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Teaching critical thinking in a developmental biology course at an American liberal arts college

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ABSTRACT We all expect our students to learn facts and concepts, but more importantly, we want them to learn how to evaluate new information from an educated and skeptical perspective; that is, we want them to become critical thinkers. For many of us who are scientists and teachers, critical thought is either intuitive or we learned it so long ago that it is not at all obvious how to pass on the skills to our students. Explicitly discussing the logic that underlies the experimental basis of developmental biology is an easy and very successful way to teach critical thinking skills. Here, I describe some simple changes to a lecture course that turn the practice of critical thinking into the centerpiece of the learning process. My starting point is the "Evidence and Antibodies" sidelight in Gilbert's *Developmental Biology* (2000), which I use as an introduction to the ideas of correlation, necessity and sufficiency, and to the kinds of experiments required to gather each type of evidence: observation ("show it"), loss of function ("block it") and gain of function ("move it"). Thereafter, every experiment can be understood quickly by the class and discussed intelligently with a common vocabulary. Both verbal and written reinforcement of these ideas dramatically improve the students' ability to evaluate new information. In particular, they are able to evaluate claims about cause and effect; they become experts at distinguishing between correlation and causation. Because the intellectual techniques are so powerful and the logic so satisfying, the students come to view the critical assessment of knowledge as a fun puzzle and the rigorous thinking behind formulating a question as an exciting challenge.

KEY WORDS: *critical thinking, developmental biology lectures, loss-of-function, gain-of-function*

Background Information

Scholarly Interests of the Author

The author's interests focus on mechanisms of morphogenesis. She has studied genetic control of *C. elegans* morphology using biomechanics to elucidate the processes by which the genetic code directs the formation of three-dimensional structures. Currently, she is studying left-right asymmetry in vertebrates.

Representative Publications

ADAMS, D. S. (1998). *Critical Thinking, the Scientific Method, and Page 25 of Gilbert.* See http://sdb.bio.purdue.edu/SDBEduca/dany_adams/critical_thinking.html

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ADAMS, D.S. (2003). *Lab Math: A Handbook of Measurements, Calculations and other Quantitative Skills for Use at the Bench.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

ADAMS, D.S. *A New Syllabus for Cell Biology: Using Evolution to Give Context and Narrative Structure to a Lecture Course*. (in preparation)

General Teaching Philosophy

What I want most is for my students to learn to think critically. That is the skill that they remember after the semester is over and carry with them into their careers and lives. Therefore, while I do teach ideas and concepts, and the facts that illustrate them, I put more emphasis on the intellectual techniques that help students take command of their ability to reason. I do that by describing, explicitly, how the philosophy of experimental developmental biology provides excellent directions for how to evaluate information and how to make a well-supported argument.

Introduction

This paper describes how I adjusted my Developmental Biology lectures so that in addition to learning facts, concepts, and certain key experiments, the students learn the principles of experimental developmental biology. Once students understand correlation, necessity, sufficiency, and the logic behind experiments, they quickly develop the ability to judge the reliability and limits of evidence, and they begin to demand appropriate support

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for claims about cause and effect. Thus, the students become critical thinkers.

Methodology

The Fundamental Idea

It is more effective to explicitly discuss the logic and the thought processes that inform experimental methods than to hope that students will develop understanding on their own if they just hear enough experiments described.

An Overview

The first lectures in my upper-division Developmental Biology course include an overview of development and some review of the essential features of cell and molecular biology. I assume that this is a fairly standard way to begin. Before launching into fertilization, however, I devote at least two lecture periods to explicit discussions of the three types of experiments that must be performed to establish a cause-and-effect relationship: observation, loss-of-function, and gain-of-function. The starting point for the discussion is the "Evidence and Antibodies" sidelight from Gilbert's *Developmental Biology* textbook (Gilbert, 2000). This short essay introduces the first principles and the vocabulary of experimental biology. Later in the semester I devote at least one lecture to experimental controls. Once these ideas have been planted in the students' minds, every piece of information can be, and frequently is, discussed with reference to those principles. Every one of those discussions, the final project, and all the examinations reinforce the idea that to prove a cause-and-effect relationship, and thus elucidate a biological mechanism, one must have evidence of necessity and sufficiency.

