Chapter 2

A Holistic Approach to Teaching a Laboratory, Using Sea Urchin Development as an Example System

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Introduction

Using the study of early sea urchin development as an example, I will attempt to demonstrate an approach to teaching labs that takes several ancient teaching techniques and combines them in, perhaps, a new way. I do not start employing this approach directly with naive freshmen; I spend an entire semester preparing them for this approach; for a description of the first-semester preparation, see Eisen et al. (1992). The approach has proved successful in this framework, and we have gone on to employ it directly in upper level laboratory courses in neurobiology, genetics, and cell physiology.

Our approach has several goals, to get students:

(1) used to working effectively in small groups,
(2) thinking independently about new problems,
(3) asking their own questions and designing ways to find answers,
(4) to improve paper writing and oral presentation skills,
(5) to use the secondary and primary literature, and
(6) a “taste” of research and the “power” of model systems.

Okay, this does not all happen in one experiment, but we use the same approach for three or four experiments on different topics in one semester.

Each unit of the approach has these eight components:

Catalyst experiment: Introduce the class to a model system with a “catalyst experiment,” designed to allow students to learn the basic techniques involved with the system.

Information packet: Include a “catalyst package” (an example package would include articles by including everything from primary literature to popular magazines; sample articles are included in the Literature Cited section) that contains: (a) background material on the system, (b) new vocabulary associated with the system (definitions not included), (c) references with which to get the students started, (d) some “hints and ideas” to get students thinking about possible experiments, (e) the protocol for the catalyst experiment, and (f) one or two relevant articles from the literature.

Brainstorm sheet: Between the class session of the catalyst experiment and the next session, we ask students to make up independently a “brainstorm sheet.” They turn it in for evaluation, but we have no set requirements for it. They can write or type them on any kind of paper. The idea is that we encourage creativity, random thoughts, and try to get the mind geared up.

Group brainstorm: In the second session of the unit, each group of four students meets, consulting and discussing each other’s brainstorm sheets. They take their time to pick one or two favorite experimental proposals.

Advise and consent: When a group is ready (that same second session), they meet with me and/or the teaching assistant to discuss their proposal. The main purpose of this session is to focus the project (realizing we have a month’s time frame in which to work) and to discuss where to find experimental materials and background literature for the experiment.

Experimental outline and schedule: After deciding on a project, before leaving class the second session, each group must have at least outlined a schedule and plan for performing their experiment. Often, they start the experiment.

The experiment and periodic updates: Over the next class sessions, the groups perform their experiments, and we have periodic brief updates from each group to the rest of the class. During this time, we serve primarily as guides and advisors.

The write-up: Students must write up a comprehensive report on the laboratory. They choose two of their three or four experiments to write up.

Clearly, this approach requires some shifts in thought from the traditional undergraduate lab presentation. My particular course has no separate lab and lecture, although this is not a prerequisite for this approach. The syllabus must allot much more time per lab, and the instructor needs to select good experimental systems with many options for student experiments. Our approach also requires access to diverse resources and/or a good imagination.

The first response to such an approach may be "This is too much and too difficult for the students I teach." My response is: "The more opportunity you give any people to think, the more they do, and the more they surprise you."

We use this lab for our Development Unit. We continually stress how we can relate the information about a process such as development discovered in one organism to that same process in other organisms. This theme should be emphasized in the articles that are used to (1) put the students' work in context and (2) show the students other parts of the spectrum of research in development (for examples, see the Literature Cited section).

Notes for the Instructor

Required Reading

The idea here is to present the students with at least one example from each of the basic science literature genres: text, popular literature, primary literature, and other examples along this spectrum. We want to pick articles that will inspire students to come up with their own ideas for experiments and to further explore the literature, and that will enable them to get a taste of the broad interest in the field throughout society. For this lab I require the students to read the basic developmental biology chapter in their text, and then select articles from newspapers, magazines, and journals; examples are provided in the Literature Cited section.

Obtaining Sea Urchins

The easiest way to get good urchins is to call Carolina Biological Supply Co. *well in advance* of the times you will need the organisms to see when they will be available. Try your best to get animals that can be maintained at room temperature. Then place your order at least 1 month in advance to ensure timely delivery. Urchins are available at most, but not all, times of the year. It is useful to order your urchins in two batches 2 weeks apart, as they don't stay superbly healthy for much longer than that.

Urchin Maintenance

I found the easiest method for keeping the animals is simply to set up a large aquarium with a filter pump. I use Instant Ocean®, following the directions on the box, to salt tap water. You should set up your tank at least 2–3 days before the urchins arrive to allow equilibration with the air. Use a heater if the supplier suggests it. Simple pH, nitrate, nitrite, and other ion tests are available from your local pet store.

