Introduction to Bean Beetles and Experimental Design Lab

1. Introduction to Bean Beetles and Lab

2. Experimental Design Concepts Jigsaw Activity
    a. Experimental Design Concepts Expert Groups Activities
        i. Experimental Design Concepts ID Activity
        ii. Identification of Concepts in Experimental Write-up Activity
    b. Experimental Design Concepts Lab Group Peer Teaching Activity
    c. Experimental Design Concepts Review Activity

3. Using bean beetles to teach experimental design and experimental variables
    a. Experimental Design and Lab Presentation Preparation

Expert Groups Worksheet Group A

1) In your expert group, come to an agreement on the identification of your terms and place that information in the table below:

Concept	Consensus Identification
Observation	
Inductive Reasoning	
Deductive Reasoning	
Biological Hypotheses	
Population	

2) In your expert group, come to an agreement on the use of your terms in biological experiments and place that information in the table below:

Concept	Consensus Description of use in biological experiments
Observation	
Inductive Reasoning	
Deductive Reasoning	
Biological Hypotheses	
Population	

3) Provide agreed upon examples of each of your terms below. If each person in your group provided a different example, you could have up to four example:

Concept	Example 1	Example 2	Example 3	Example 4
Observation				
Inductive Reasoning				
Deductive Reasoning				
Biological Hypotheses				
Population				

Expert Groups Worksheet **Group A**

4) In your expert group, come to an agreement on the relationship between terms and place that information in the table below:

Concept 1	Concept 2	Consensus Relationship
Observation	Inductive Reasoning	
	Deductive Reasoning	
	Biological Hypotheses	
	Population	

Inductive Reasoning	Deductive Reasoning	
	Biological Hypotheses	
	Population	

Deductive Reasoning	Biological Hypotheses	
	Population	

Biological Hypotheses	Population	

5) Finally, come to an agreement on why each concept is an important component of designing an experiment and place that info in the table below:

Concept	Explain why each concept is an important component of designing an experiment
Observation	
Inductive Reasoning	
Deductive Reasoning	
Biological Hypotheses	
Population	

Expert Groups Bean Beetle Experimental Write-up Activity

An experimental write-up with background information, methods and results are attached to this sheet. In part I of this activity, identify where and how your concepts have been applied in this experiment. Once the class has finished in expert groups, you will return to your normal lab groups to work on part II.

Part I

Identify where and how your concepts have been applied in the attached experiment:

Concept	Identify where and how your concepts have been applied in the attached experimental write-up of a bean beetle experiment

Part II

Once you have completed part I and the class has returned to regular lab groups, each expert should take turns explaining their concepts to the group while the group members fill out the table that follows. You should also explain where and how your concepts were used in the attached experimental write-up while they complete the table that follows.

Expert Groups Bean Beetle Experimental Write-up Activity

Concepts	Provide your understanding of each of the concepts presented by your lab group partners in the spaces below. Then briefly explain their concept use in the attached experiment.

Discrimination in Male Bean Beetle Mounting Behavior

Objectives

- Consider various factors that could influence male mounting behavior
- Design and perform a set of experiments to test whether male mounting behavior is random with respect to sex

Introduction

Bean beetles, *Callosobruchus maculatus*, spend their short adult life span (1- 2 weeks) mating, with females laying (ovipositing) single fertilized eggs on the surface of various bean species (Talukder & Howse, 1994). Following oviposition, a beetle larva will burrow into the bean and ingest the endosperm portion of the seed before emerging as an adult. The later stages of this process can be readily observed through the appearance of a window where the beetle is pupating. Upon emergence, adults of this species do not require any food or water to survive.

Mating behavior in this species is devoid of courtship and is characterized by a male climbing on a female (mounting) and attempting the transfer of a spermatophore. Mating is also characterized by traumatic insemination of the female due to the male's spiny genitalia (Crudgington and Siva-Jothy, 2000). Indeed, the female response to male mounting in some cases is to attempt to dismount a male during copulation before a spermatophore is deposited (Eady, 1994), presumably to prevent damage to her reproductive tract (Crudgington and Siva-Jothy, 2000).