The Specifics

I. In the course syllabus is a paragraph that explains to the students that they will be expected to think about the experimental basis of knowledge. I read the following statement out loud during the first class:

"Another important component of this course will be the emphasis on putting information in the context of the scientific method. In other words, we will structure our study with reference to the processes of making observations, formulating hypotheses, testing those hypotheses, analyzing the results of experiments, and forming both conclusions and new questions on the basis of those results. In fact, all of the examinations will have one task in common: there will be an unfamiliar observation, and you will be asked to formulate a hypothesis, describe experiments to test that hypothesis, make predictions about the results of those experiments, and discuss possible results. We will also use this framework as a guide to interpreting data generated by experiments that have actually been performed. Our goal will be to understand how experimental evidence contributes to our current understanding of the broader issue of how organisms develop."

II. Early in the semester, approximately two class periods are devoted to a discussion of the experiments described on page 43 of Gilbert's *Developmental Biology* 6th Ed[1] (Appendix A). I begin with the life cycle of *Dictyostelium discoideum,* and we discuss observations concerning stage-specific cell-cell adhesion. Immunocytochemistry has shown that a 24 kDa protein is present on the surfaces of cells that can adhere, but it is absent from cells that do not adhere. Thus, there is a correlation between the presence of the protein and the ability of cells to adhere. Class time is also devoted to defining "observations" and "hypotheses," and discussing how they differ from "experiments" and "facts."

Next, I explain how antibodies and Fab fragments are used to label proteins and to interfere with protein function. The students then discuss the experiments described by Gilbert, focusing on the logic used to design the project. The goal is to give names to the types of experiments, and to see how every experiment is used to establish either correlation, necessity, or sufficiency.

First, we discuss correlations. We give the name "show it" to the category of experiment that establishes correlation (for example, the immuncytochemistry result described above), and class time is devoted to an explicit discussion of the difference between correlation and causation. Specifically, I challenge the class to propose alternative hypotheses that can explain why the 24 kDa protein is present only on cells that can adhere. I let the discussion continue until the students have collectively recreated Gilbert's summary:

Hypothesis 1: The 24 kDa protein causes adhesion.
Hypothesis 2: Adhesion causes the expression of the 24 kDa protein.
Hypothesis 3: Adhesion and the expression of the 24 kDa protein are both caused by another signal.
Hypothesis 4: The correlation is coincidental.

This summary causes the students to wonder how they might use experiments to determine which hypothesis is correct.

I now introduce the idea of "loss-of-function evidence" and give the name "block it" to that category of experiment. I explain how "block it" experiments demonstrate necessity and allow the experimenter to begin to distinguish among the hypotheses. At this point, I describe how the experimenters used Fab fragments to interfere with the function of the 24 kDa protein with the result that adhesion was prevented, thus indicating that the 24 kD protein is necessary for adhesion. At this point we again discuss alternative explanations for the data:

Hypothesis 5: The Fab fragments killed the cells.
Hypothesis 6: The 24 kD protein works with something else that is also necessary for adhesion.

The students quickly understand the idea of "necessary but not sufficient."

"Necessary but not sufficient" leads directly to a discussion of "gain-of-function evidence." We use "move it" as our name for that category of experiment. We talk about how a "move it" experiment shows sufficiency. The example from Gilbert concerns a different, 80 kDa protein, that, like the 24 kDa protein, is present only on cells that can adhere. The experimenters used molecular techniques to modify the gene for the 80 kDa protein, causing the protein to be present all

TABLE 1

EXPERIMENT TYPES

Type	Explanation	Examples
Show it	Observations to establish correlation	Reporter gene to localize activity of a promoter Probe for ion by using selective dyes
Block it	Loss-of-function experiment to establish necessity	Gene knock out Laser ablation
Move it	Gain-of-function experiment to establish sufficiency	Insert bead soaked in exogenous chemical Tissue transplant

the time, even on cells that normally do not adhere. They found that when the 80 kDa protein was expressed at the wrong time, the cells adhered at the wrong time: the effect "moved" when the protein was "moved." Thus, the 80 kDa protein is sufficient to cause adhesion. The last topic I cover is the idea that a cause can be sufficient but not necessary for an effect if there is redundancy in the system[2] . The experiment types are summarized in Table 1.

