**Natal Bean Discrimination by Bean Beetles**

**Learning Objectives**

* Design and perform a set of experiments to evaluate whether female bean beetles (*Callosobruchus maculatus*) discriminate between suitable species of beans.

**Introduction**

Read the following setup from Smith and Jones, 2014:[[1]](#footnote-1)

Bean beetles, *Callosobruchus maculatus*, are herbivorous pest insects that are found in Africa and Asia. Females lay their eggs on the surface of beans. Eggs are layed singly, and hatch into larvae (maggots) several days later. The larva then burrows into the bean and will form a pupa 21-30 days after the egg was deposited. They mature 24-36 hours after emergence from the pupa and do not need to feed.

Adults typically live for 1-2 weeks. Mating and oviposition occur during this time period. Females will choose the best substrate (bean) to lay their offspring on, since the larvae cannot move. By choosing a substrate for oviposition, the female chooses the food resource available to her offspring (Brown and Downhower 1988). This is a critical choice for the female, as it influences the growth, survival and future reproductive success of her offspring (Mitchell, 1975, Wasserman and Futuyma, 1981). Females can lay eggs on a wide range of bean species, but very few bean species will result in normal development and the successful emergence of adults. Some species of beans are toxic to larvae (Janzen 1977).

**Materials**

In class, you will be provided with live cultures of bean beetles containing adults that have been raised on mung beans ( *Vigna radiata* ).

Female beetles are easily identified in the live cultures because they have two dark stripes on the posterior of the abdomen, whereas the posterior abdomen of males is uniformly light in color.

You will also have access to petri dishes and several types of beans.

**Experimental Design**

Since the oviposition choices of females influences the survival and future success of their offspring, females may be very sensitive to the species and condition of the beans on which they are depositing eggs.

Each group should design a set of experiments to address whether female bean beetles discriminate between suitable species of beans. We will re-visit the experiment next week and collect our data set. We will choose one experiment to perform as a class.

As a group, list several ways we might examine female bean choice for oviposition. We will pick one experiment from the class to perform. Write your ideas below.

**\*\*Stop here. We will go over ideas for our experiment as a class and choose one to perform\*\***

Outline the agreed on experiment below.

1. **Formulate a hypothesis for this week’s experiment. Be specific!**
2. **We will re-visit the experiment in a week to collect data on the number of eggs laid. Formulate a hypothesis for this experiment.**
3. **Identify the independent variable(s).**
4. **Identify the dependent variable(s).**
5. **What variables will you keep standard?**
6. **What is your control?**
7. **Design data collection tables for both of your experiments on a separate sheet of paper.**

For this data, it is most useful to perform a Chi Square analysis. Chi Square statistical test evaluate whether this is a significant difference between groups of data. The null hypothesis (Ho) is the hypothesis that states that there is no difference between the groups of data. The alternative hypothesis, (Ha) is the hypothesis you outlined above.

1. **Restate your Ho hypothesis:**
2. **Ha hypothesis:**
3. **If the null hypothesis is correct, what would we expect to see?**
4. **If our alternative hypothesis is correct, what would we expect to see?**

To do this statistical test, we need to calculate observed and expected values for the bean species and for our control. Our expected values are based on our null hypothesis (that there is no difference).

For example, if we had 40 eggs and four different treatment groups, we would expect there to be 10 eggs on each group.

|  |  |  |
| --- | --- | --- |
| **Treatment** | **Observed number of eggs** | **Expected number of eggs** |
| Mung beans | 5 | 10 |
| Pinto beans | 25 | 10 |
| Chick peas | 10 | 10 |
| Control | 0 | 10 |

**Fill in the data table below with our observed (what we actually found) and expected values.**

|  |  |  |
| --- | --- | --- |
| **Treatment** | **Observed number of eggs** | **Expected number of eggs** |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |

To calculate the chi-square value, or χ 2 , we simply add the square differences, divided by the expected, of all the observed and expected. In mathematical terms:

χ 2 = Σ (O-E)2

E

So for our example from the previous sample table, the first (O-E)2 / E would be (5-10)2

10 = 2.5

We would then add to this value all of the other (O-E) 2 / E in the table and then add to get the χ 2 value.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment** | **Observed number of eggs** | **Expected number of eggs** | **Observed-Expected** | **(Observed-Expected)2** | **(Observed-Expected)2/Expected** |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

**Compute the χ 2 value for your data by adding all of the (Observed-Expected)2/Expected values.**

In order to find something to compare this number with, we need to calculate the degrees of freedom, or the number of different comparisons that can be made within the table. The degrees of freedom are the number of columns (M) minus one times the number of rows (N) minus one. Degrees of freedom = (M-1) (N-1)

**A B**

**C D**

How many ways can this two by two table be broken into individual comparisons? Hint: Use the formula above.

**Calculate the degrees of freedom in your experiment.**

Now we can compare against the Chi distribution for the likelihood that our data is generated by chance. Remember, we are looking at the column labeled 0.05 for our p value. This means that at this level, we are 95% sure our results are real. The Chi distribution table gives us a critical value to compare our test value to.

* If test>critical value @ p level, **reject** null hypothesis
* If test<critical value @ alpha level, **fail to reject** null hypothesis

**Compare to the Chi-distribution table at the end of the lab. Did your results come about by chance?**



We will now use this data and information to do a lab write up.

