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Using Pill Bugs and Barriers to Explore the Scientific Method in the First Introductory Laboratory Session

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To examine the scientific method in their first laboratory session, students design their own experiments to assess the innate behaviors used by pill bugs to navigate around barriers. Students will have access to barriers of various shapes, sizes and consistencies and so will have essentially an unlimited number of hypotheses that they can test. We also added a short exercise looking at the effects of nematodes on fungi to compensate for former unsuccessful exercises examined on the effects of one protist species on another's population numbers.

Keywords: pill bugs, experimental design, locomotion and barriers, fungi capturing nematodes

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Introduction

There are several labs devoted to studying the scientific method in our first semester of introductory biology, which covers ecology, evolution and diversity. One of our scientific method activities involves different groups of students monitoring for two weeks the effect of one protist species on another. The objectives of this exercise is to introduce students to experimental design, sampling and data analyses with activities that treat species interactions, a topic they will explore in some detail later in this course.

Recently we have experienced problems with this activity. Students find it most difficult to set up experimental populations with equipment they have not used before, such as micropipettes. They have the same problems the following week, attempting with small samples of their population on a few slides, to draw conclusions as to the effects of one species on another. For the past few semesters, the protist cultures have simply failed and students have had to work with simulated data.

This summer we replaced these activities with exercises involving pill bugs and fungi responses to nematodes. The first allowed the students more control over experimental design with animals easy for them to manipulate and to culture in the laboratory. The latter allowed them to quantify with non-destructive sampling the effects of one species on another. Since nematodes and fungal fruiting bodies were much larger than the protists used previously, a larger sector of the population (petri dish culture) could be easily observed under the stereoscope.

Student activities: pill bugs

Students are given very little directions as to how to design their examination of pill bug behavior. They are simply told that they are to design an activity that compares pill bug locomotion in arenas that do and do not contain barriers to forward movement by pill bugs. Trays are provided containing sand, which students may moisten. To give students practice at collecting observational date students observe at least three pill bugs moving in open barrier free arenas. Then students observe the same animals in arenas with two large round barriers. They then discuss as a class their findings before every group designs their own experiments with the different types of barriers available to them. Groups may after the discussion use the same barriers as before, as long as they change the distance between barriers, or the number or type of barriers used.

Students find that observing animals is not the easy task they first assumed it would be. It is difficult for students to track every turn, turn direction, and distance covered per turn, as the animals move. Different groups use different techniques to do this. Often one individual of a group records turns, another the distance between turns, while a third student records the location or estimates the speed at which the animal is moving as they try to recreate the animal's path. Some students using only a thin layer of dry sand and the larger species of pill bug, were able to "trace" the