I finish the discussion of "show it," "block it," and "move it" by summarizing these ideas and distributing the "toolbox," an empty chart that the students fill in as they learn about experimental techniques (Appendix B). The toolbox comprises three pages, one for each type of experiment. The columns are for techniques and appropriate controls. The rows are dedicated to different possible "causes," from ions to tissues. Every time a technique is mentioned in class, the students add to their toolboxes by writing notes about the technique in the appropriate boxes on the appropriate pages. By the end of the semester, the students have a record of an impressive number of techniques, and they understand the power and the limitations of those techniques. They have also gathered additional information about the goals of different experiments. On a very practical level, they end up with a list that they can consult and refine in the future.

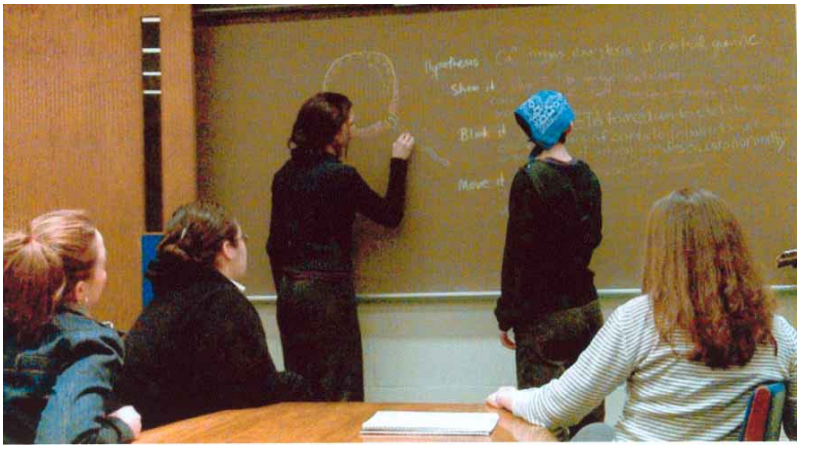

Fig. 1. A group of students designing experiments to test a hypothesis about egg activation.

III. For the rest of the semester, the appropriate category of every experiment discussed in class is immediately identified (and the technique is added to the appropriate page of the toolbox)[3] . This accomplishes many things: [1] it saves a great deal of class time as I do not have to explain the motivations for or limitations of every experiment; [2] the students *understand* the experiments discussed and their context, and therefore the meaning, of the results; [3] the students quickly learn to think about what kind of experiment should have been done for any particular statement to be meaningful; and [4] it gives the students examples of, and practice at, critical thinking.

Later in the semester, usually about the time the students bring up the idea on their own, I devote at least one lecture to control experiments, including why and how they are performed. From then on, when we talk about a technique, we also talk about the appropriate controls, and we add them to the toolbox. The resulting discussions of appropriate controls are excellent exercises in critical thinking.

This approach also provides the best strategy I have ever found for dealing with a question that I cannot answer. Previously, I was honest about my ignorance, said "What a good question," and asked "Where do you think you could find the answer to that?" Now, I am honest about my ignorance, and then I turn the question into a class discussion about how to design an experiment to answer the question. It turns a potentially flat moment into an opportunity for the students to practice thinking.

Implementing the Technique

The techniques and exercises described above are simple to incorporate into an already existing course. My course is an upper-division elective that requires a course in either genetics or cell biology to have been taken previously. All students have already completed an introductory biology course and at least one semester of general chemistry as well. The course usually has between 15 and 20 third-year and fourth-year undergraduate students. An associated laboratory course may be completed concurrently, but is not required. The course, which I teach alone, meets three times a week for 70 minutes at a time. For the most part, I use overhead projector transparencies and the chalkboard to illustrate the lectures which comprise most classes, and I encourage frequent interruptions for questions. From time to time, the class is divided into groups of three to five students for discussions. Below, I describe my own experiences in adopting these techniques, beginning with the amount of work involved in making each change.

Advance Preparation: Some Work during the First Two Years, then None

I spent about 15 minutes writing the descriptive paragraph in the course syllabus. Initially, writing and revising the lectures on experimental evidence and controls took some hours; now those lectures are a standard part of the course and preparation is minimal. It took about 2 hours to create the toolbox, and about an hour to create the Project Discussion forms described below.

During Lecture: Less Work than Before

Like all biology teachers, I was already discussing experiments in lecture, so I merely needed to modify *how* I talked about experiments. The "show it," "block it," and "move it" vocabulary in

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[1] There are many projects that could be used in place of those described by Gilbert. One particularly nice example is the work described in Nishida and Sawada (2001).