When the urchins first arrive (usually individually wrapped in plastic bags), do not simply dump them in your tank. This shock can lead to premature gamete emission, which usually induces all neighbors to do the same. This is, of course, a disaster. Just set the individual bags of urchins in the tank and allow temperature equilibration for at least 30 minutes. Urchins will eat lettuce or specific food available from a pet store.

Student Outline

Introduction

“For this, be sure, tonight thou shalt have cramps,
Side-stitches that shall pen thy breath up; urchins
Shall, for that bast of night that they may work,
All exercise on thee.”

Bill Shakespeare
The Tempest I, ii

> Genesis, however, is not a simple activity of Nature, but is composed of alteration and of shaping. That is to say, in order that bone, nerve, veins, and all other tissues may come into existence, the *underlying substance* from which the animal springs must be *altered*. In order that the substance so altered may acquire its appropriate shape and position, its cavities, outgrowths, attachments, and so forth, it has to undergo a *shaping* or formative process. One would be justified in calling this substance which undergoes alteration the *material* of the animal, just as wood is the material of a ship, and wax of an image.
>
> Galen (c. 160 A.D.)

Galen, one of the first experimental physicians we have records of, describes above what we have come to know as differentiation or the process of the alteration or development of starting stuff into the final organism. All the processes of eukaryotic life must get their start at the beginning, when there is only some yolk, a few membranes, and a bunch of DNA. Speaking of eggs, I suppose one could boil the field of developmental biology down to one question:

How does this blob of membranes, nutrients, and DNA—a fertilized egg—become a mature organism?

This question encompasses much: from descriptive studies of what is seen happening to the proteins making the things happen to the genes that make the proteins that make the things happen. Each of these incredibly complex problems changes with time and in time and space.

Aristotle waffles on for quite awhile about the general concept of development, and he even extensively described the organism that we will use to study the developmental process, the sea urchin. The urchin which gets its name, meaning “sea hedgehog” from the Greek and Latin due to its similarity to the land hedgehog, has been used as a food source and a medicinal organism since long before the time of Christ. Hedgehogs were thought to be the bearers of evil spirits (see Bill’s quotation above) and thus the word “urchin” also managed to etymologically weasel its way into the term “street urchin” (i.e., a slimy bum).

Anyway, getting back to the sea urchin proper, there is also a long history of investigation into the development of this animal. As with most else intellectual or scientific, after Aristotle and his pals, there was at least a good *1000 years* before anyone else (in the Western world at least) thought about development or about any other significant scientific problem.

After translations of the Greeks’ writings became available, people began to think a bit more about scientific questions. Some of the early ideas in development, for example the homunculus or little man that was thought to reside in every human sperm, seem odd now, but aren’t really, considering the time and knowledge base from whence they came.

The story of the study of sea urchin development goes back to the mid to late 1800s, when scientists already had begun to realize that the urchin would be a good system for studying development. We have talked about how to choose the best system for a study and how crucial it is to make a good choice before starting to explore your particular question. Think about some of the things we would want in a system to most efficiently study early development. Some ideas off the top of my head are: easy access to the organism, easy access to a lot of the organism’s gametes, easy visualization of gametes and their labors, plenty of gametes to work with, and a high probability that the organism can develop in the lab. As I hope we will see, sea urchins fit most of these requirements and more pretty well.

As you think about and work on this lab, consider how the field of development and your particular experiment relate to the central themes of biology: unity and diversity, regulation, communication (see especially the article on social amebas by Kessin and Van Lookeran Campagne, 1992), the use of model systems, natural selection and evolution, and that the way to understand normal processes is to alter them and study the results. A good exercise would be for you to include a table of such considerations in your lab write-up.

Urchins are ideal for studying early development—from gametes to advanced larvae. It is difficult to raise the larvae through metamorphosis and into adults in the lab. Therefore, we will concentrate on early stuff, which, as you’ll see, is more than enough to keep us busy.

Based on our work thus far in the course, not our factual work but our conceptual work, think about this question before you go on:

What are some general strategies for best going about studying development?

Terms to Know

Know these terms and their definitions as well as how each fits into the “big picture” of development of an organism:

Differentiation	acrosome reaction	fertilization
Blastula	gastrula	jelly coat
vitelline membrane	cortical granules	pluteus
ecto- , meso- , endoderm	hyaline layer	cortical layer

Readings

Read (1) Chapter 43 on Animal Development by Campbell (1993), (2) the article entitled "How does a fertilized egg turn into a fly, a chicken, you or me?" by Kanigel (1992), and (3) the article by Maden (1985).