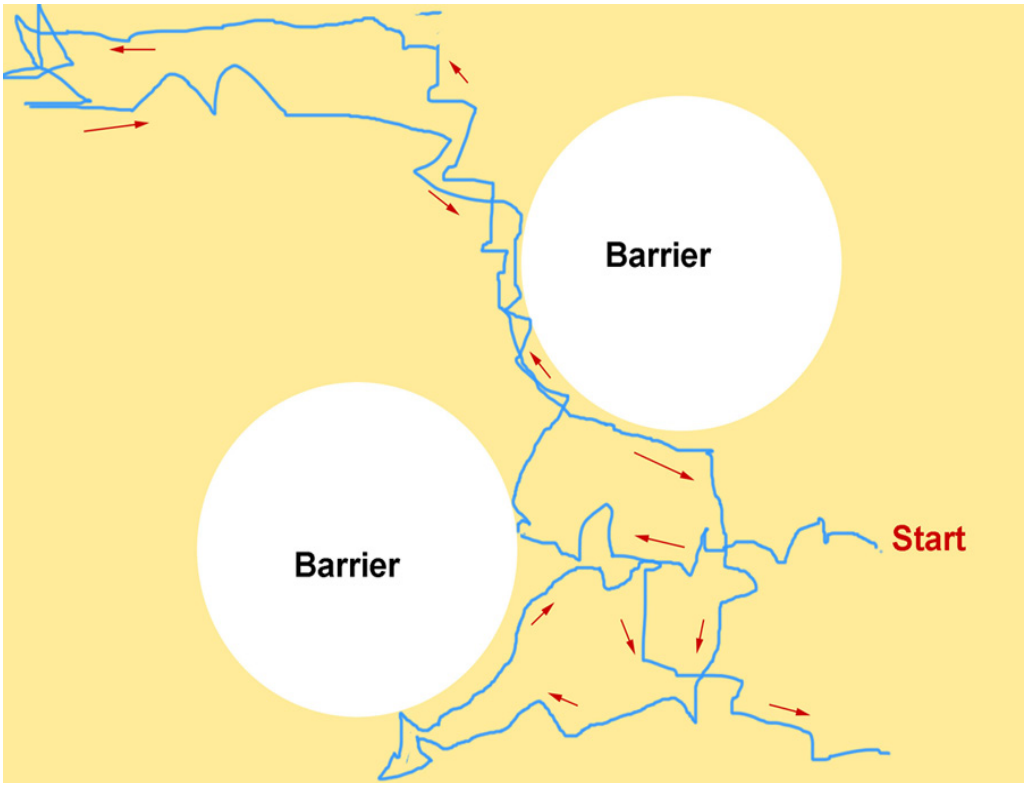

Figure 1. Path of one pill bug in an arena containing two barriers.

movements of an animal from the tracks left behind (Fig. 1). Others used the camera in their phones to record specimens moving, but then learned a bit about how camera angle, etc. could affect their estimates. Students are exposed to differences between species and individuals, and so the benefits of multiple trials.

Most groups collected enough information so that the amount of turning or relative speeds for animals in both types of arena could be compared via tables or bar graphs. We also finally had an activity where groups of students could share insight and yet copying of actual reports was discouraged as results varied so much among groups. In fact we found that the reports from different individuals of a group, since each student also could chose the focus of their report (five pages maximum), also varied Information on the scientific method and the differences between exploratory and experimental science is presented in lecture, and our laboratory manual has sections on graphing and writing reports.

Notes for the Instructor

Materials Needed

- Trays of sand
- Barriers of various shapes and sizes,
- Large plastic or wooden blocks.
- PC pipe cut into sections.
- Animal huts sold in pet shops with openings in them
- Pill bugs of two species, varying in size (*Porcello* sp. and *Armadillidium* sp.)

Trays must be large relative to the size of the barrier and pill bug. Animals and barriers must be placed in the center of the tray because animals will track the rims of the trays. We use the large metal dissecting trays used for fetal pig dissections.

Some students, since we supply pipe cleaners to encourage pill bugs to move, created tracks out of the pipe cleaners to see if the pill bugs would follow them. They do.

Less and dry sand leads to better "tracks", although most students choose to record movement using phones or simply pen and paper. This is probably because tracks do not accurately record the number of turns and this is one of the parameters that varies the most between differently staged arenas.

Barriers must be high enough and big enough to discourage animals from climbing up them. So big wooden or plastic blocks at least 8 cm in diameter work best. Round blocks work better than square blocks. We use several different diameters of PC pipe cut into 10 cm, high sections and the bigger plastic blocks from sets for toddlers. Some students got interesting results when they turned small animal huts initially entrance away, and then, entrance toward the pill bugs. Pill bugs so conditioned more often simply ignored the entrance on their second encounter with the hut, naïve pill bugs more often moved into the interior of the hut and sometimes attempted to burrow in the sand there.

This laboratory takes a well coached or seasoned laboratory instructor to keep students motivated and focused, and know when to have them group for discussion.

