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Practical Note

Exploring College Biology Students' Understanding of Experimental Design

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INTRODUCTION

Experimentation is one of the key features of scientific methods and science teaching. Designing of experiments forms a crucial part in this process. As the design of an experiment reflects the objective and the research question that is being worked on, to design the experiment well is to yield data that either supports or rejects a hypothesis. It is essential for students to develop this ability as well as to understand various parameters, such as control and treatment and to assign variables which involve in their experimentation.

The present study is situated in a lab-based program called Collaboratively Understanding Biology Education (CUBE) that engages students in hands-on experimentation using simple model organisms (Arunan, 2011, 2013: *see Websites*; Arunan and Nagarjuna, 2013; Kharatmal, 2021). Model organisms, such as *Escherichia coli*, *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Caenorhabditis elegans* and *Danio rerio*, have been used in the cutting-edge research in biology (Fields and Johnston, 2005), and even in laboratory exercises at school and college levels (Nagasawa and Katayama, 2021). Model organisms are well suited for biology learning because they can be easily maintained and bred in laboratory settings, have a shorter life span, can produce a large number of offspring, and have a sequenced genome in addition to being well-known among students (Jacobs-McDaniels *et al*., 2013).

The objective of the present study is to find out in what ways students formulate experimental designs while working on their experiments with two well-known model organisms, fruit fly (VijayRaghavan and Rodrigues, 2008) and snail (Balakrishnan, 2001). The fruit fly, *Drosophila melanogaster*, is probably the most popular model organism for studying genetics (Ray and Lakhotia, 2014). Though the sea snail, *Aplysia californica*, has been used as a model organism in neuroscience research studies, for the present study, we worked with *Achatina fulica*, a terrestrial snail, instead of *A. californica*, due to ease of availability in the local surroundings.

MATERIALS AND METHODS

Subject and schedule of the study

The sample for the current study was eight students involved in two groups of four each for two experiments. They had already been in the

CUBE lab's network and attended part of the discussion on experiments related to model organisms. They were three postgraduates, three second-year undergraduates, and two first-year undergraduate, with an age range from 19 to 23 year-old; five females and three males. Each group had a mix of all genders with a combination of first-year, second-year undergraduates and postgraduates. The postgraduates (microbiology or zoology major) had a research methodology course in their syllabus, while the second-year undergraduates had one paper on research methodology. One staff or a teacher, who was not research member, was assigned to a mentor for each group to guide the students in designing the experiment, doing hands-on activities, *etc*.

On each day of the workshop, the students worked on their experiments for about four hours in the morning and the following four hours in the afternoon were allotted to a discussion session with all the students. During their experimentation, students also worked on trapping, culturing and feeding their model organisms. The discussion session focused on inquiry- and conversation-based approach wherein all the peers took part in questions based on the objectives, hypothesis, and experimental design. As part of their exercise, we asked students to articulate their experiments on the following items:

- *Draw and label your experimental design setup.*
- *Write in detail about the following in the context of your experimental design: Control, Treatment, Dependent Variable, and Independent Variable.*

In order to provide their responses to the items, students required progressing in all along their experiment. As we were interested in understanding how students thought about their experimentation process, we sought students written responses after 10 days of their working in lab. Given the time constraint during the workshop, students were not able to practically complete the experiment to meet the objectives of their study. However, given their interaction period during the workshop, we could study about their understanding about experimental design. All these students provided their individual responses using the paper and pencil task over a period of one and a half hour.

Fruit fly experiment

In the fruit fly group, the students' assigned task was to find out an answer to "*Is there difference in olfactory receptor present in lab-bred and native flies?"*

In this experiment, the olfactory receptor is being tested using a chemical Isoamyl Acetate (IAA) of a specific concentration, 1000-fold dilution with distilled water (DW), on two strains – lab-bred and native fruit flies, *D. melanogaster*. The native flies were collected from the wild. The lab-bred *D. melanogaster*, Canton Special Benzer (CsBz) wild type stock, which was originally procured from Canton's lab, was available to the CUBE lab from Tata Institute of Fundamental Research.

In the particular design of this experiment, the most important parameter that students need to identify first and foremost is the state of larva being in the second stage, or the second-instar larva. Extensive preparedness is ensured by the students where they need to trap the fruit fly first from the local surrounding and culture it by using a medium. Once the fruit flies mate and produce larvae, the students need to identify various stages of the larvae and procure those which are in the stage of second-instar.

The second most important parameter in the design is about the setup of the Petri plates (diameter 9 cm) on which the larvae are placed and DW (control) and IAA (treatment) will be introduced to observe the movement of larvae. This experimental design is for measuring the olfactory response index (ORI), adapted from Khurana and Siddiqi (2013), of both the strains of larvae. The experimental design requires a setup of four Petri plates, two per each strain, one for control plate (DW on both sides) and the other for treatment plate (DW and IAA on each side). Then approximately 30 second-instar larvae are placed in the center of each Petri plate. After 2 minutes, which side the larvae have moved towards, DW or IAA, is observed. On the control plate, the larvae are expected to move toward both sides at random. The ORI is calculated by using the following formula: **treatment – control / treatment + control**. This is repeated for both the strains.