The following books are available in class (others, of course, are in the libraries): *Developmental Biology of the Sea Urchin Embryo* by Giudice (1973), *The American Arbacia and Other Sea Urchins* by Harvey (1956), and *Science as a Way of Knowing: IV–Developmental Biology* by Moore (1987).

Hints and Ideas

Here are some ideas, based on the catalyst experiment, to get you started:

1. Sea water is made up of certain ions. What is the effect of altering the concentration of some of these ions on development? When the [ion] is altered at different times in development?
2. Attempt to answer the question: Is protein synthesis necessary for all stages of development after fertilization?
3. People have always been fascinated with the idea of parthenogenesis (e.g., see the New Testament). Do urchins possess the ability to start development without fertilization occurring?
4. What is the purpose of the jelly coat that forms around the egg?
5. Come up with a hypothesis to test the effects of these or any other reagents you can think of on development: ethanol, HCl, KCl, lithium, caffeine, etc.
6. Do the types and amounts of proteins change during development? Which ones?
7. What effects do UV light or other mutagens have during different times after fertilization?

More ideas for papers and projects:

8. Does human development parallel urchin development? To what degree?
9. Limb regeneration, such as occurs in salamanders and starfish is an amazing, nearly unbelievable phenomenon? How does it work? Why doesn't it happen in humans or does it?
10. Parthenogenesis.
11. How does an organism "sets itself up" to develop so that it is vertically a mirror image, but not horizontally, for example?
12. How important is what happens during development to the adult in the brain, behavior, etc.?

Catalyst Experiment:
Sea Urchin Fertilization and Early Development

Keep in mind that urchins come from all the world's oceans and that all their life processes work best at the temperature of the waters from which they originate. Each urchin has more than enough gametes for the whole class; in fact, for the whole world. So, to prevent wasting urchins, just use one of each sex for the entire class, please. Unfortunately, there is no way to sex urchins without inducing the release of, and then examining, their gametes.

1. Obtain two or three urchins from me. *Do not reach into the tank yourself to get them.*

2. Perch the urchin upside-down (with its mouthparts up; a diagram will be provided in the lab) on the rim of a beaker full of *sea water*. Make sure that the water is touching the underparts of the urchin.
3. With a needle and syringe, carefully inject 0.5–1 ml of 0.5 M KCl to the side of the mouthparts.
4. The hope is that ribbons of white sperm or clouds of orangish eggs, depending on the sex of the urchin of course, will immediately emerge from the animal into the sea water.
   (a) *If white sperm emerge, immediately shift the animal to a dry petri dish for gamete collection.* Dry sperm are best for optimal fertilization.
   (b) *If the orangish eggs emerge, allow the gametes to finish releasing into the sea water.* Then follow these steps to wash the eggs:
      (i) Pour the eggs and water into two 50 ml centrifuge tubes (the size of the tubes is not important).
      (ii) Spin the tubes at 1000–2000 rpm for 2 minutes
      (iii) Carefully remove the clear sea water supernatant from above the eggs .
      (iv) Add a small volume (1–2 ml) of fresh sea water to the eggs.
5. In your groups, prepare some microscope slides with thin strips of tape on the top and bottom of the slide in the middle so as to support a coverslip, but not squish the gametes.
6. Add a drop of concentrated eggs and find the eggs at 10X (with the light disc set at 10) and then examine them at 40X (with the light disc set at 40). Identify all the parts of the egg you can.
7. Add a drop of sperm to the side of the coverslip so that surface tension pulls the sperm under the slip with the eggs. Observe what happens in the field of view. Find the sperm. Observe the process of fertilization and the behavior of the sperm. *Make any drawings and records of observations you feel are necessary.*
8. The microscope light might alter the temperature and kill the eggs on the slide, but observe them as far as they can go.
9. Consult the diagram of the sea urchin developmental process that will be provided in the lab. Keep timing and track of your fertilization.
10. To better record the process, set up one fertilization reaction in a beaker or petri dish at the “prime” temperature (in an incubator or water bath) for your species of urchin. Periodically remove a drop of the fertilization mix and observe it under the microscope.
11. Observe the fertilization throughout the lab period, and once on each of the following 2 days.
12. During breaks and after the experiment, discuss possible extensions of the experiment your group might do over the next weeks. Be especially aware of the time frame of things (e.g., how long the process of development you want to look at takes and how long the “simple” fertilization reaction you just performed takes). Let me know of specific materials you might need.

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