ONLINE INQUIRY & INVESTIGATION

Measuring Animal Movements in a Natural Ecosystem:

A Mark-Recapture Investigation Using Stream-Dwelling Snails



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ven relatively sedentary animals exhibit some mobility, enabling them to obtain essential resources, avoid predators, and reproduce (Krebs, 2001). By following movements of individuals within an animal population, we improve our understanding of factors essential to the survival of that species, including food and habitat requirements (Zollner & Lima, 2005). From a conservation perspective, this knowledge is needed to predict effects of habitat destruction or restoration on this species and organisms it interacts with through predator-prey or symbiotic relationships (Wiegand et al., 2005).

Here I present an investigative exercise that allows students to observe, measure, and describe animal movements in a natural ecosystem. Stream-dwelling snails are collected, marked with nail polish, and released. During a subsequent visit to the study site, students find marked snails and record distances and directions they moved. Simple statistical techniques are used to quantify movements in the snail population, and answer specific research questions. Finally, students use knowledge of ecological and evolutionary concepts and snail biology obtained from lectures and readings to propose explanations for movement patterns they observe.

The investigation is intended to enhance skills in research methods and supplement several major lecture topics, including animal behavior, evolution, and population ecology. I use this exercise in a college-level introductory ecology course, but it could be easily applied in introductory biology courses at high school and college levels. Alternatively, The Regents of the University of California (2005) developed their own procedures for a mark-recapture study of aquatic snails. Their aquatic animal behavior exercise, titled "Water Snails," is one of several Outdoor Biology Instructional Strategies (OBIS) available for purchase that specifically target elementary, junior high, and high school students and teachers.

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Preparing for the Study

Materials

• Nail polish

I have used two different techniques to identify individual snails; both involve marking the dorsal region of the shell. Bee tags (available from The Bee Works; http://www.beeworks.com) are numbered plastic tags that occur in many colors (Figure 1). Once attached with Krazy Glue®, a tag can remain on a shell for months or years. Bee tags are somewhat expensive, however. For large introductory-level classes, I recommend the simpler, low-cost method of marking snails with nail polish (Figure 2). Marks made with nail polish are not permanent, but sufficient numbers of snails retain marks for the time needed to complete this investigation. Additionally, the rich variety of available nail polish colors makes it possible to apply distinct marks to snails released from different locations in the stream. Finally, marks made with nail polish appear to have no effect

Figure 1. A snail (*Leptoxis carinata*) with bee tag attached to the dorsal region of its shell.



on snail behavior (Burris et al., 1990). In this exercise, each research team of three to four students will need its own unique color of nail polish.

Painted rocks

Each research team will need a small rock to identify the location where snails were originally captured and released. Stones usually suffice, but cobbles are required in streams with strong currents that move small rocks. Paint each rock with fluorescent spray paint, then use a permanent marking pen to write a different team name (i.e., the team's nail polish color) on each rock.

• Containers for holding snails

Containers are needed to collect and hold snails. Plastic dishpans are ideal because they are shallow enough to enable handling of snails, yet deep enough to prevent them from crawling out.

• Paper towels

Towels are used to dry snail shells before applying nail polish.

• Measuring tape (30 m)

This is needed to measure distances traveled by snails.

• Clipboard, pencils, and several copies of Table 1

Use a pencil to record data so they will not be lost to smudging if Table 1 gets wet.

• Calculators

Calculators are helpful for obtaining descriptive statistics and conducting statistical tests, especially if large numbers of snails are recaptured.

Time & Weather Conditions Required for the Investigation

This exercise requires two field sessions separated by one to seven days. Students collect, mark, and release snails during the first field session. Dry weather is required for the first session, because only dry shells can be marked. Students recapture marked snails during the second field session, and directions and distances traveled by individuals are recorded. Time required for each class session ranges from one to two hours, depending on class size and snail abundance at the study site. An additional one to two hours is needed in the classroom to analyze data and discuss results.

Study Site & Time of Year

Before the first field session, the instructor must find a suitable section of stream. The study site should contain large numbers of snails, and water that is shallow and clear so that snails on the streambed are easily seen. The activity works best from late spring through mid-fall, when snails are abundant, active, and easily seen. Marked snails are difficult to relocate when leaf litter is abundant in the stream, so late fall is generally a poor time of year for this exercise.