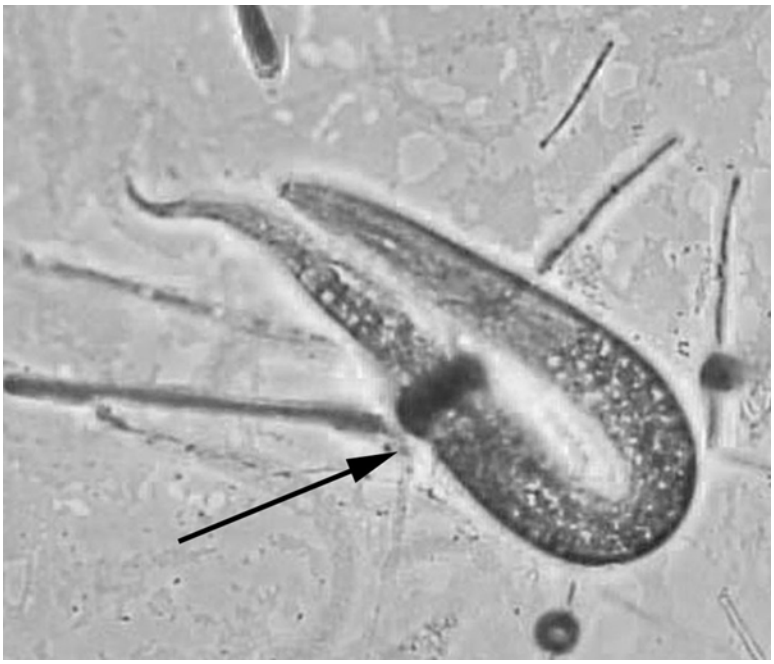

Figure 2. Trapped nematode

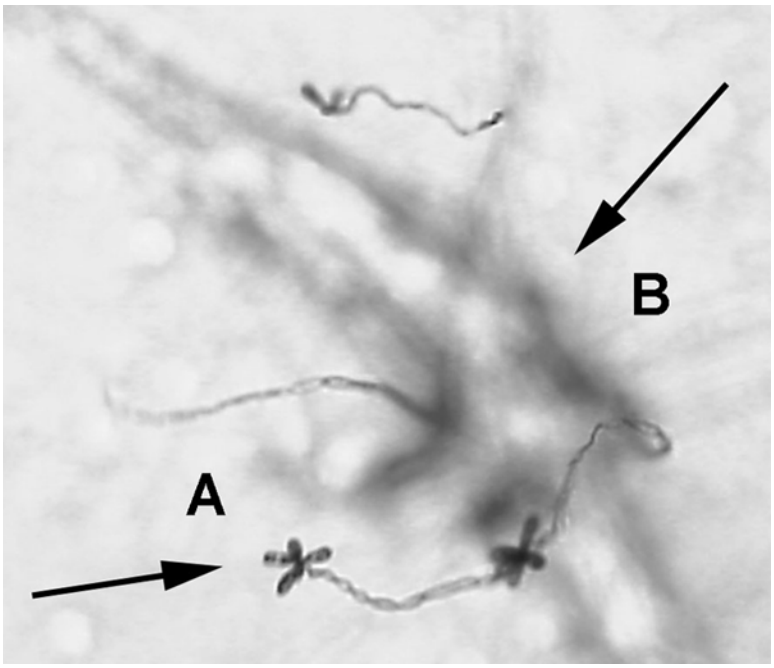

Figure 3. Fungal fruiting bodies (A) that developed over the digested remains (B) of a nematode.

Student activities: nematodes and fungi

This is an activity that uses a commercial kit available form Wards Natural Science or Carolina Biological that supplies media, Petri dishes, fungi and nematodes. We also use Petri dish bottoms with grids from Carolina, catalog number 741470.

We used this activity to introduce students to sterile technique as each group prepared its own fungal culture (*Arthrobotrys conoides*), transferring a small block of living to corn meal agar plates that they have poured themselves. A week later they add nematodes (*Rhabditis* sp.) to the cultures with sections inoculating plates with nematodes for next day's laboratory sections to score. Instructors start the process by inoculating some of the students' fungal cultures with nematodes one and two days before the first day that laboratories meet the second week of the experiment.

Students replace their round petri top with a top (actually the bottom of another type of dish) that has an embedded grid The grid divides the dish into quadrats that students can sample (Fig 2). Students record the number of trapped nematodes (so indirectly fungal traps), traps without nematodes (Fig. 2) , moving (so not trapped) nematodes, and fruiting bodies per quadrat (Fig. 3).

About half of the students examine plates one day after nematodes have been introduced, the other half of the students should examine plates two days after nematodes have been introduced. We encourage pairs to work together to sample 4-5 quadrats. Different quadrats will be sampled in different laboratory sections throughout that day, so at days end, we have at least three counts for 9-14 quadrats on five to six plates.

In discussions that follow the next time they meet, students develop an appreciation for descriptors such as average, mean and sampling error. Since the student cultures were started with a block placed in the center of new medium, students are also encouraged to think about how that protocol might affect their counts and why they were told to count one center (green) quadrat, three middle (blue) and five outside (yellow) quadrats on each plate (Fig. 4).

Materials Needed

- Kit is bundled with media, petri dishes, and transfer loops
- Live material can be ordered with the kit or sent later.
- Petri plates with grids are bundled ten per package, somewhat expensive, but these can be used form semester to semester.

We have run this experiment twice with quite varied results, due to the condition of the nematode cultures received. We have opted to obtain live materials with the rest of the kit (containing media, petri dishes and loop for spreading nematodes) both times, but in one case received nematode cultures in bad condition. By the time the fungi cultures were dense enough to inoculate with nematodes, most of our nematode cultures were dead. In the future, we will simply order the culture media, fungi and petri plates initially, all as separate items, and the nematode cultures a week later.

Student must be encouraged to keep careful records. We supply excel spreadsheets clearly labeled with date since nematode inoculation and type of count (such as fruiting bodies or trapped nematodes) to be recorded. Rows identify

quadrats by letter and number, with columns serving to identify different samples or counts of a variable for a group. This is an activity that almost yields too much information, given that the required report may be a student's first attempt at serious data analyses. Laboratory instructors will have to guide students with regard to meaningful graph construction.

