# Behaviorial choice experiments using true bugs

Grades 3-12

Updated January 13, 2016

## Summary

This protocol will explain how to use milkweed bugs in behavior choice experiments. (Other insects or small animals can easily be substituted in place of these bugs.)

## Learning goals

After this activity, students should be able to

- Appreciate that insects are animals with complex behaviors.
- Think about the behavioral motivations of insects.
- Learn some terms related to animal behavior. (Potentially unfamiliar terms appear in bold.)

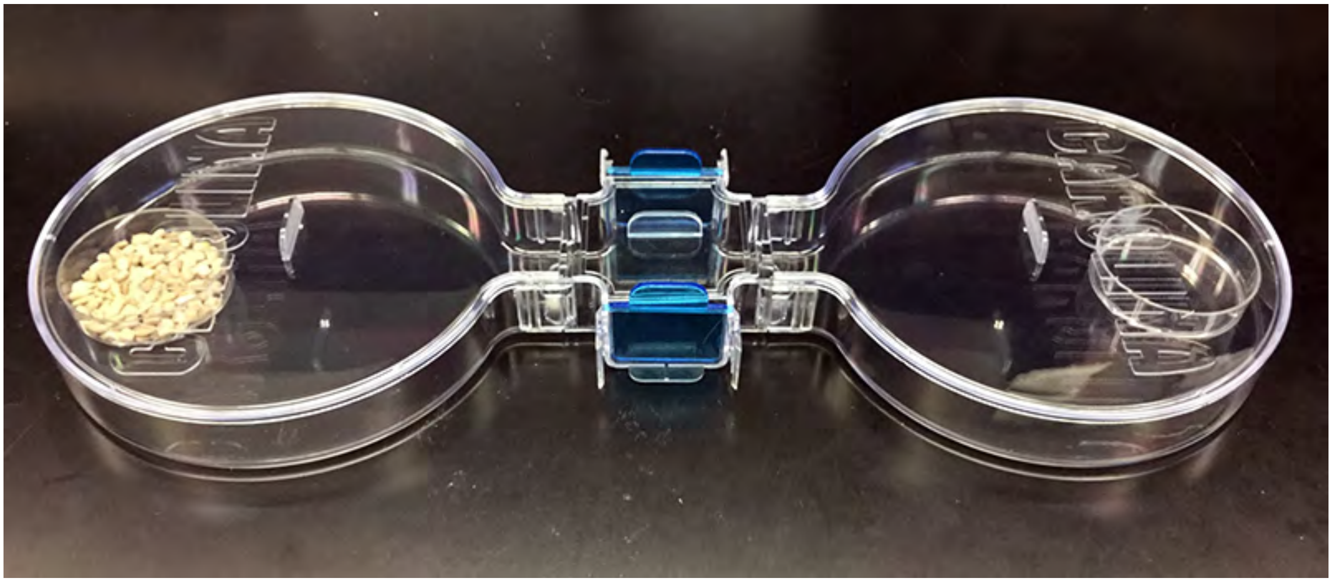
Optionally, teachers may guide more advanced students through experimental design and statistical analysis of results.

## Background information

The study of animal behavior is called **ethology**. One of the simplest behaviors to observe is an animal's movement through the environment. Since natural environments can be very complex, **ethologists** often design simple lab experiments in order to more clearly identifying the innate behaviors of animals.

A simple behavioral test you can do in the lab is a choice test. In this type of experiment an animal is placed in the middle of a **choice chamber** with two alternatives to either side. The sides of the chamber could hold different foods, different colors or intensities of light, or the smells of different attractant or repellant chemicals.

Typically, when you first put an animal in the chamber it's first reaction to run randomly in some direction out of fear. This kind of random, undirected movement, is a type behavior called **kinesis**. What we're typically more interested in is **taxis**, a type of behavior where an animal orients its body toward a stimulus, such as one of the offerings in the choice chamber.



A suitable chamber can be purchased from Carolina Biological Supply Company (http://www.carolina.com/elementary-lab-supplies-equipment/carolina-large-choice-chamber-kit/143051.pr). It's possible to build your own chamber using either plastic Petri dishes or disposable plastic food containers. The basic idea is that the chamber should have 3 parts. The two choice areas should be as similar as possible and equidistant from the middle chamber. Ideally it's also easy to get things in and out of all areas of the chamber. (This is where Carolina's chamber is excellent.)

Since we're not interested in where the animal goes as a result of fear-induced kinesis, it's helpful to repeat the experiment several times, so that if it randomly goes left or right, that bias is averaged out over many trials. It's also helpful to give the animal some time to make a choice that might reflect taxis toward one side of the chamber.