What Snails Should You Mark?

The objective of this exercise is to describe movements in a single population or species of snail. Therefore, students need to distinguish the focal species they will mark from other snail species that will be rejected. This is usually a simple task because most co-occurring snail species exhibit obvious differences in shell architecture. Snails with similar looking shells generally belong to the same species. Identification guides (e.g., Brown, 2001) can help interested students and teachers assign correct names to different kinds of snails, but such taxonomic detail is unnecessary in this investigation.

Figure 2. Snails marked with nail polish. Marks should be applied to the dorsal region of the shell, and be large enough to be seen by students standing over the streambed.



I use two criteria to decide which species of snail to mark. First, large numbers of individuals should be available. Secondly, adults should be large enough that highly visible marks can be made on shells, and snails are easily seen on the streambed.

Conducting the Investigation

Procedures: Field Session One (Marking Snails)

- 1. To ensure involvement of all students, the class should divide into research teams of three to four students. One student not affiliated with any team will also be needed for recording class data. Each team will need a container of nail polish in a color that is distinct from other teams, and the painted rock bearing the name of the nail polish color. Each team will also require paper towels and two containers for holding snails. The data recorder will need a clipboard, a pencil, and several copies of Table 1.
- 2. Each team selects a location in the stream where snails occur, and places their rock on the streambed to mark this location. Team members proceed to pick up snails located near the rock. Collecting continues until at least 50 snails of the correct species and a size suitable for marking are collected by all teams combined. I find that by marking 50-100 snails, and recapturing 20-50% of these snails one week later, my class collects sufficient data to describe movements in the population.
- 3. Each team marks captured snails. A large mark is applied to the dorsum of each shell so that it is easily seen by a person looking down at the streambed (Figure 3). Snails are prepared for marking by drying the dorsal region of each shell with a paper towel, then placing snails in a pan lined with dry paper towels. The latter practice facilitates shell drying by pulling residual water away from snails. Nail polish is applied to each shell with a brush, then snails are returned to a dry, towel-

Table 1. Movement data for stream-dwelling snails.

Study site location (e.g., stream name, township, county, and state):
Date snails were marked:
Color of marks (with number of individuals that were marked):
1() 2() 3() 4()
Recapture date: Number of recaptured snails (n):
Total linear distances traveled (sum of values in column 3; ΣX_i): m

	1. Color of mark on recaptured snail	2. Direction snail moved (downstream or upstream)	3. Linear distance snail moved (m)
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Table 1. continued

	1. Color of mark on recaptured snail	2. Direction snail moved (downstream or upstream)	3. Linear distance snail moved (m)
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lined holding container until marks are dry to the touch (Figure 4). Due to water retention within shells, I find that few if any snails die during the approximately 10-15 minutes needed for nail polish to dry. Last, the data recorder enters the total number of snails marked by each team in Table 1.

4. Each team releases snails at the original capture location by carefully placing them on the streambed near their painted rock (Figure 5).

Procedures: Field Session Two (Recapturing Snails)

1. Return to the study site one to seven days after marking and releasing snails, and thoroughly search the area for marked individuals. Working as one group, the class determines the most extreme downstream and upstream points to which any snail traveled. To accomplish this, students begin far downstream of release locations, and walk slowly in an upstream direction while carefully scanning the entire streambed for marked

Figure 3. A marked snail (center of photograph) in clear, shallow water. Snails are easy to relocate in this type of habitat, making it favorable for markrecapture investigations.



Figure 4. A student marking a snail with nail polish.



snails. If a marked snail is found shortly after beginning this upstream walk, additional individuals likely occur farther downstream. In this case, the class should reverse directions and walk in a downstream direction. Students continue walking downstream until an extensive section of stream has been traveled without anyone finding a marked snail. Once the class is confident no snails occur farther downstream, students turn around again and proceed upstream. The search ends upstream of locations where marked snails were released, after a considerable distance has been traveled without a marked snail being sighted, and it appears that no more marked snails are likely to be found.