Observation of a bean beetle (*Callosobruchus maculatus*) colony reveals that males, easily identifiable by a lack of white stripe on the caudal end of the abdomen (Figure 1), are regularly seen mounting multiple individuals within the colony, regardless of sex. This interesting animal behavior raises the question of whether or not the mounting is random. More specifically, is male – male mounting an example of intrasexual selection (i.e., competition between males that ultimately leads to greater access to females by one of the males)?

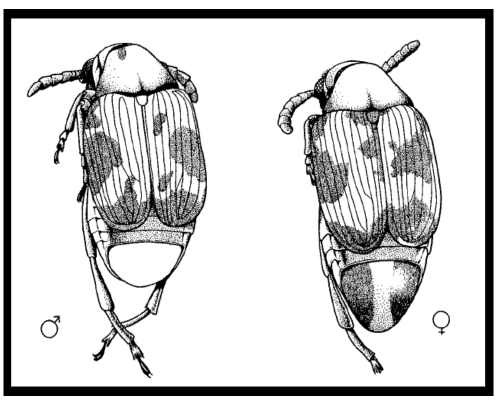


Figure 1: Dorsal view of *Callosobruchus maculatus* **(**From Brown and Downhower, 1988).

Based on and modified from Merry Clark and Lisa Blumke , 2012. Discrimination in Male Bean Beetle Mounting Behavior. https://www.beanbeetles.org/protocols/mounting/

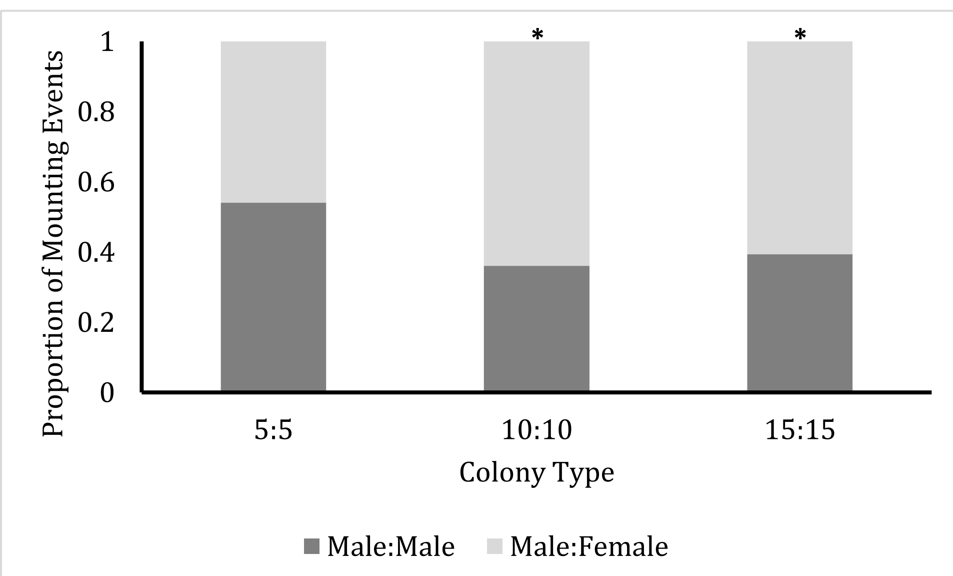
Experimental Design

Students observed plates (60 mm) of bean beetle colonies, containing ratios of 5:5, 10:10, or 15:15 male to female beetles. Each colony consisted of randomly chosen males and females from a larger stock culture of beetles. A marked (white, non-toxic acrylic paint) male was tracked by multiple students within a single group. The number of times that the marked male was seen mounting any other beetle was recorded in a tally format and the mounted beetle's sex was noted. After 10 minutes, the same plates were redistributed to different groups of students and another tracking period began. This method was continued for the duration of 1 hour. After all the data were collected (Table 1), binomial tests were used to compare the frequency of male-male versus male-female mounts to see if they differed significantly from an equal proportion of mounting events. An equal proportion would indicate random mating within that colony.

Results

The following results were observed after the experimental time ended. The ratio of male to female beetles within a colony had a marked effect on the randomness of mounting behavior observed when the data were pooled across trials (Table 1). Colonies of 5:5 males:females exhibited random mounting behavior. In contrast, colonies with larger number of beetles showed a bias toward male-female mounting behavior.