True bugs, such as the milkweed bug *Oncopeltus fasciatus*, can be good subjects for behavioral experiments. It's helpful to have a colony with many individuals so that you can repeat behavioral experiments with different individuals. (Check out the other online teaching resources at *Bugs in our Backyard* for instructions on raising milkweed bugs in the classroom.)

## Materials

| **item** | **vendor information** | **or try this...** |
|---|---|---|
| choice chamber | Large Choice Chamber Kit (Carolina Biological cat# 143051, $15.25 each) | Cut 1-inch square holes in plastic containers. Line up holes and hot-glue 3 in a row. |
| bugs | Milkweed bugs are available from Carolina Biological | Collect bugs or other arthropods, such as pill bugs, from your own backyard! |

## Procedure

Here is a basic procedure. Later I'll offer some ideas for variations.

- Decide on your **experimental design**. Depending on the age or level of your students, you may ask them to design their own experiments or agree upon on after group discussion. Can they describe the experiment in terms of a hypothesis that will be tested? At a minimum, you should decide

- which bugs (species, sex, age, etc.) you'll use in your experiment. Does it matter whether you separate males from females?
- what your choice chambers will offer the bugs.
- how many replicate trials the experiment will include. Should you test the same bug multiple times? Or different bugs? Each approach has its merits, and which you choose might depend on how many bugs you have to work with!
- how you minimize any confounding influences on your experiment. For example, if you repeat your experiment, vary the side (left or right) of each offering so the bug isn't attracted to something in that direction (such as light).

- **Set up a table to record your results**. Fill in the table in this hand-out, or make your own. Have the students ready to record observations.
- **Set up each side of the choice chamber**. Add whatever food, etc. you will be using to test the bugs' behavior.
- **Gently move one bug into the middle of the chamber**. The more stressed an animal is, the longer it will take for your to get a clear result. (If you're using Carolina chambers, beware that small bugs might fit between the gaps in the lids. You can cover these with Petri dish lid or some other transparent cover.)
- **Wait and observe** while the bug makes its choice. If a bug isn't too stressed from being moved, it should calm down and go about it's regular behaviors within about 10 minutes. During this time is helpful to make close, qualitative observations of the bug's movement. (These are especially helpful if your experiment doesn't work the way you expect it to. For example, if the bug isn't moving, what *is* it doing?)
- **Record the result** (which side of the chamber the bugs ends up on).
- Ask the students to **summarize** their data in some way. Depending on your students' experience, this might mean making a bar graph of the number of times bugs went in each direction. Does this show a preference? How might the result of one experiment compare to another experiment that is similar but different in some way?
- **Optionally**, more in advanced classes, a teacher can guide students through use of the $\chi^2$ **test** to help in forming conclusion. (Instructions below.)
- **Share** your results in comments on bugsinourbackyard.org or tweet to @Jhaematoloma!

## Ideas for choice experiments

Choice experiments are a classic activity in the science classroom because teachers and students can adapt them to a wide range of questions. Think about how you can fit this activity into other content and concepts you're already teaching. Here are some ideas!

- **Food choices**
  - The lab strain of milkweed bugs has been in captivity since they were bred in the 1970s to survive on sunflower seeds. However, another (now lost) strain had been bred to feed on pumpkin seeds. Wild milkweed bugs feed on the sap and seeds of milkweed plants. How does the preference of bugs differ for these foods? Does the preference differ in wild vs. captive-bred milkweed bugs?
  - If you have red-shouldered soapberry bugs in your area, what plant have you found them on? There are populations in different parts of the US that have adapted to living on goldenrain trees vs. balloon vines. What is the preference of bugs in your area for these two plants? (Seeds are available from outsidepride.com and treeshrubseeds.com.)

- In general, bugs may make their choice faster if you keep them overnight without food or water. You could also test how depriving them of water and/or food for different time periods affects their food choices or how quickly they go to a food source when they're added to a chamber with one. (Be careful! Milkweed bugs will die if they don't have food or water for more than about 3 days, sooner if the air is dry.)

- **Smells** - Insects have a great sense of smell and experience much of their world through scent. Can you test whether bugs are attracted to or repelled by certain smells? You can extract the smell from many things just by crushing it into water or alcohol and then letting it dry on paper. Or by wiping it with a paper towel dampened with water or alcohol. Are bugs attracted to (or repelled by) the smell of
  - Water or alcohol? -- This may be an important **control experiment**!
  - Foods
  - Other bugs? (Males to females? Females to males? Other species of bugs?)
  - Bug spray?
  - Predators… Can you find a mantis, spider or bird to wipe some scent off of?
- **Light** - Do bugs prefer bright light vs. cover? Use lamps, flashlights, dark construction paper, aluminum foil, or colored cellophane to create differently lit areas.
- **Social cues** - Many bugs are **semisocial**, meaning that they will hang out with other individuals of their species when stressed (or bored). This is especially true of **aposematic** bugs like milkweed bugs and soapberry bugs. Have a group of bugs relax in one side of the choice chamber, leaving the other side empty. Then introduce a new bug. Does the newbie join the group? Does it matter if the group contains males or females and if the new bug is male or female? Does their age make a difference?