2. When a marked snail is encountered, a student will identify its original release location by matching the color of its mark and the painted stone bearing the color name. Based on relative positions of the snail and its release location, the student determines if the snail moved upstream or downstream. Two students then stretch a measuring tape between the snail and its original release location, so that linear distance traveled is measured (Figure 6). A student data recorder will enter the color of this snail's mark, and both direction and linear distance traveled in Table 1. This procedure is repeated for each recovered snail. The total number of recaptured snails is entered at the top of Table 1. Total distance traveled by all snails combined is also summed and entered at the top of Table 1; it might be practical to perform this calculation in the laboratory if large numbers of snails are recaptured. See Tables 2-3 for completed copies of Table 1 that contain real movement data for Leptoxis carinata, a common stream-dwelling snail of the eastern United States. These data were collected by students in general ecology classes I taught at Longwood University, located in Farmville, Virginia. Data contained in Table 2 were collected from a section of streambed dominated by sand and other small particles. Data in Table 3 were obtained from a streambed dominated by large rocks.

Using Statistical Analysis To Answer Research Questions

Simple statistical techniques are used to describe movements in the snail population. Some questions my classes address are provided below, along with examples of calculations used to answer each question.

Distance

1. What was the average (i.e., mean) distance that a snail traveled? The sample mean, or average, is perhaps the most commonly used statistic to estimate a population characteristic (Zar, 1999). The mean linear distance traveled by snails can be calculated using the formula and examples in Table 4.

Figure 5. Marked snails being placed on the streambed at the original release location. A painted rock (near center of photograph) is used to reference the release location.



Figure 6. Students measuring the linear distance traveled by a marked snail from its original release location.



2. What was the range of distances traveled by snails? Although the mean is a useful descriptor of a population characteristic, it is also important to recognize the degree to which individual measurements deviate from this average. The range is a simple measure of variability that accounts for differences in linear distances traveled by individual snails. Examples of how to calculate the range are also provided in Table 4.

Direction

1. Did snails move randomly, or did they tend to move in one direction? I use a Chi-square test to determine if numbers of snails moving downstream and upstream differed significantly from what would be expected if

Table 2. Movement data for stream-dwelling snails (habitat = sandy streambed).

Study site location (e.g., stream name, township, county, and state): Fishpond Creek, Appomattox County, Virginia Date snails were marked: September 23, 2002

Color of marks (with number of individuals that were marked):

1. White (25) 2. Red (25) 3. Orange (25) 4. Yellow (25) Recapture date: September 30, 2002 Number of recaptured snails (n): 50 Total linear distances traveled (sum of values in column 3; ΣX_i): 93.30 m

	1. Color of mark on recaptured snail	2. Direction snail moved (downstream or upstream)	3. Linear distance snail moved (m)
1	White	Upstream	0.60
2	White	Upstream	1.13
3	White	Upstream	1.51
4	White	Upstream	1.09
5	White	Upstream	1.54
6	White	Upstream	2.32
7	White	Upstream	2.10
8	White	Upstream	1.27
9	Red	Upstream	0.37
10	Red	Downstream	0.25
11	Red	Upstream	0.72
12	Red	Upstream	2.00
13	Red	Upstream	2.41
14	Red	Upstream	1.26
15	Red	Upstream	1.81
16	Red	Upstream	3.02
17	Red	Upstream	1.91
18	Red	Upstream	2.94
19	Red	Upstream	2.73
20	Red	Upstream	0.27
21	Red	Upstream	0.72
22	Red	Upstream	0.65
23	Red	Upstream	1.17
24	Orange	Upstream	2.93
25	Orange	Upstream	2.95
26	Orange	Upstream	2.71
27	Orange	Upstream	2.58
28	Orange	Upstream	2.86
29	Orange	Upstream	2.74
30	Orange	Upstream	2.97
31	Orange	Upstream	4.58
32	Orange	Upstream	4.37
33	Orange	Upstream	5.10

Table 2. continued

\square	1. Color of mark on recaptured snail	2. Direction snail moved (downstream or upstream)	3. Linear distance snail moved (m)
34	Orange	Upstream	5.88
35	Yellow	Downstream	0.95
36	Yellow	Downstream	0.72
37	Yellow	Downstream	0.52
38	Yellow	Downstream	0.62
39	Yellow	Downstream	0.25
40	Yellow	Downstream	0.27
41	Yellow	Upstream	1.06
42	Yellow	Upstream	0.80
43	Yellow	Upstream	
44	Yellow	Upstream	1.29
45	Yellow	Upstream	1.58
46	Yellow	Upstream	1.59
47	Yellow	Upstream	2.05
48	Yellow	Upstream	2.32
49	Yellow	Upstream	2.47
50	Yellow	Upstream	2.55

movements were random (Zar, 1999). See Table 5 for examples of how to conduct this test using data in Tables 2-3.