[2] The only example of "sufficient but not necessary" that I cover during the semester is MyoD, which is sufficient to induce many types of cells to differentiate as muscle, yet MyoD null mice have normal muscle due to upregulation of the myf5 gene (Kalthoff, 2001). (MyoD is sufficient but not necessary for muscle differentiation; it is not sufficient for normal development, as MyoD null mice have other abnormalities).

[3] Almost every experiment can be put into one of these categories; however, it is not always obvious how to make the assignment, especially during a lecture. Rescues, for example, can be considered gain-of-function experiments and/or controls for loss-of-function experiments. Overexpression experiments are also difficult to classify, although I usually categorize them as "move its." Even when an experiment cannot be categorized, however, it often stimulates interesting discussions that nevertheless help the students to further understand the experiment.

fact saves time. What used to take 10 minutes to describe now takes about 5 minutes: the students understand the whole picture much more quickly since they already understand what experiments can, and cannot, tell you.

Exams: Less Work to prepare, More Work to grade

Preparing examinations requires half the time previously needed. Half of every exam is a description of an appropriate[1] observation. After six years, I now have a collection of usable observations. Grading exams does take longer. Although I strongly encourage students to write succinctly, there is, admittedly, more to read and interpret; this is the one disadvantage of this teaching approach.

Assessment of Student Progress

I use two techniques to evaluate a student's ability to create an experimental design that demonstrates critical thinking: examinations and a research proposal.

Examinations

Every exam has one question that asks the students to formulate a hypothesis about an unfamiliar observation and then to design three experiments: a "show it," a "block it," and a "move it" experiment. The experiments chosen can be expensive and even unethical, but they must be, in theory, possible. For the first exam, the students must describe only the hypothesis and the experiments. For the second exam, they must also describe possible results for each experiment, both consistent with and not consistent with the hypothesis. For the third exam, the students must include a control experiment. The fourth exam and the final exam require that the students include alternative hypotheses.

Because the question becomes longer and more complex gradually over the course of the semester, and because the early exams count for a smaller percentage of the final grade, the students quickly recover from their anxiety about a "new kind of exam" and actually begin to enjoy solving the puzzle. Thus, not only do the students practice their critical thinking skills in a meaningful way, that is, to earn a high grade, they also do so in a way that they find to be memorable and fun.

Sample Exam

This examination question is from an actual exam that was administered approximately 9 weeks into a 13-week course. The answers were written by an undergraduate with no research experience (reproduced with permission).

The Observation

During the first two cleavage divisions of the nematode *Parascaris aequorum*, special cytoplasm, termed the germ plasm, is segregated into particular daughter cells. Cells that do not inherit the germ plasm undergo a process called chromosome diminution (the chromosomes fragment). All germ cells are descended from the cell that *does* inherit the germ plasm and that retains its full complement of DNA.

The Tasks

[1] Offer a hypothesis about a process (the cause) that might be responsible for some aspect of the phenomenon (the effect) described above.
Student answer: "There is a protein [that I will call] PCFP, [that is] found in germ plasm [and] that prevents chromosomal fragmentation."
[2a] Describe an experiment to determine if the process and the phenomenon are correlated, either in time or in space (correlation; "show it").
Student answer: "Produce an antibody to PCFP and expose cells of the germ plasm, and the cells that do not inherit germ plasm, to the antibody. [Use a] secondary antibody conjugated to a [fluorophore to image the primary]."
[2b] Describe a result that is consistent with your hypothesis.
Student answer: "Germ plasm is stained with antibody and the cells that do not [inherit germ plasm] are not stained."
[2c] Describe a result that is inconsistent with your hypothesis.
Student answer: "Germ plasm does not stain with antibody, or, both germ plasm and cells without germ plasm stain."
[3a] Describe an experiment to determine if the causative process you have hypothesized is necessary for that aspect of the phenomenon to occur (loss-of-function; "block it").
Student answer: "Identify the gene that encodes PCFP and perform site-directed mutagenesis on that gene."
[3b] Describe a result that is consistent with your hypothesis.
Student answer: "Germ cells have fragmented DNA."
[3c] Describe a result that is inconsistent with your hypothesis.
Student answer: "Germs cells still have no chromosomal fragmentation."
[4a] Describe an experiment to determine if the causative process you have hypothesized is sufficient to cause that aspect of the phenomenon to happen (gain-of-function; "move it").
Student answer: "Introduce a plasmid into cells that don't inherit germ plasm. This plasmid will contain the gene encoding PCFP adjacent to [a] galactose-inducible promoter."
[4b] Describe a result that is consistent with your hypothesis.
Student answer: "In the presence of galactose, these cells will not have chromosomal fragmentation; in absence of galactose, they will have fragmentation."
[4c] Describe a result that is inconsistent with your hypothesis.
Student answer: "In presence of galactose, the cells will have chromosomal fragmentation."
[5] Describe a control experiment for ONE of the above experiments, and state what you are controlling for.
Student answer: "Positive control to determine if antibody is working: Identify cells that are known to contain PCFP; expose these cells to the antibody."