Beetle Male:Female Ratio	Mounting Behavior	Observations	Total	Binomial p-values
5:5	Male:Male	20	37	p < 0.74
5:5	Male:Female	17		
10:10	Male:Male	22	61	**p < 0.02**
10:10	Male:Female	39		
15:15	Male:Male	39	99	**p < 0.02**
15:15	Male:Female	60		



Based on and modified from Merry Clark and Lisa Blumke , 2012. Discrimination in Male Bean Beetle Mounting Behavior. https://www.beanbeetles.org/protocols/mounting/

Experimental Design Review Activity

As a final learning activity, in your normal lab groups, read the attached experimental write-up of a second bean beetle experiment conducted at a different university. Work together to determine how each concept you have learned today was applied. Then, provide the requested information in the table below. Once you have completed the table, inform your instructor. You will be provided with permission to begin designing your own experiment using bean beetles based on what you learned reading the Bean Beetle Handbook and the information provided on the handout. You will use the remaining lab time to design your experiments based on a question you wish to answer regarding the beetles. In next week's lab, you will briefly present your question and experimental design to the lab for comments and suggestions. After any changes have been made and with the permission of the instructor, you will then set-up your experiments.

Concept	Identify where and how the concepts have been applied in the attached bean beetle experimental write-up.
Observation	
Inductive Reasoning	
Deductive Reasoning	
Biological Hypotheses	
Population	
Sample	
Independent Sampling	
Paired Sampling	
Randomization	
Replication	
Variation	
Bias	
Statistics	
Null Hypothesis	
Alternate Hypothesis	
Hypothesis Testing	
Control Group	
Experimental Group	
Independent variable	
Dependent variable	

Substrate Size Selection by Bean Beetles

Objective

Perform an experiment to evaluate whether female bean beetles (*Callosobruchus maculatus*) discriminate between different size beans of the same species.

Introduction

Bean beetles (cowpea seed beetles), *Callosobruchus maculatus*, are agricultural pest insects of Africa and Asia. Females lay their eggs on the surface of beans (Family Fabaceae). Eggs are deposited (oviposition) singly and several days after oviposition, a beetle larva (maggot) burrows into the bean. At 30°C, pupation and emergence of an adult beetle occurs 25-30 days after an egg was deposited. Adults are mature 24 - 36 hours after emergence and they do not need to feed. Adults may live for 1-2 weeks during which time mating and oviposition occurs. Since larvae cannot move from the bean on which an egg was deposited, the oviposition choice of a female determines the future food resources available to their offspring (Brown and Downhower 1988). As a result, it is the most critical choice a female makes for her offspring, because it will influence their growth, survival, and future reproduction (Mitchell 1975; Wasserman and Futuyma 1981). Although females can be induced to lay eggs (oviposit) on a wide range of bean species, very few bean species result in normal development and the successful emergence of adults. Some bean species are very clearly toxic to *Callosobruchus maculatus* larvae (Janzen 1977). To test the hypothesis that females can discriminate beans of the same species based on size; the following experiment was conducted by several groups of students in a midsized university biology laboratory.

Experimental Design

A female and a male bean beetle from maintained cultures stored in a lab were introduced to each of 76 petri dishes (60 mm size) containing 10 small and 10 large mung beans. Each pair of beetles were randomly assigned to either the small or large been treatment groups and the female was permitted to lay eggs for 7 days. The difference in the quantity of the bean resource was documented at the start of the experiment by weighing the beans in each size category. The average per bean mass of 10 large beans was 89mg and that of the small beans was 37mg. After the 7-day ovipositing period, the beetles were removed from the dishes and the number of eggs laid on each of the 20 beans were counted. The data were subjected to a paired t-test to test for significant differences in the number of beans laid on each size class.

Results

The following results were observed after the experimental time ended. Female beetles were biased to larger beans over small ones. The data are summarized below and the results of the paired t-test are also given.