Interpreting & Discussing Results

Results can indicate variability in directions and distances traveled by individual snails, yet also reveal movement patterns in the population. For example, if the streambed consists of distinct habitat types (e.g., both sandy and rocky areas), relationships between snail movement and habitat occupied can be observed and quantified. In one investigation at my study site, students found that snails inhabiting a streambed dominated by sand generally moved upstream at a relatively rapid rate (see Table 2 and mean distance moved and Chi-square test results in Tables 4-5). In another study conducted at the same site, snails in rocky habitats moved very little and in random directions (see Table 3 and mean distance moved and Chi-square test results in Tables 4-5).

From knowledge of snail biology and ecological and evolutionary concepts obtained from lectures and reading materials, students can propose explanations for these and other findings. Most species of snails are adapted for grazing on microorganisms that inhabit surfaces of rocks and other solid substrates (Brown, 2001). Additionally, crevices between adjacent rocks provide shelter from predators and strong currents (Stewart & Garcia, 2002; Turner & Montgomery, 2003). Natural selection should therefore favor a snail that crawls rapidly across a sandy streambed after encountering this low-quality habitat. However, a snail in a resource-rich rocky habitat should attempt to remain there, and will therefore show little movement (Chapman, 2000). Patterns of upstream movement in snails have been documented often (Huryn & Denny, 1997). Upstream movement may occur for both mechanical and adaptive reasons. Drag imposed by currents can prevent a snail from crawling downstream by causing its shell to rotate until the anterior end faces upstream (Huryn & Denny, 1997). Structural shell characteristics that facilitate upstream movement might result from natural selection. Snails can be displaced and transported downstream by high-energy events, including floods (Huryn & Denny, 1997). Snails crawling upstream to compensate for this drift have an increased likelihood of remaining in or returning to favorable habitat.

Concluding Remarks

A value of this exercise is that it provides a large, time-limited class an opportunity to study animals in a natural ecosystem, and still collect enough data to definitively answer research questions. This inquiry-based investigation also provides real-life illustrations of a variety of ecological and evolutionary concepts that are addressed in many biology courses. A summary of how this activity aids in implementing science teaching components of the *National Science Education Standards* (National Academy of Sciences, 1995) is provided in Table 6.

Snails are ideal models for animal movement studies; they respond actively and consistently to variation in resource availability and predation risk, yet move slowly enough that movements can be observed and patterns quantified (Huryn & Denny, 1997; Turner & Montgomery, 2003). Additionally, students really seem to enjoy working with these organisms. Many students initially find the idea of tracking snails amusing, yet get excited when recovering a snail they or a classmate previously marked.

Table 3. Movement data for stream-dwelling snails (habitat = rocky streambed).

Study site location (e.g., stream name, township, county, and state): Fishpond Creek, Appomattox County, Virginia

Date snails were marked: September 11, 2000

Color of marks (with number of individuals that were marked):

