amphibians, trematodes, and deformities: An overview from southern Michigan. *Comparative Parasitology* 69: 81-85.
- Johnson, P. T. J., K. B. Lunde, et al. 1999. The effect of trematode infection on amphibian limb development and survivorship. *Science* 284: 802-804.
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