Based on and modified from Lawrence S. Blumer and Christopher W. Beck, 2011. Substrate Size Selection by Bean Beetles. https://www.beanbeetles.org/protocols/substrate-size/

Treatment	N	Mean	SD	SE
Small Beans	76	11.32	5.05	0.58
Large Beans	76	23.5	11.17	1.28

N = Sample Size
SD = Standard Deviation
SE = Standard Error

Paired Samples T-Test

			statistic	df	p	Mean difference
Small Beans	Large Beans	Student's t	-12.67	75	< .0001	-12.18

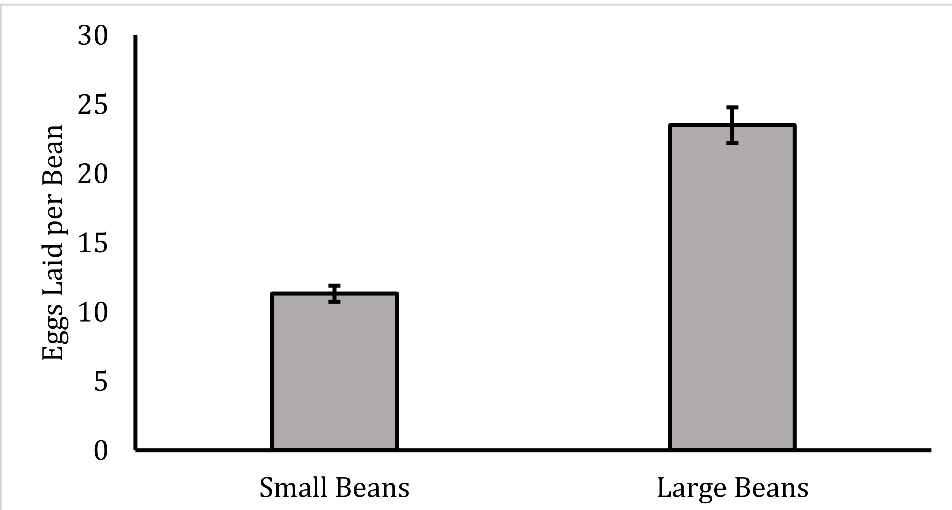


Figure 1. Female discrimination of bean size in long-exposure experiments. The mean +/- SE of the number of eggs laid by female bean beetles per replicate are shown for 76 replicates containing 10 large and 10 small mung beans. The difference in eggs laid on large versus small beans was highly significant (paired t-tests, $p<0.000001$).

Based on and modified from Lawrence S. Blumer and Christopher W. Beck, 2011. Substrate Size Selection by Bean Beetles. https://www.beanbeetles.org/protocols/substrate-size/

Using bean beetles to teach experimental design and experimental variables[1]

Objectives

1) To formulate a hypothesis and design an experiment to test your hypothesis

2) Communicate hypothesis and experimental design to your peers

Bean Beetle Experiment[2]

Goal: To formulate a hypothesis and design an experiment to test your hypothesis.

Today, each lab group will choose one of the observations listed below and develop a question to pursue further. Next week, you will perform an experiment designed by you to test the hypothesis to the question that you have developed. Prior to beginning the experiment, each group will give a 5 minute presentation on their question, hypothesis and the experiment that was designed by the group. This will be followed by a brief period of peer review and discussion in order to make suggestions or changes to your protocol.

OBSERVATIONS ABOUT BEAN BEETLES

Males are driven to find females and mate with them. Typically, males find females and begin mating in 15 minutes in small containers. Male beetles have been observed attempting to mate with other male beetles.

It is claimed that adult bean beetles do not need to eat or drink.

Populations cannot persist on kidney beans or black beans because of the high, lethal amounts of Phytohaemagglutinin.

Females prefer to lay eggs on their natal bean (the bean from which they emerge).

Females prefer to lay eggs on larger beans within the same species.

Supplies Available
Non-virgin Male and Female Beetles
Mung Beans (natal bean for some), Others Bean Types (Kidney Beans, Black-eye peas, Chick-peas, Black Beans) Petri Dishes, Scissors, Microscopes, Electronic Balances
Beetle "storage" containers (sweater boxes) include: (a) dark area (b) light area

[1] Activity is based on Lab Activity from Allison D'Costa and Mark Schlueter, 2014. J.W.Felts edited and added certain observations and materials to be used in General Biology as well as to help transition to CURE based project.
[2] See beanbeetles.org for handbook and other background information (L.S. Blumer and C.W. Beck, 2005-2018).