**Lab Report Template**

**The report should be typed and single spaced.** See grading rubric for clarity on formatting.

**Title Page:**

*\*Should include Title (a brief, concise, yet descriptive title), your name, lab instructor’s name, and lab section.*

*\* Note: this is a separate sheet*

**Body of Report**

**Identify the different sections of the body of the report with headings.**

The report should begin with a brief paragraph (complete sentences) that includes a statement of the problem and your hypothesis.  This should be under the heading of Introduction.

* Statement of the problem:
  + What question are you trying to answer?
  + Include any preliminary observations or background information about the subject (in this case the bean beetles) such as reproductive cycle, life span etc. Be sure to cite any sources.
* Hypothesis:
  + Write a possible explanation/prediction for the problem/question you are asking.
  + Make sure this possible explanation/prediction is a complete sentence and not a question.
  + Make sure the statement is testable. In other words, can you perform an experiment that will either support or refute your prediction.  If you cannot not think of a way to test your prediction, then it is not testable.

Next heading should be **Materials**:

* Make a list (this does not need to be in paragraph form) of ALL items used in the experiment and their quantities.  Of the materials used, **identify which are dependent and independent variables, constants (standardized variable) and control group (you will lose points if you do not identify ALL dependent and independent variables, constants and controls)**.

Next heading should be **Procedure**:

* Write a paragraph (complete sentences) which explains what you did in the experiment.
* Your procedure should be written so that anyone else could repeat the experiment.  For instance, what beetle did you use? How was your petri dish set up? What beans did you use?   That means that **even some of the most obvious steps need to be stated** so there is no ambiguity.
* **When designing the procedure, be sure to include replicating the experiment to ensure data is reproducible and valid.**

Next heading should be **Results**:

* Write a paragraph (complete sentences) describing the results and observations of your experiment.  Here you will compare results for controls and variables and not simply list the numbers.
* This section also includes data tables, graphs or charts to illustrate the results of you experiment.  **Be sure to include calculated averages of *t*.**
* All tables, graphs and charts should be labeled appropriately (a title, labels for *x* & *y* axis, legend etc.) so the reader will be able to understand.

Next heading should be **Conclusions**:

* Write a paragraph restating your hypothesis and whether you accept or reject your hypothesis
* In this paragraph, **explain** why you accepted or rejected your hypothesis **using data from the experiment**. Include a brief summary of the data—averages, highest, lowest, etc., to help the reader understand your results and why you have come to particular conclusions.
* Discuss your thoughts about the possible reasons for your results (for example, if you chose salt water as a variable, give a possible reason why salt water, in particular, may have generated your results).
* Discuss possible errors that could have occurred in the collection of the data (experimental errors) and describe how these errors may have impacted the data.

**The following rubric will be used to grade these reports:**

**Lab Report Grading Rubric**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Excellent | Satisfactory | Unsatisfactory | Points awarded |
|  | **5.0 points** | **2.5 points** | **0 points** |  |
| Title Page | Contains title, student name, instructor name and section | Missing either instructor name or section | No title page |  |
| Formatting: typed, spacing | Typed and single spaced | Typed, but not single spaced | Not typed |  |
| Grammar and Spelling | No errors; contains complete sentences and no misspellings | A few minor errors in grammar and spelling | Several major errors in grammar and spelling |  |
| Formatting: headings | Each section has a heading as described in template | Some sections lack headings | No headings |  |
| Hypothesis | Predictions are clearly stated and written as a testable statement | Predictions/expected outcomes are not clearly stated | Not written as a testable statement |  |
| Materials | All equipment and materials described; identify variables, controls and constants | Materials incompletely described | No identification of variables, controls and constants |  |
| Procedure | Clear step-by step description | Description missing details making it difficult for another scientist to repeat experiment | Description missing so much detail it would be impossible to repeat |  |
| Results | Clearly written description of results comparing controls and variables | Results are presented but no comparison between controls and variables are made | No written description of results |  |
| Data tables, graphs or charts | Easy to interpret, clear labels, all data, including calculated averages, included | Disorganized (not easy to understand, missing labels) but all data included | Disorganized and or data clearly missing |  |
| Conclusion | Clearly explains acceptance or rejection of hypothesis using data to support conclusion; identifies sources of error | Accepts or rejects hypothesis but does not use data to explain why; or does not identify sources of error | Does not explain conclusion and does not identify sources of error |  |
|  |  |  |  | Total \_\_\_\_\_\_out of 50 points |

**Literature Cited**

Brown, L. and J.F. Downhower. 1988. Analyses in Behavioral Ecology: A Manual for Lab and Field. Sinauer Associates, 194 pages.

Janzen, D.H. 1977. How southern cowpea weevil larvae (Bruchidae *Callosobruchus maculatus*) die on non-host seeds. Ecology 58:921-927.

Mitchell, R. 1975. The evolution of oviposition tactics in the bean weevil, *Callosobruchus maculatus* F. Ecology 56:696-702.

Wasserman, S.S. and D.J. Futuyma. 1981. Evolution of host plant utilization in laboratory populations of the southern cowpea weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae). Evolution 35:605-617.

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1. *Blumer, Lawrence S., Beck*, *Christoper W*., *Natal Bean Discrimination by Bean Beetles*, [[http://www.beanbeetles.org/protocols/natal\_preference/student.html](http://www.beanbeetles.org/protocols/natal_preference/student.html" \t "_blank)]. November 24, 2012. [↑](#footnote-ref-1)