It has happened more than once that an observation given on an exam has turned up in the primary literature soon thereafter. In all cases, the students have designed experiments that matched the published work. When I bring those published papers into class and show the students that their proposals match science that is actually being performed and published, not only are they pleased to find out the actual result, they also get very excited, their confidence increases, and they begin to see themselves as scientists.

[1] I define an appropriate observation as one containing at least one phenomenon that is an obvious candidate for a hypothesis. I have found that about half the class will suggest the obvious hypothesis; the rest look for a more subtle aspect of the observation to address.

The Research Proposal

The students also write a research proposal for the class. The proposal is an extended version of the test question: in addition to designing the experiments, the students choose the observation and do background research. Early in the semester, I ask them to choose a subject that is particularly interesting to them; I sometimes provide some direction by mentioning topics that are covered in the text but will not be covered in lecture. They begin their library research early in the semester. Once they have read the primary literature, they devise an original hypothesis, and plan experiments to test that hypothesis.

Despite the similarity between this exercise and the examination questions, the students initially struggle because they lack confidence and direction, and because they are nervous when their original hypothesis needs modification. To help them organize and understand their topics, I devote a class period to group discussions of projects; this session is scheduled for a time after the students have done a significant amount of background research, but before the first draft of the proposal must be submitted. I divide the class into groups of three students; each student has one-third of the class time to describe her project and then plan experiments with help from the other two students. To help the students focus and achieve this goal in such a short time, I hand out "Project Discussion" forms for the students to follow and complete (Appendix C). These forms give structure to the within-group conversations and force the students to be both clear and concise with their experimental plans. I move from group to group during the discussions to provide extra help as needed. By the end of the class period, every student has a proposal outline.

This exercise has a number of positive effects. First, each student has the benefit of two other similarly trained minds working on her question; all students' proposals are improved. Second, each student gets two chances to help another student, which boosts confidence and provides good practice at thinking critically and an opportunity to practice providing helpful criticism. Third, the students learn about each other's projects; as a result, the class becomes more cohesive, and the students begin to see each other as sources of ideas and information. Fourth, the students remind themselves why they are interested in their topics, and this provides motivation for completing the project. Finally, by the end of the class period, every student is on schedule to complete the paper on time. The students, without exception, find this exercise very useful and very exciting.

Results

What the Students gain

I am favorably impressed, over and over again, by the improvement in my students' ability to *understand* the primary literature, to *assess* the validity of claims, and to *think* about how to answer questions. The following list summarizes the most important kinds of progress I have witnessed in my students:

1. They understand the experimental foundations of information.
2. They have an intellectual technique to use when asked to think.
3. They understand how to interpret the results of experiments performed with either classical or modern techniques.
4. They can read the primary literature and comprehend it much more, more quickly.
5. They can judge the validity of conclusions.
6. Every student seems to understand, even those who are not at the top of the class.
7. As their confidence grows, they become more active participants in class.
8. They are *aware* that they are thinking well, and most find that very exciting. That awareness also enhances their self esteem and builds their confidence.

There is at least one more, very practical benefit for the students. Many graduate schools and fellowship-granting agencies ask for research proposals in their applications or during interviews. This past year, three students told me that the proposal they wrote in my course, and the practice they got by writing it, contributed directly to the success of their applications for graduate school or a fellowship.

Student Response

The majority of students respond very positively to this course; a few are neutral. Not one student has found it a negative experience. On their mid-semester self-evaluations, students wrote the following statements in response to the question "Where would you say you have shown the most change for the better?"

"I believe that I am gaining a real understanding of how to go about asking questions.... The experimental design techniques and problem-solving approaches have really strengthened my critical thinking skills."