EXPERIMENTAL DESIGN

You need to answer each of these questions in your experiment design presentation. Each group will give a five minute presentation on their question and the experiment they will perform to answer that question. After your group presents, others in your lab, including the instructor will ask questions and comment on your proposal. If any experimental design issue is identified, your group will need to address it BEFORE you set-up your experiments.

1. State your question (or reword the question).

2. State your purpose.

3. State your hypothesis.

4. List your variables.

   a. What is your independent variable(s)?

   b. What is your dependent variable(s)?

   c. What is your controlled variable(s)?

5. Design your experiment.

   a. What materials and/or organisms will you need? How many? How many replicates?

   b. Write out a step-by-step procedure.

   c. Does your experimental design include the concepts from our Jigsaw activity? Do you have a control? How do you plan to control for any biases or confounding factors?

   * Consult your "Available Supply List"

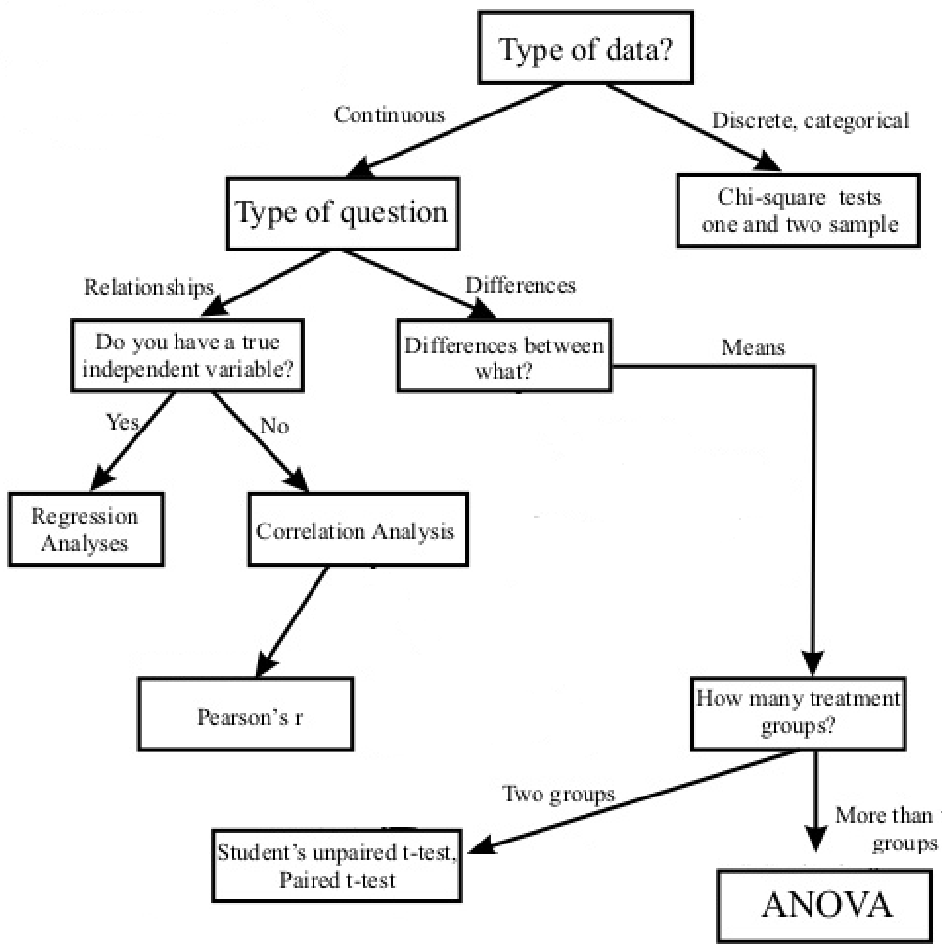
   * Remember to include a control group

   * Remember to include replicates in your experimental design

   * Statistics will be useful (e.g. t-test, Correlation)

Use the flow chart below to help determine the type of analysis you will need to conduct on the data you collect. Focus on the dependent variable (what you plan to measure) as you move through the chart.

Flow Chart for Selecting Commonly Used Statistical Tests



- Continuous data—measurements can take on any value along a continuous range. Examples: weight, mass, height, temperature
- Categorical data—values are categories that can be used to divide the dataset into groups. Examples: species, phenotypes, behaviors