1. White (25) 2. Red (25) 3. Orange (25) 4. Yellow (25)

Recapture date: September 18, 2000 Number of recaptured snails (n): 38

Total linear distances traveled (sum of values in column 3; ΣX_i): 21.55 m

	1. Color of mark on recaptured snail	2. Direction snail moved (downstream or upstream)	3. Linear distance snail moved (m)	
1	White	Upstream	0.09	
2	White	Upstream	0.40	
3	White	Downstream	0.25	
4	White	Upstream	0.57	
5	White	Downstream	0.33	
6	White	Downstream	0.15	
7	White	Upstream	0.15	
8	White	Upstream	0.19	
9	White	Upstream	0.19	
10	Red	Downstream	0.08	
11	Red	Downstream	0.70	
12	Red	Upstream	0.19	
13	Red	Downstream	0.85	
14	Red	Upstream	0.24	
15	Orange	Downstream	1.10	
16	Orange	Downstream	1.30	
17	Orange	Downstream	0.48	
18	Orange	Downstream	0.42	
19	Orange	Downstream	0.70	
20	Orange	Downstream	0.01	
21	Orange	Upstream	1.25	
22	Orange	Upstream	0.70	
23	Orange	Upstream	1.25	
24	Orange	Upstream	0.70	
25	Orange	Upstream	0.56	
26	Orange	Upstream	0.97	
27	Yellow	Downstream	0.64	
28	Yellow	Downstream	0.95	
29	Yellow	Downstream	0.40	
30	Yellow	Upstream	0.48	
31	Yellow	Downstream	0.12	
32	Yellow	Upstream	0.02	
33	Yellow	Upstream	1.25	

Table 3. continued

\bigcap	1. Color of mark on recaptured snail	2. Direction snail moved (downstream or upstream)	3. Linear distance snail moved (m)
34	Yellow	Upstream	0.80
35	Yellow	Upstream	0.88
36	Yellow	Upstream	0.90
37	Yellow	Upstream	0.57
38	Yellow	Upstream	0.72
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Table 4. Calculating the sample mean and range (formulas and descriptions modified from Zar, 1999; examples based on data in Tables 2-3).

 $Mean = \Sigma X_i \div n,$

where in this investigation

Mean = the average linear distance (m) traveled by a recaptured snail, and

 ΣX_i = the sum total of distances traveled by recaptured snails, and

n = the total number of recaptured snails.

Range = the difference between the largest and smallest value in the data set, where in this investigation Range = the difference between the longest and shortest distance traveled by an individual snail.

Examples:

Based on data from Table 2 (sandy streambed)

 $Mean = 93.30 \div 50 = 1.87 \text{ m}$ Range = 5.88 - 0.25 = 5.63 m

Based on data from Table 3 (rocky streambed)

Mean = 21.55 ÷ 38 = 0.57 m Range = 1.30 - 0.01 = 1.29 m

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Table 5. The Chi-square goodness of fit test (formula and description modified from Zar, 1999; examples based on data in Tables 2-3).

The Chi-square test is used to objectively determine if the distribution of data among distinct categories is nonrandom. For example, each recaptured snail in this study is found downstream or upstream of its original release location. If snails moved randomly (i.e., purely by chance), we would expect approximately 50% of snails to occur downstream, and 50% to occur upstream. The Chi-square test reveals whether numbers of snails moving downstream and upstream differs significantly from this 1:1 ratio.

Formula and example:

The formula for calculating the Chi-square test statistic (χ^2) is $\chi^2 = \Sigma$ (observed frequency – expected frequency)² ÷ (expected frequency)

Expected frequencies are determined from the total number of observations (e.g., number of recaptured snails) and the null hypothesis. Our null hypothesis (hypothesis of no difference) is that equal numbers of snails moved downstream and upstream. Our alternative hypothesis is that numbers of snails moving downstream and upstream were not equal.

For example, one of my classes captured and marked 100 snails from a section of stream with a sandy bottom. They recaptured 50 of these snails one week later (Table 2). If equal numbers of snails moved upstream and downstream, then 25 recaptured snails should have occurred upstream and 25 snails should have occurred downstream relative to their original capture and release location. Therefore, our expected frequency in each of the two data categories is 25.

Observed frequencies are the number of snails actually found in each category. From a summary of data in Table 2, we see that 7 snails moved downstream and 43 moved upstream.

We can summarize results from Table 2 (sandy streambed) as follows:

	Downstream	Upstream	n
Observed frequencies	7	43	50
Expected frequencies	25	25	50

Now we calculate our Chi-square test statistic.