## Optional: Considering your results with the $\chi^2$ test

Choice tests are a great way to introduce statistical analysis to students. Typically the appropriate statistical test for this sort of experiment will be the **$\chi^2$ (chi-squared) test for independence**, which is a simple test that can be worked using a basic calculator or several free websites.

Students (and the general public!) often have a hard time understanding the point of statistics, and view their use suspiciously. Again, choice tests and the $\chi^2$ test are a great opportunity to illustrate how statistics can be helpful. Here's an example: Imagine you test 10 bugs preference for sunflower seeds vs. pumpkin seeds. If the bugs really have no preference, you should expect them to go to each side equally often-- 50/50. For 10 tests, that would mean results that are 5 choices of sunflower and 5 of pumpkin. But if the bugs are just randomly going left or right without really caring about the seeds, then it's not that unlikely that you'd get 6 of one and 4 of the other. (This is just likely flipping a coin 10 times-- you won't *always* get 5 heads and 5 tails.) A statistical test tells you how much confidence you should have that an outcome is random, that there's no real difference. Most statistical tests result in a number called a *p*-value. It is helpful to think of a ***p*-value** as the probability that the results you get from an experiment are just due to chance, and that there really is no difference (in the preference of your bugs, if we continue our example). In other words, the test can also be used to tell if results are different from expectations. Similarly, if you run two related experiments, a statistical test can give you some measure of confidence whether or not the results of the two are different from one another. Finally, it's important to remind students that by running a statistical test, they are not "giving up" thw power or responsibility to think and make conclusions for themselves! (See the section below on interpreting *p*-values.)

To use the $\chi^2$ test, your results need to be in the form of a **table**. It's helpful to organize the different results as rows. For example, if 12 bugs ended up in chamber A and 3 bugs ended up in chamber B, make rows labeled A and B. The table also needs at least 2 columns. However one of these can be your expected results rather than actual counts. If bugs have no preference for chambers A or B, then we'd expect them to go into each with equal likelihood, giving us a 50/50 outcome. If we ran 15 bugs through the chamber, then we'd expect 7.5 bugs in A and 7.5 bugs in B. (Yes, you can't have half a bug! But odd numbers here help underscore that this is an expectation, not a real outcome.) So you're table might look like this:

| | observed results | expected results |
|---|---|---|
| chamber A | 12 | 7.5 |
| chamber B | 3 | 7.5 |
| total | 15 | |

To make the test "by hand," you need to figure out two things: the degrees of freedom in your data and their $\chi^2$ statistic. The **degrees of freedom (df)** is just one less than the number of outcomes in your table. So, if you have two outcomes, then df =1. Calculating **the $\chi^2$ statistic** involves some simple arithmetic. First, find the deviation of the two outcomes (observed - expected) for each outcome (chamber A and chamber B). Doing this on paper, it's convenient to write these values down in another column labeled "deviations", "d", or "O-E". Next square the deviations. (Have students use the $x^2$ key on their calculators or just multiply the number by itself.) Then divide the squared deviations by the expected values for each outcome. (It's helpful to add columns to the table for each of these steps.) Finally, add up each of these last values. The result is a $\chi^2$ statistic!

From here you'll need to consult a **$\chi^2$ distribution table**, such as the one on this page, to determine the corresponding *p*-value for your $\chi^2$ statistic and degrees of freedom. But here's a short-cut: if df = 1, and your $\chi^2$ is more than 3.84, then your *p*-value is below 0.05.

In the end, you should have a table in your notes that looks something like this:

| | observed results | expected results | deviations (O-E) | squared deviation | ((O-E)^2)/E | |
|---|---|---|---|---|---|---|
| chamber A | 12 | 7.5 | 4.5 | 20.25 | 2.7 | |
| chamber B | 3 | 7.5 | -4.5 | 20.25 | 2.7 | |
| total | 15 | | | | 5.4 | $= \chi^2$ |
| df = 1 | | | | p = | 0.02013 | |

The same calculations will work if you'd like to compare the results of two experiments. So rather than having "observed" and "expected" results, you have results from "experiment 1" and "experiment 2".