Snail experiment

In the snail group, the students' assigned task was "*To study whether the snail has learnt the bitter taste of quinine associated with the good smell of cucumber.*"

The most important parameters are terrestrial snails, a sheet of paper on which 18 cm diameter circle is drawn, a glass plate placed on the paper, DW (control), and a cucumber extract/slice (treatment). For the control plate, DW is placed on both sides of the glass plate and a snail is placed in the center. For the treatment plate, DW is placed on one side and cucumber slice is placed on the other side of the glass plate, and a snail is placed in the center. As the conditioning part, the snail is fed with a cucumber slice laced with quinine extract in order that the snail associates the bitter taste with cucumber smell. This conditioning is done between the two trials. Thus, the experimental sequence is as follows: control – pre-conditioning trial – conditioning – post-conditioning trial. This experimental design to measure the ORI of snail was adapted from Lemaitre and Chase (1997). The ORI is calculated by the following formula: **treatment – control / treatment + control**. In the snail experiment, the ORI is measured for both the pre-conditioning and post-conditioning trials.

RESULTS

The independent and dependent variables are the two key variables in a science experiment. In the case of present two experiments, the independent variables are IAA in fruit fly experiment, cucumber/quinine in snail experiment, whereas the dependent variable is ORI in both experiments. A compilation of students' responses on listing the parameters of control, treatment, dependent variable, and independent variable from their experimental designs is shown in Table 1.

Most students (5/8) could think appropriately about control. This could be because, in all the experiments on model organisms, the control was DW. The rest, students F2, F3 and F4, seemed to be confused. Regarding the treatment, most students (6/8) could mention it appropriately. The other two students' (F2 and F4) responses indicate confusion. They mentioned both control and treatment incorrectly: in their drawings of the experimental design, DW and IAA were placed on the same Petri plates instead of two separate plates. Two students (S2 and S3) mentioned the dependent variable in the experiment on snail correctly, possibly without understanding it, because they mentioned the independent variable incorrectly. It was observed that all the students could not understand the independent variable and dependent variable. From the parameters mentioned in regard to the dependent variable, the students were aware that various factors can cause alterations in an experiment, but they did not understand that one factor has to be tested on one occasion.

Table 1 Students' listing parameters in their experimental design.
The parameters that students noted correctly are underlined. Each student is mentioned as a code with respect to the model organism group.

Student	Control	Treatment	Dependent Variable	Independent Variable
Fruit fly Experiment	**Larvae with DW**	**Larvae with IAA**	**ORI**	**Extract**
F1	<u>DW</u>	<u>IAA</u>	IAA, agar plate, sucrose solution, Ringers solution, larvae, banana peel, temperature, time	DW, number of flies
F2	Ringer's solution, acids	media and bottles autoclaved	time, temperature, light intensity	pH, temperature, humidity
F3	larvae's movement	<u>IAA</u>	temperature, duration of day and night, humidity, light intensity, type of waste	blank response
F4	media preparation	blank response	humidity, temperature, food	water
Snail Experiment	**Snail with DW**	**Snail with cucumber extract**	**ORI**	**Extract**
S1	<u>DW</u>	<u>extract</u>	using various different samples	using same snail
S2	<u>DW</u>	<u>extract</u>	<u>ORI</u>	sound, place, or location of experiment
S3	<u>DW</u>	<u>extract</u>	<u>ORI</u>	size of setup
S4	<u>DW</u>	<u>extract</u>	animal's orientation	odour of other food stuff, perfume, etc.

DISCUSSION

The explicit understanding of assigning variables in students' experiments seemed a challenging task. In the present study, the usage of the terms, independent variable and dependent variable, possibly got the students confused. The problem was that the students were not able to understand the relationship between independent variable, dependent variable, and control. In order to address this problem, the terms such as independent variable and dependent variable can be replaced with cause and effect in the context of each experiment. This may help students to understand the relation between variables.

There is a need for integrative approach in teaching and research in all areas of biology in our country (Lakhotia, 2002) through the initiatives of universities-institutes interactions (Arunan, 2011: *see Websites*). Most of the undergraduate colleges in our country provide theory-based courses on research methodology and have separate labs or practicals that have no connection of understanding of experimentation with the theory classes (Vale and Dell, 2009). Apart from this, problems due to absence of basic laboratory exercises form a major limitation for teaching science in universities/colleges. These problems can be resolved by designing and developing simple experiments that can stimulate analysis and thinking abilities of the students and also are feasible even in remotely located colleges (Lakhotia and Mukunda, 2008). The *National Curriculum Framework on Teaching of Science* recommends for science teachers to emphasize science process skills and scientific method (NCERT, 2006: *see Websites*). The teachers can play a critical role in collaboration with students (AAAS, 2011: *see Websites*; Arunan and Nagarjuna, 2013) in developing modules on the designing of experiments, assigning of variables, *etc*. that complement the research methodology course.

The experimentation not only provides students with a firsthand experience of doing real science, but also enables them to connect with the real world contexts (Nyutu *et al.*, 2019). The understanding of experimental process can be useful for students in developing critical thinking and evaluating the process of science. The practical skills of framing a research hypothesis about the questions, formulating a research design, and assigning parameters, such as control, variables, *etc.* can enable citizens to question pseudoscience and to value the process of science.

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