"It's becoming easier to read complicated journal articles with understanding."

"I ... like the experiment section of the test because I can apply my knowledge."

"The research proposal was really difficult for me ... but that's good, it means it's a challenge."

"[My] critical thinking has expanded.... Experimental thinking has made science in general more clear for me. I feel less overwhelmed by all the research and knowledge by understanding how to break it down into manageable questions."

I have also received the following spontaneous comments:

- "Empowering"

- "I am studying pathogenic *E. coli* for one of my other classes and am reading this book on the microbes. I came across this paragraph, part of which I have to share with you!! It talks about how ... 'the intimin of *E. coli* was shown to be NECESSARY BUT NOT SUFFICIENT to induce lesions.' I just thought it was so cool that I am reading this highly scientific book and can make sense of concepts that would have been so foreign to me not all that long ago!!"

One student actually expressed regret that the fourth exam was the last.

Summary

The teaching methods described here, which grew out of my own attempts to guide students through the literature and through the lab, have been successful in the classroom and useful to me as well; my "grade" from the students went from 70% to 97% in one year. Moreover, I have received notes from colleagues who have tried these ideas in their own classrooms and were so pleased with the result that they have incorporated them permanently. One colleague formally presented the techniques to other scientists at his institution. What is most important, however, is that the students learn critical

thinking, they are aware that they have learned, and they proudly continue to use their new intellectual skills in new classes and new contexts.

Acknowledgements

I am very grateful to the students of Bio 346. Thank you to L. Moran for her contribution to this manuscript. Thanks also to Philip Hertzler, Scott Gilbert, Mary Montgomery, and the other developmental biologists who have let me know about the success of these techniques in their classes. Some of these ideas were originally presented as a poster for the 1999 annual Society for Developmental Biology meeting. A version of the poster was subsequently posted on the SDB Web site (http://sdb.bio.purdue.edu/SDBEduca/dany_adams/critical_thinking.html).

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APPENDIX A

Sidelights & Speculations

Evidence and Antibodies

BIOLOGY, like any other science, does not deal with Facts; rather, it deals with evidence. Several types of evidence will be presented in this book, and they are not equivalent in strength. As an example, we will use the analysis of cell adhesion in *Dictyostelium*. The first, and weakest, type of evidence is **correlative evidence.** Here, correlations are made between two or more events, and there is an inference that one event causes the other. As we have seen, fluorescently labeled antibodies to a certain 24-kDa glycoprotein do not bind to dividing myxamoebae, but they do find this protein in myxamoeba cell membranes soon after the cells stop dividing and become competent to aggregate (see Figure 2.19). Thus, there is a correlation between the presence of this cell membrane glycoprotein and the ability to aggregate.

Correlative evidence gives a starting point to investigations, but one cannot say with certainty that one event causes the other based solely on correlations. Although one might infer that the synthesis of this 24-kDa glycoprotein caused the adhesion of the cells, it is also possible that cell adhesion caused the cells to synthesize this new glycoprotein, or that cell adhesion and the synthesis of the glycoprotein are separate events initiated by the same underlying cause. The simultaneous occurrence of the two events could even be coincidental, the events having no relationship to each other.*

*In a tongue-in-cheek letter spoofing such correlative inferences, Sies (1988) demonstrated a remarkably good correlation between the number of storks seen in West Germany from 1965 to 1980 and the number of babies born during those same years.

How, then, does one get beyond mere correlation? In the study of cell adhesion in *Dictyostelium*, the next step was to use the antibodies that bound to the 24-kDa glycoprotein to block the adhesion of myxamoebae. Using a technique pioneered by Gerisch's laboratory (Beug et al. 1970), Knecht and coworkers (1987) isolated the antibodies' antigen-binding sites (the portions of the antibody molecule that actually recognize the antigen). This was necessary because the whole antibody molecule contains two antigen-binding sites and would therefore artificially crosslink and agglutinate the myxamoebae. When these antigen-binding fragments (called Fab fragments) were added to aggregation-competent cells, the cells could not aggregate. The antibody fragments inhibited the cells' adhering together, presumably by binding to the 24-kDa glycoprotein and blocking its function. This type of evidence is called **loss-of-function evidence.** While stronger than correlative evidence, it still does not make other inferences impossible. For instance, perhaps the antibodies killed the cells (as might have been the case if the 24-kDa glycoprotein were a critical transport channel). This would also stop the cells from adhering. Or perhaps the 24-kDa glycoprotein has nothing to do with adhesion itself but is necessary for the real adhesive molecule to function (perhaps, for example, it stabilizes membrane proteins in general). In this case, blocking the glycoprotein would similarly cause the inhibition of cell aggregation. Thus, loss-of-function evidence must be bolstered by many controls demonstrating that the agents causing the loss of function specifically knock out the particular function and nothing else.