You can find the precise *p*-value by using Excel. The function CHI.TEST(R1, R2) will start from your original results, while CHI.DIST.RT(x, df) uses your $\chi^2$ statistic and degrees of freedom. Both functions will return the corresponding *p*-value.

In addition, several websites will allow you to run the $\chi^2$ test starting from your data. These can be used if you just want to introduce the use of statistics without having students go through the math. A good one is [http://graphpad.com/quickcalcs/chisquared1.cfm](http://graphpad.com/quickcalcs/chisquared1.cfm). The GraphPad website also lets you use the $\chi^2$ test for more than 2 groups of results (and more than 2 outcomes), if you need to do that.

## *p*-values and biological significance

So what does a *p*-value mean? And how should it be interpreted? One useful way of thinking about *p* is that it is the probably that there is no real difference between the two groups of results. So, **a low *p*-value means it's unlikely that no difference exists**. Traditionally, 0.05 is taken as the critical *p*-value for making conclusions. If a test produces a $p < 0.05$, the results are said to be significantly different.

What the p-value cannot encapsulate is whether the result is *biologically significant*. It may certainly be the case that values that do not reach statistical significance still matter to organisms. Conversely, some measurable differences may be irrelevant in real life. This is not to dismiss the usefulness of statistics. But the results of tests must be considered in context.

**Critical values for the $\chi^2$ distribution**

| | degrees of freedom | | |
|---|---|---|---|
| p-value | 1 | 2 | 3 |
| 0.9 | 0.0158 | 0.2107 | 0.5843 |
| 0.5 | 0.4549 | 1.386 | 2.365 |
| 0.2 | 1.642 | 3.219 | 4.642 |
| 0.1 | 2.706 | 4.605 | 6.251 |
| 0.06 | 3.537 | 5.627 | 7.407 |
| 0.05 | 3.841 | 5.991 | 7.815 |
| 0.04 | 4.218 | 6.438 | 8.311 |
| 0.03 | 4.709 | 7.013 | 8.947 |
| 0.02 | 5.412 | 7.824 | 9.837 |
| 0.01 | 6.635 | 9.210 | 11.34 |
| 0.001 | 10.83 | 13.82 | 16.27 |
| 0.0001 | 15.14 | 18.42 | 21.11 |

There are also situations where a person may not want to use 0.05 as the critical value for decision-making. What if you were testing the effectiveness of a new drug to treat cancer? What if you were testing the performance of an airplane engine? Ask students what situations might require more stringent (in other words, smaller) *p*-values.

# Template for Choice Chamber Experiment

## Name ________________________________________

## Research Question

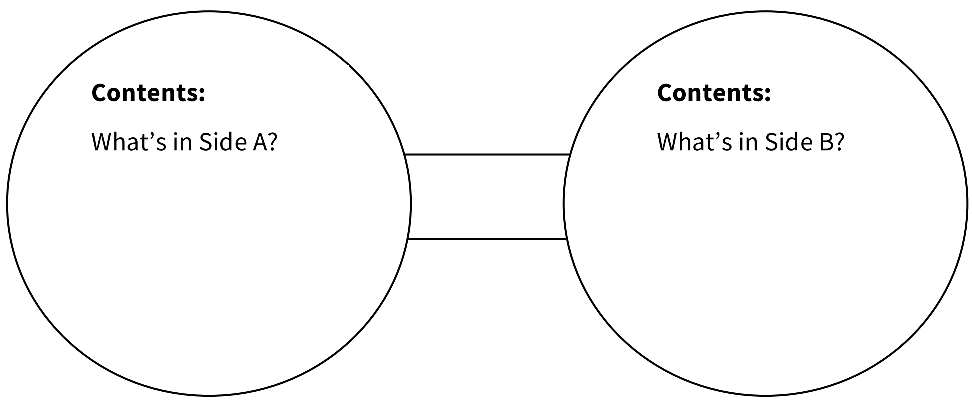
What are you trying to learn more about?

## Hypothesis and Predictions

What do you think is going to happen and why?

## Experimental Setup

1. What's in each side of the Choice Chamber?
   Please write down the contents of each side of the choice chamber in the spaces below.




2. Are there other factors of this experimental setup that are important to write down? If so, write them in the space below.

3. What kind of data will you collect? Use the space below to write down what kind of results you will look for and record in your data sheet.

4. How many separate trials do you plan to run in your experiment? ___________

5. Is there anything else that you would like to make note of before beginning your experiment?

## Sample Data Collection Sheet

**Name ______________________________________**

**Side A:** **Side B:**

**Purpose of this experiment:**

| Time | Outcome | Other notes |
|---|---|---|
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