Student Response

Students chose which lab to write up for their formal report. Seventy five percent chose to write up the pill bug activity. In the summer we have a number of non-life science majors taking the first semester of our course for science majors. The engineering and chemistry majors chose more often than life science majors to write their reports on the nematode fungal interactions, justifying their choice by citing this activity's clear cut objectives and analyses. The students who preferred the pill bug activity enjoyed designing their own experiments and working with behavior. The success of a student's efforts did not seem at all related to subject chosen (based on average grade).

More experienced laboratory instructors also preferred the pill bug activity. New instructors felt somewhat intimidated at leading the discussions necessary to keep students focused on objectives in the pill bug exercise. Some instructors also preferred the amount of information collected, and so the more clear cut analyses and graphs, discussions and conclusions, that students would submit as part of their formal write up if they chose the nematode exercise.

Acknowledgements

We would like to acknowledge the help of Christa Jones and Saad Haque, graduate students who pretested pill bug protocols. They helped us determine the types of barriers and arenas to use in this activity.

About the Authors

Marianne Niedzlek-Feaver received her Ph.D. from the University of Michigan. As an evolutionary ecologist, she is interested in identifying factors that shape the mating systems of grasshoppers and katydids. She currently teaches Evolution, Invertebrate Zoology and Introductory Biology

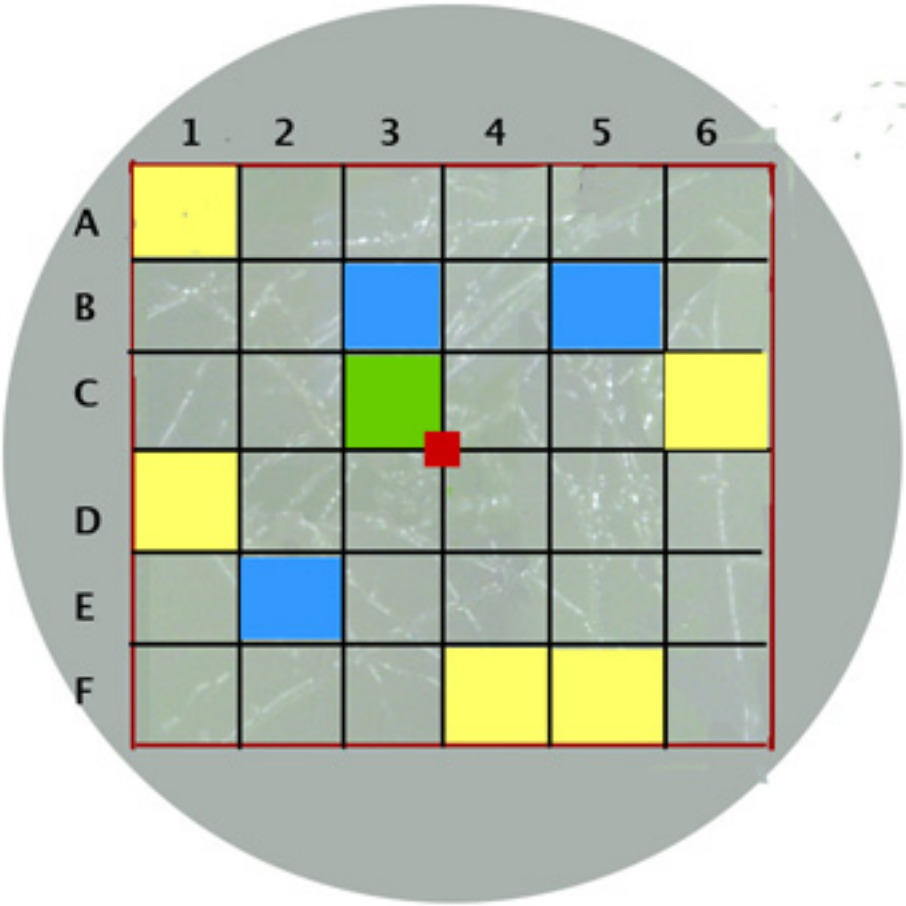

Figure 4. Sampling grid: Red square indicate inoculate of fungus. Other colors denote possible sampling quadrats in areas of various fungal densities.

courses. She has received various grants to improve the laboratory experience, and is a member of the NCSU Academy of Outstanding Teachers.

Betty L Black received her Ph.D. from Washington University (St. Louis). She teaches a course in Developmental Anatomy plus distance education courses in Introductory Biology, Histology, and Animal Diversity. She has received two University awards for "Innovative Excellence in Teaching and Learning with Technology" and has 12 publications on teaching technology topics.

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