 $\chi^2 = (7 - 25)^2 \div (25) + (43 - 25)^2 \div (25) = 25.92$

Finally, we must compare our Chi-square test statistic to the appropriate critical value of the Chi-square distribution (hereafter critical value). The critical value that we use is based on our 1) degrees of freedom and 2) the chosen significance level. Definitions, formulas, and detailed descriptions of critical values, degrees of freedom, and significance levels are found in most basic statistics and experimental design textbooks, as well as statistics-based Web sites (e.g., Zar, 1999; Kuehl, 2000; TimeWeb, 2005). Briefly, degrees of freedom is equivalent to the number of data categories minus the number of statistics calculated from them. In our example, we have two data categories (upstream and downstream), and calculated one Chi-square test statistic. Consequently, we are left with one degree of freedom. Our significance level is equivalent to our accepted probability of Chi-square test results erroneously revealing that snails moved nonrandomly, when direction moved was actually random (i.e., erroneously rejecting the null hypothesis that snails move randomly; Type I error). Consistent with most biological investigations, my classes use a significance level of 0.05. In other words, there is at most a 5% chance that a class would erroneously conclude that snails moved nonrandomly.

Finally, to reject the null hypothesis and conclude that snails move nonrandomly, the magnitude of the Chi-square test statistic must be equal to or larger than the appropriate critical value found in a table of critical values of the Chi-square distribution (e.g., Zar, 1999). Using a 0.05 significance level and one degree of freedom, the critical Chi-square statistic will always be 3.841 (Table B.1 in Zar, 1999). Since the Chi-square test statistic calculated from data in Table 2 (25.92) is greater than the critical value (3.841), we conclude that snails moved nonrandomly. In other words, most snails inhabiting a sandy streambed moved in the same direction. Examination of data in Table 2 reveals a pattern of upstream movement in these snails (43 snails moved upstream and only 7 moved downstream).

Did snails inhabiting a rocky streambed also move upstream? We can use data from Table 3 and a second Chi-square test to answer this question. One of my classes collected and marked 100 snails inhabiting a rocky section of streambed, and recaptured 38 snails one week later. If snails moved randomly, 19 of these snails should have been recaptured downstream and 19 snails upstream from the original mark and release location. Using data in Table 3, we see that 16 recaptured snails actually moved downstream and 22 moved upstream.

We can summarize results from Table 3 (rocky streambed) as follows:

	Downstream	Upstream	n
Observed frequencies	16	22	38
Expected frequencies	. 19 ,	19	38

Now we calculate our Chi-square test statistic.

 $\chi^2 = (16 - 19)^2 \div (19) + (22 - 19)^2 \div (19) = 0.94$

Because the magnitude of this Chi-square test statistic is less than the critical value (3.841), we cannot reject the null hypothesis that snails moved randomly. Approximately equal numbers of snails moved upstream and downstream (Table 3), suggesting that snails inhabiting the rocky streambed did not tend to move in the same direction.

Table 6. How this activity aids in implementing *National Science Education Standards* (National Academy of Sciences, 1995).

STANDARD	VALUE OF THIS ACTIVITY
Teachers should plan an inquiry-based science program.	Through guided inquiry, teachers improve student knowledge of the entire scientific process, including formulating questions, study design, data collection and analysis, and making objective conclusions. The exercise requires that students set specific goals. Additionally, student understanding of science course content is facilitated through active learning.
Teachers should guide and facilitate learning.	This exercise enables teachers and students to focus on specific questions and answer them objectively (i.e., using statistical analysis). Objectives and methods of this activity can be modified to satisfy curiosity of students and teachers interested in additional aspects of animal biology and ecology. Students are responsible for collecting original data, and therefore share responsibility for their own learning. Finally, students work in teams, encouraging collaborations and participation by all class members.
Teachers should engage in ongoing assessment of their teaching and student learning.	The exercise provides alternative methods of assessing student learning and achievement. Students can be assessed on extent of participation in the activity, accuracy of calculations, interpretation of results, and/or the quality of a written report based on the study. Teachers can use student achievement, particularly in regard to interpretation of conclusions based on statistical analysis results, to assess their teaching effectiveness.
Teachers should design and manage learning environments that provide students with resources needed to learn science.	By incorporating a field site into their science program, teachers will increase abundance and diversity of student learning resources.
Teachers should develop communities of science learners that reflect intellectural rigor of scientific inquiry and attitudes and social values conducive to learning.	This exercise nurtures (and requires) collaborations among students. By using an experiment to gather quantitative evidence, and statistical analysis to make objective conclusions, the activity models skills and values of scientific inquiry and facilitates further discussion of scientific rules.