The strongest type of evidence is **gain-of-function evidence.** Here, the initiation of the first event causes the second event to happen even in instances where neither event usually occurs. For instance, da Silva and Klein (1990) and Faix and co-workers (1990) obtained such evidence to show that the 80-kDa glycoprotein of *Dictyostelium* is an adhesive molecule. They isolated the gene for the 80-kDa protein and modified it in a way that would cause it to be expressed all the time. They then placed it back into well-fed, dividing myxamoebae, which do not usually express this protein and are not usually able to adhere to one another. The presence of this protein on the cell membrane of these dividing cells was confirmed by antibody labeling. Moreover, the treated cells now adhered to one another even in the proliferative stages (when they normally do not). Thus, they had gained an adhesive function solely upon expressing this particular glycoprotein on their cell surfaces. This gain-of-function evidence is more convincing than other types of evidence. Similar experiments have recently been performed on mammalian cells to demonstrate the presence of particular cell adhesion molecules in the developing embryo.

Evidence must also be taken together. "Every scientist," writes Fleck (1979), "knows just how little a single experiment can prove or convince. To establish proof, an entire system of experiments and controls is needed." Science is a communal endeavor, and it is doubtful that any great discovery is the achievement of a single experiment, or of any individual. Correlative, loss-of-function, and gain-of-function evidence must consistently support each other to establish and solidify a conclusion. ■

APPENDIX B

THE TOOLBOX: Techniques for testing hypotheses about cause and effect

The students are provided with an empty chart. Below, certain rows have been filled in to demonstrate how the chart is meant to be used.

Page one:

OBSERVATIONS (TO ESTABLISH CORRELATION); LABELING

Cause	**Methodology**	**Positive Controls** (Methodology works —the truth)	**Negative Controls** (Methodology has no confounding side effects—nothing but the truth)
Ion DNA RNA Protein Cell Tissue	Immuncytochemistry	Western blotting; stain cell type known to contain protein	Use mock primary antibody; use secondary antibody only; adsorb primary using antigen

No-treatment control (the whole truth)—Leave some subjects untouched.

Page two:

LOSS-OF-FUNCTION EXPERIMENTS (TO SHOW NECESSITY)

Cause	**Methodology**	**Positive Controls** (Methodology works —the truth)	**Negative Controls** (Methodology has no confounding side effects—nothing but the truth)
Ion DNA RNA Protein Cell Tissue	Surgical removal	Look; histology; probe for tissue-specific protein	Remove and replace tissue

No-treatment control (the whole truth)—Leave some subjects untouched.

Page three:

GAIN-OF-FUNCTION EXPERIMENTS (TO SHOW SUFFICIENCY)

Cause	**Methodology**	**Positive Controls** (Methodology works —the truth)	**Negative Controls** (Methodology has no confounding side effects—nothing but the truth)
Ion DNA RNA Protein Cell Tissue	Inject mRNA	Northern blotting; *In situ* hybridization; reporter construct; probe for protein	Mock injection; injection of neutral mRNA species (i.e., same nucleotides, different sequence)

No-treatment control (the whole truth)— Leave some subjects untouched.

APPENDIX C

Proposal Discussion form:

1. The general topic is:
2. Some unexplained observations associated with this topic are:
3. Of particular interest is this ONE observation:
4. The components (ions, molecules, genes, gene products, cells, tissues) thought to affect this particularly interesting phenomenon are:
5. Which leads to the following hypothesis: _____ causes _____.
6. I plan to use _____as the experimental organism because:
7. To establish correlation, this experiment will be performed:
8. The negative control will be:
9. The positive control will be:
10. To establish necessity, this experiment will be performed:
11. The negative control will be:
12. The positive control will be:
13. To establish sufficiency, this experiment will be performed:
14. The negative control will be:
15. The positive control will be:
16. The title of the proposal will be: