



INQUIRY EXERCISES for the
COLLEGE BIOLOGY LAB

40 INQUIRY EXERCISES for the COLLEGE BIOLOGY LAB

A. Daniel Johnson

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Preface

In 1998, the Howard Hughes Medical Institute (HHMI) issued *Beyond Bio101: The Transformation of Undergraduate Education*. The report outlined several strategies used by faculty at various institutions that are changing the way undergraduate science is taught. The section on laboratory experiences begins with a snapshot of the current situation on many campuses:

Biology students approach teaching laboratories with mixed emotions. For some, laboratory courses are windows on the world of science, allowing them to gain experience with the techniques, concepts, and emotions that go with real research. For others, laboratories are exercises in preordination, a tedious derivation of answers that are already known to questions that do not seem important.

Often the best laboratory experience is one in which students pursue their own research under faculty guidance. In fact, given the success of undergraduate research, more and more faculty members have begun asking: Why not make teaching laboratories more like research projects? Instead of just showing students what it is like to do science, why not confront them with real problems and ask them to come up with their own solutions? (Olson et al. 1998, p. 30; © Howard Hughes Memorial Institute. Used with permission.)

HHMI, the Carnegie Foundation for the Advancement of Teaching, the National Research Council (NRC), and numerous other groups have repeatedly called for fundamental changes in how undergraduate biology is taught (Kenny et al. 1998; NRC 2003). Each set of recommendations is based on the same theme: Students learn more, retain knowledge longer, and are better able to apply it if they are taught using active, inquiry-based strategies that let them participate in the discovery of knowledge. The advantages of inquiry-based instructional methods are not just a matter of expert opinion; numerous studies have shown they lead to significantly greater gains in student learning outcomes (for reviews and examples, see Arce and Betancourt 1997; Bain 2004; Chickering and Gamson 1987; Coppola, Ege, and Lawton 1997; Gardiner 1994; Hofstein and Lunetta 1982; National Institute of Education 1984; NRC 2003; National Survey of Student Engagement 2000).

Unfortunately, most undergraduates continue to be taught by traditional didactic exposition. They listen passively to lectures, and then in lab perform exercises that reiterate principles they supposedly just learned. Given the evidence that active, inquiry-based instructional practices increase learning gains, why do faculty continue to lecture? Why do demonstration-oriented exercises hang on as the dominant style of lab instruction? Some insight can be gained by looking at high school teachers, who have experienced similar calls for reform.

Efforts to incorporate more inquiry into high school science curricula go back more than a century. In 1892 the National Education Association (NEA) asked a panel of education leaders to examine the structure, content, and organization of the high school curriculum. The “Committee of Ten” (as it came to be called) made numerous recommendations in its final report. The following excerpt from the report shows that many of its recommendations reflect an inquiry-based approach to teaching science (emphases are this author’s):

*The Conference on Natural History unanimously agreed that the study of botany and zoology ought to be introduced into the primary schools at the very beginning of the school course, and be pursued steadily.... In the next place they agreed that in these early lessons in natural science **no textbook should be used**; but that the study should constantly be associated with the study of literature, language, and drawing.... Like the report on Physics, Chemistry, and Astronomy, the report on Natural History **emphasizes the absolute necessity of laboratory work by the pupils on plants and animals**, and would have careful drawing insisted on from the beginning of the instruction.... [T]he Conference on Natural History recommends that the pupils should be made to **express themselves clearly and exactly in words, or by drawings, in describing the objects which they observe....***
(NEA 1893)

Efforts to incorporate more inquiry-based instruction continued through the following century. John Dewey (author of *Democracy and Education*) called for more inquiry-based instruction in a 1916 speech to the National Education Association (Dewey 1916). By the 1950s and 1960s, the National Science Foundation had made progress toward actually implementing some changes; it sponsored several inquiry-based K–12 curriculum improvement projects, including the Biological Sciences Curriculum Study (BSCS), Chemical Education Materials (CHEM) Study, and the Science Curriculum Improvement Study (SCIS). Unfortunately the changes were short-lived and fairly localized. By 1980, exposition had returned as the dominant mode of instruction at all grade levels (Hurd et al. 1980).

During the 1980s, concerns grew that K–12 students in the United States lagged behind students in the rest of the world in science and math proficiency (NCEE 1982), and from 1989 to 2003, the American Association for the Advancement of Science and National Research Council repeatedly challenged science educators

from kindergarten through college to integrate active, inquiry-based instructional methods into their classrooms (AAAS 1989, 1993; NRC 1996, 1999, 2003). Yet in 2005, a survey of state science curriculum standards found that most of the standards still emphasized content coverage; few integrated inquiry-based teaching in any systematic way (Gross et al. 2005). In short, after more than a century of work by education leaders, passive exposition remains the dominant method of instruction for most K–12 programs.

Why is there so much resistance to inquiry-based methods? In *Science Teaching and Development of Thinking*, Lawson (2002) lists 10 reasons precollege instructors commonly gave for not using inquiry-based teaching methods; most could easily apply to undergraduate instructors as well.

- Development and implementation take too much time (the most-cited reason).
- There is insufficient content coverage.
- The reading level required is too high/difficult for my students.
- The risk is too high; I do not know how the instructional units will turn out, and administration will not understand what I am doing and will penalize me.
- There are no strong students in the regular biology class (or in the nonmajors course for undergraduates).
- Students are too immature and waste too much time to use it successfully.
- I have been teaching this way for too long to change now.
- The textbook/manual/ancillary materials restrict the order in which we cover topics.
- The students and I are too uncomfortable with it.
- It is too expensive. My teaching lab is not equipped properly, and there is no budget to buy necessary materials.

It has been further suggested that undergraduate instructors are less likely than high school teachers to adopt inquiry-based instruction because there is less central oversight of the curriculum on the college level (NRC 2003 [especially see references]; Tanner and Allen 2006). A central administrative directive for K–12 reforms can help ensure that inquiry-based methods will be used systematically in several courses or across an entire curriculum. The college-level instructor, on the other hand, must make changes with minimal guidance or support.

Because there is no central source for materials and resources that meet these various needs, college instructors must cobble together solutions from a variety of sources. Simply modifying materials originally designed for high schools is not a solution because the two audiences are fundamentally different. Students' maturity, intellectual skills, and attention span differ, as do instructional goals.

The number of students served also is a factor. College instructors routinely have dozens to hundreds of students enrolled in a single large introductory course. The amount of time available for lab-related work also differs greatly.

The relative scarcity of resources designed specifically for college instructors is another source of resistance. There is an immense body of validated curricula, lesson plans, instructional resource guides, textbooks, and lab exercises available to K–12 instructors. The amount of material developed specifically for college instructors is orders of magnitude smaller.

PURPOSE AND STRUCTURE OF THIS BOOK

To incorporate more inquiry at the undergraduate level, faculty need a practical introduction to the general theory and best practices of inquiry-based teaching; tools to help them create, administer, and evaluate new inquiry-based courses and update existing courses so they can follow a more inquiry-based approach; instructional supporting materials designed specifically for their audience; and laboratory exercises that can be adapted to a variety of inquiry-based teaching and learning strategies.

This book was designed to be a general resource guide for college faculty who want to add inquiry-based methods to their biology laboratory courses. It focuses mostly on the laboratory setting, but many of the principles and methods described in Chapters 1–4 can be applied to a variety of course situations. For those who are new to this topic, Chapters 1, 2, and 4 provide a basic introduction to inquiry as an instructional practice and offer guidelines for developing an inquiry-based course using an outcomes-oriented approach. Chapter 3 reviews assessment methods and provides guidance in how to teach novice instructors (such as teaching assistants, for example) to use inquiry. Those who are already familiar with these topic areas can skip the early chapters with little loss of continuity.

Some readers may want to supplement an existing lab course with just one or two of these lab units. Others may decide (as the author's department did) to reorganize their entire lab program around inquiry. Chapter 2 discusses strategies for making this transition. Again, I have only provided an overview; details are in references at the end of each chapter and unit.

The main body of this book consists of 16 modular lab units; some are inquiry-oriented adaptations of well-established exercises, while others are entirely new. An introduction preceding the units (p. 81) summarizes the differences between them and explains the conventions used. The units are self-contained as much as possible, so they may be arranged in any order. Each unit was developed for students in either a nonmajor or introductory majors biology course. They span a range of topics and vary in length, overall structure, and difficulty. **The Summary of Units on page 86 provides information on each unit's intended audience, model and questions, major concepts, prior skills and knowledge needed, and degree of difficulty.**

The units have undergone extensive testing with undergraduates and been revised repeatedly, so they are very robust. It is important to remember, though, that every student group is unique; what one particular audience finds difficult, another may find straightforward. Inquiry requires instructors to be flexible and responsive to students' needs. Readers should not be afraid to experiment and modify exercises to fit their particular instructional goals.

Each unit contains classroom-ready student exercises, plus Instructors' Notes and Preparatory Notes. The student exercises are at the level at which they are used at the author's institution. They range in complexity from exercises that are best suited for nonmajors just starting in biology, to exercises designed for majors in sophomore-level courses. However, most can be revised to fit a different target audience. The background material that precedes the student exercises has been written so it can be revised, rearranged, or stripped down as needed to fit the target audience and the level of inquiry and difficulty desired by the instructor.

The Instructors' Notes provide supplemental background information and instructional goals and typical outcomes. They indicate where students may struggle with a unit and suggest how the instructor can guide them. Suggestions for modifying units to fit a range of course formats and audiences are included, as are suggested modifications if a small lab section (fewer than 12 students) would have difficulty completing a unit.

The Preparatory Notes for each unit list specific equipment and quantities of supplies needed for a single lab section of 20 students. Quantities are simply scaled up for larger or multiple lab sections. Detailed instructions are provided for obtaining and maintaining model organisms (if any) and for preparing and storing reagents.

Key terms and concepts appear in **boldface type**.

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About the Author

A Daniel (Dan) Johnson is a North Carolina native who obtained his BS in biology from the University of North Carolina at Charlotte. After three years in the pharmaceutical industry he entered Wake Forest University School of Medicine where he earned a PhD in cell biology in 1992. He subsequently completed postdoctoral fellowships at the Texas Heart Institute in Houston and the University of Virginia in Charlottesville.

In 1998 he returned to Wake Forest University, this time to the Department of Biology as core curriculum coordinator, where he currently holds the rank of senior lecturer. He teaches general biology for nonmajors; introductory cell biology and physiology courses for premajors; and graduate courses in instructional methods, professional skills development, and bioethics on both the undergraduate and medical school campuses. He leads faculty development workshops through the campus Teaching and Learning Center and has served as an instructional methods consultant on several awarded national grants. Dr. Johnson is an active member and regular workshop leader for the Association of Biology Laboratory Education (ABLE), and in 2008 he was voted to its governing board.

Dr. Johnson and his wife volunteer their free time to Historic Bethabara Park in Winston-Salem, North Carolina. They are the principal caretakers for the Hortus Medicus, the park's historically accurate restoration of its circa 1761 Moravian medical garden.

CHAPTER 1

A Brief Introduction to Inquiry

When the term *inquiry* comes up in conversations about science curriculum reform and improvement, it usually is shorthand for **inquiry-based learning (IBL)** and, by extension, inquiry-based instruction. But what exactly is meant by inquiry-based learning? How does it differ from “traditional” learning? What is the difference between the way most of us teach (and were taught) and inquiry-based instruction?

First and foremost, inquiry is more than a collection of teaching techniques and classroom principles; it is a mind-set. The instructor focuses on developing the abilities and skills of the learner to use knowledge effectively. In contrast, traditional, didactic instruction focuses mainly on accumulation of content knowledge; it is highly fact- and content-oriented. Halonen, Brown-Anderson, and McKeachie (2002) describe the two philosophies this way:

Content-centered teachers tend to define their primary objective as sharing important facts and concepts with students, with limited attention to the process of learning itself and the thinking that learning requires. Many content-centered teachers believe that merely providing exposure to the ideas of the discipline will

cause students' thinking to evolve naturally over time. Some believe that the capacity to think is innate, and that spending valuable class time promoting changes in thinking seems unnecessary or even misguided.

In contrast, learner-centered teaching elevates the process of learning by requiring students to grapple with ideas, not just passively receive them. Teachers with this pedagogical philosophy accept and relish their responsibility for fostering changes in how students think by emphasizing active learning strategies. Cognitive scientists report that underlying brain structures change to support enduring learning when students think about the course material in more meaningful ways.... Knowledge about how memory functions bolsters the viewpoint that students can improve their thinking skills through well-designed college courses." (2002, pp. 284–285. Copyright 2002 Cengage Learning, Inc. Reproduced with permission.)

The central objectives of inquiry are to (a) encourage students to be active participants in discovering knowledge for themselves and (b) provide them with legitimate opportunities to do so. The following scenarios illustrate these objectives. Two different teaching assistants are leading two general biology laboratory sections. The topic of the day is enzyme function in both classes, but the teaching assistants proceed very differently. One follows a more traditional approach, while the other uses inquiry-centered instructional methods.

Scenario 1: The instructor starts the lab with a 30-minute lecture on biological functions of enzymes and reviews some ideas covered in lecture the previous day. At the end of the lecture, the instructor tells students that their goal for the day is to demonstrate the correlation between enzyme activity of purified beta-galactosidase and temperature. Students have a detailed assay protocol to follow and so are turned loose to complete the exercise. The worksheet that summarizes students' results for the exercise is due next week. Two hours after lab began, the last student leaves for the day.

Scenario 2: The instructor starts class with a question: "What are some common methods we use to prevent food spoilage, and why do you think each one works?" Students are given one minute to come up with their own answers, then they turn to a lab partner and share their answers. After another two minutes, the instructor asks pairs to share their explanations with the class while he or she collates the explanations on a whiteboard. From time to time, the instructor asks quieter students for their ideas and probes others with follow-up questions. For example, when one pair suggests that refrigeration slows microbial growth, the instructor asks, "How specifically does the cold slow growth? What is cold doing to the organisms?" The class fumbles with this question for a few minutes until the quiet pair at the back table suggests that cold somehow inhibits enzymes that microbes use for metabolism and energy production.

After the class has compiled a list of ideas to test, the instructor points out that they cannot test them all but that the lab does have materials available to measure the speed at which bacterial enzymes break down sugar. Then—about 45 minutes after lab started—the instructor gives the class its challenge for the day: Working in groups of three to four, students are to find out whether cold could slow down enzyme activity enough to prevent spoilage. The instructor gives the students some purified beta-galactosidase (one of several enzymes that bacteria use to hydrolyze sugar for energy), a substrate solution and a one-page handout outlining the general steps for measuring enzyme activity. Students must devise the specific procedures they will use and include proper controls. A two-page written summary of their methods and observations is due next week. Three days after the lab has ended, this instructor still is answering questions by e-mail.

These two scenarios are at different points on the continuum that runs between traditional, purely didactic methods at one extreme and purely open-ended, inquiry-based learning and instruction at the other. Scenario 1 exemplifies a demonstration-type lab, in which most of the time students assume a passive role in learning. Note the following elements of Scenario 1:

1. Lab begins with a review of basic course content that many students likely understand already from the earlier lecture and that all students are personally responsible for knowing.
2. The goal of the lab is to demonstrate/confirm (yet again) a well-established piece of general knowledge (hence the pejorative label, “cookbook lab”). Students know what to expect from the exercise and have well-defined procedures to follow. Little thought is necessary to finish the exercise, so students never become cognitively engaged in it.
3. Students work in isolation. They are not required to demonstrate their thinking processes openly to peers or to defend their thinking against challenges. They have no opportunity to test and revise their ideas against the thinking of others.
4. The lab provides no applicable context for the content knowledge. Why should students care that enzyme activity changes with temperature?
5. The worksheet eliminates the need for students to think about how to communicate their results effectively.
6. There is no in-progress assessment of learning. Students receive no feedback regarding their knowledge and skills before the final graded assignment is handed in.

The lab structure in Scenario 2 requires students to actively participate in their learning processes. It begins with an obvious intellectual challenge that builds continuously. To succeed, each student must be deeply engaged with the topic at hand. Some other features worth noting are the following:

1. The instructor asks follow-up questions that uncover students' ongoing thinking processes. This strategy keeps thinking processes out in the open, so students see and learn to model successful patterns. The strategy also helps students move forward when their thought processes have stalled or branched off in unproductive directions.
2. The instructor encourages students to stay actively engaged anytime interest wanes and to think beyond their initial responses.
3. Multiple testable hypotheses are shared and discussed with peers. The "right" ideas are not the only ones considered.
4. Students are required to devise their own procedures and to communicate their rationales. This forces students to think about *how* their new knowledge will be obtained.
5. There are no predetermined results that students must come to; making their own observations and interpreting them is the priority. Moreover, students will not be able to completely answer the challenge question. There is room for interpreting experimental data as well as for further experimentation.
6. Students' understanding is assessed informally several times before the final grade is given.

Neither of these two approaches (didacticism versus inquiry) is fundamentally better than the other. Used properly, both have their place in the classroom. However, they are not interchangeable; at certain times one approach meets instructional goals and students' needs better than the other. To understand why requires looking at how humans learn.

CONSTRUCTIVISM PREDICTS MANY STUDENT LEARNING PATTERNS

Constructivism is a model of human learning that emerged from the work of John Dewey in the early 1900s, Jean Piaget in the 1950s, and David Ausubel in the 1960s and 1970s. Recent research in the fields of cognitive neuroscience and human behavior has confirmed most of the basic tenets of constructivism. According to the constructivist model, thinking patterns and knowledge cannot be transferred unchanged from one person to another because a learner is not a blank slate. As information (in the form of content knowledge or thinking-process skills) is transmitted, the receiving individuals construct their own mental models with it; the models reflect their unique life experiences and past learning. An individual's constructed knowledge exists as two major elements:

- A series of compartmentalized mental models that consist of both content knowledge (i.e., factual information) and related thinking process skills
- A larger-scale mental scaffold that links together the various mental models and determines which models are used most often

When individuals are challenged with learning new content knowledge or process skills, they will attempt to do so by using one of their preexisting mental models. Learners strongly resist developing new mental models as long as an existing model can solve the challenge. If an extant model is used successfully, any new knowledge or skills gained become closely associated with that particular mental model only. In the future, learners will tend to use their newly acquired content information only in the context of that particular mental model, using just the cognitive processing skills associated with it. This process of associating new content and basic process skills with existing mental models occurs routinely and is a necessary component of learning. However, new knowledge and process skills gained this way tend *not* to be applied to other situations, leading to what is often called “shallow learning.”

Deeper learning occurs when a learner faces a question, problem, or situation that his or her current mental models fail completely to resolve. Once all prior mental models fail, the individual begins (usually unconsciously) to assemble one or more new “provisional” mental models and to test them against the current unsolved problem. While provisional models are in play, the person is particularly receptive to learning new content knowledge and process skills. In addition, links to potentially relevant content knowledge and process skills from the person’s other preexisting mental models are established as part of the new provisional mental models. Once a new mental model has been constructed that appears to solve the current problem satisfactorily, it is reinforced and becomes stronger. The new mental model is placed within the larger mental scaffold, and other provisional models are abandoned. If the new mental model is not used regularly after it is created, it fades and is lost. Conversely, a new model that is used subsequently becomes even more stable and grows as additional content knowledge and skills become associated with it. Formation of these new but highly stable mental models is referred to as “deep learning.”

Within the constructivist model, the mental scaffold is a manifestation of the underlying principles that guide a person’s thinking processes *in toto*. This scaffold largely determines which mental models will be used first and how frequently. It is also the mechanism by which connections between mental models are made and by which multiple mental models are brought to bear on a problem simultaneously. Thinking patterns that make up the scaffold include learned priorities, early developmental and educational experiences, and habitual behavior; the remainder of the scaffold is linked to basic personality traits, fundamental belief systems, and one’s sense of self. Because it is so deeply ingrained, the mental scaffold is the component of learning that is most resistant to change and requires the most concentrated effort to do so.

When traditional didacticism and inquiry are compared from the constructivist perspective, the inherent advantages and disadvantages of each approach to teaching become more apparent. Certain student behaviors that regularly frustrate instructors also begin to make more sense. For example, many general education courses are disciplinary surveys that are taught in a strictly didactic, content-centered style. The emphasis is almost entirely on acquisition, memorization, and direct recall of the central facts and content knowledge that underlie the discipline.

To an instructor, this task may seem to be simple and straightforward. After all, many students have already developed several mental models and process skills with which to place that mass of factual information in proper context. However, some percentage of students at the college level will not yet have developed a mental model that allows them to accomplish this apparently simple task. The instructor is unlikely to ever model the thinking-process skills that students should use to accomplish the required task. These students never have an opportunity to learn the necessary skills or to develop a successful mental model that can accomplish the goals of the course. As a result these students take required general education courses two, three, or more times without ever receiving a passing grade.

Even when students pass their required survey courses successfully, they often do not develop the process skills that are intended. Try this experiment: Ask a large group of students to explain a moderately complex concept from a lecture in a didactic survey course taken the preceding semester (or even material covered by a prior exam); then have the students apply it to a novel situation. For the majority, the relevant content knowledge remains highly compartmentalized and is unavailable for recall. Others may recall the information erroneously but have significant misunderstanding of the details or misapply it. A few students will be able to apply their prior knowledge to the novel problem, but reluctantly or with great difficulty.

These outcomes are disheartening, but should not be surprising. A typical general education class is structured to ensure they will occur. Remember that according to the constructivist model, an individual challenged with learning new knowledge or skills will try to accomplish the task using a preexisting mental model. Unlike the students who cannot complete their general education requirements, a majority of traditional-aged college students have a robust mental model that they use to identify relevant facts and retain them for a short time, then recognize correct and incorrect statements relating to those facts on a multiple-choice test. Most are drawing on the same mental models they used in high school to get into college initially. These students were never challenged, so now their existing mental models fail to accomplish the required tasks at the college level.

The outcome would not be different if the same application challenge were issued to students in a typical teaching laboratory instead of a lecture hall. As stated in the Preface, the laboratory experience of most undergraduates is highly scripted and content-centered. Despite claims that the teaching lab is where students get

to “learn by doing,” in reality most students do not gain as much as they could from laboratory experiences because their preexisting mental models are not challenged in a way that fosters deep, meaningful learning. In short, they are not being taught using inquiry methods.

INQUIRY ENCOURAGES DEEPER, MORE FUNCTIONAL LEARNING

In the constructivist view, the ultimate responsibility for learning rests with the learner. The responsibility of the instructor is to serve as a facilitator of the *process* of learning, not as the final source of authority or information. This includes providing learners with achievable challenges, that is, challenges that are beyond their current mental abilities and skill sets but that the learner still has a reasonable chance of accomplishing. When learners succeed in meeting challenges, it builds their confidence and increases their motivation to take on more advanced challenges.

Let's return to Scenario 2, where the instructor used an inquiry-based approach in a general biology lab, and see how learning is being facilitated. Scenario 2 depicts an idealized situation and not every student will respond equally well. Compared to the traditional demonstration lab in Scenario 1, however, even highly resistant students are likely to show significantly greater learning gains.

Students were asked several leading questions:

- *What are some common methods we use to prevent food spoilage?*
- *How does cold slow growth? What is cold doing to microbes?*
- *Will cold slow down enzyme activity enough to prevent spoilage?*

Most students in a general biology course already know that enzyme activity usually rises with temperature; this fact is part of an existing mental model. Using their existing mental models, students also can make reasonable predictions about how cold will affect enzyme activity. However, the final answer to the last question cannot be determined with certainty. From the constructivist viewpoint, students are faced with a challenge that (for most of them) their current mental models cannot solve. These are a few questions students might raise in response to this challenge, for which they cannot provide answers:

- *How cold does it have to be to stop enzyme activity entirely?*
- *Does cold affect all enzymes exactly the same way?*
- *If just one metabolic enzyme is inhibited by cold, is that enough to stop microbial metabolism completely?*

At this point, students will begin unconsciously assembling provisional mental models that might allow them to answer this question. Normally this activity happens within each individual, but because learners are particularly receptive to new content knowledge and process skills at this stage, the instructor requires them to share their thinking processes with peers, then the class, rather than allowing them to work alone. Multiple ideas are considered, and students are asked to look beyond their initial responses and evaluate their provisional mental models more carefully. Students see multiple thinking processes modeled and learn which ones are most successful. There also is the opportunity to build links to potentially relevant content knowledge and process skills that their peers discovered, but they themselves did not.

As they conduct their experiments, students must cooperate in designing an enzyme assay that includes the proper experimental variables and controls. They also must discuss how to interpret their results and present their data. Each of these behaviors reinforces new content and knowledge-processing skills within students' new provisional mental models. As a result, the new provisional model becomes more stable and is linked through the mental scaffold to other existing models.

Inquiry-based instruction does much more than just promote formation of new mental models though. If well executed, inquiry encourages students to revisit and test connections among their preexisting mental models, thus strengthening and consolidating prior knowledge as well. Advanced students can be encouraged to engage in **metacognition**, that is, thinking consciously about how they are thinking and learning. For most students, what happens during learning occurs at an unconscious level. If an instructor poses the appropriate questions, receptive students can be trained to follow their own learning process on a conscious level. Once students become aware of their own learning processes, they can be introduced to formal metacognitive strategies that give them direct access to their mental scaffolds and that heighten the ability to deploy their mental models more flexibly. In the general population, this level of cognitive self-regulation is uncommon and develops well after the undergraduate years. Using inquiry, though, it can be developed much earlier. Metacognitive teaching methods are beyond the scope of this discussion. Those who are interested in knowing more should consult Pintrich, Brown, and Weinstein (1994) and Weinstein (2000, 2002) as starting points for further information.

A FEW WORDS IN DEFENSE OF DIDACTICISM

The preceding discussion may leave readers with the mistaken idea that content-centered instruction should be abandoned. That is not the case; neither didacticism nor inquiry is fundamentally better. They represent two different sets of teaching tools, and each set can achieve certain instructional goals very effectively. The key is to employ each one at the appropriate time. A validated strategy for choosing instructional methods is discussed in Chapter 2.

A traditional lecture remains a good choice if an instructor's goal is content dissemination only. For instance, a lecture is probably the better approach when teaching students the phylogenetic relationships among classes of invertebrates. The amount of time needed for students to develop their own mental models would be considerable, and it is unlikely they would complete the challenge successfully. Similarly, many programs provide undergraduates with straightforward lab safety training. There simply is no need to use inquiry to explain the established procedures for chemical waste disposal, handling and disposing of sharps, use of safety glasses, or similar routine procedures. In the same vein, demonstration laboratories are the best way to train students to perform technically difficult operations or assays. One good example would be teaching students sterile technique in a microbiology laboratory. On the other hand, a more inquiry-oriented approach is likely to be better for introducing students to the concept and goals of phylogenetics and for exploring how that information could be applied.

In the author's experience, didacticism and inquiry can be mixed in a single course (or even a single lab session) very effectively. Imagine that a new two-semester introductory biology course is being developed for training undergraduates to be K–12 teachers. State licensing regulations mandate that these students know a predefined set of content, but the faculty know from experience that these teacher trainees need assistance in building thinking-process skills. The new course might start by laying out the conflict as a constructivist-style challenge to the students:

- *Why is the content mandated by the state considered vital for all students?*
- *How can that content be presented to K–12 students using an inquiry-based approach?*

The challenge to the student teachers would be to develop a general strategy and specific methods for providing state-mandated content to K–12 students while using inquiry-based methods appropriate for each age group. As the teacher trainees identify areas where their current content knowledge of biology is below that mandated by the state standards, faculty instructors would provide didactic minilectures that review the relevant content.

WHY IS DIDACTIC TEACHING SO PREDOMINANT?

Very few instructors operate entirely at either of the two extremes shown in the earlier scenarios. Most use both didactic and inquiry-based instructional methods in their classrooms. That said, college science instructors overwhelmingly rely on didactic methods. Most faculty claim that they encourage deep learning and critical thinking in their classroom. Yet data provided by an observation protocol or other objective evaluation tool usually show otherwise. When pressed to explain why, faculty give reasons that tend to fall into three categories: personal history, predictability, and ease of development and assessment.

Personal History. Biology as a discipline has long emphasized breadth of content knowledge, with the assumption that thinking skills develop naturally. Most current faculty learned successfully in this environment, so they tend to assume others do as well. Moreover, novice teachers tend to emulate techniques and methods they experienced personally.

Predictability. In a content-centered classroom the instructor is the central figure. One individual controls the pace, so the quantity of content delivered can be predicted accurately. Frequently an instructor has been teaching long enough to predict which topics will be most difficult for students and what questions will be asked. Contrast this with inquiry, where the instructor must change and adapt to students' needs and questions. Instructional outcomes are less predictable than they are with didacticism. Inquiry-based teaching requires instructors to be more flexible in their expectations of students. Furthermore, students are already comfortable with their passive role in learning; active learning makes them uncomfortable (especially when it is first introduced), and they are more likely to complain.

Ease of Development and Assessment. Most textbooks and laboratory manuals for undergraduate biology are designed for a content-centered curriculum. Question banks (either from publishers or from the faculty member's old exams) make the process of assessing student learning fairly straightforward. In contrast, inquiry-based teaching often means an instructor must develop new lab exercises, find alternative textbooks, and write new homework assignments. Assessment becomes a more significant problem as well: If the course emphasis is not on gain in content knowledge, how can students be assessed for grades?

Collectively, these elements produce a sort of pedagogical "natural selection" that strongly favors continued use of traditional didactic teaching methods.

WHAT METHODS AND PRACTICES QUALIFY AS INQUIRY?

Fortunately, building an inquiry-based teaching practice is not as arduous as it first looks. Remember that inquiry is defined as any teaching method that encourages students to construct or discover knowledge for themselves, as practicing scientists do. Any teaching practice or exercise that mimics or models the behavior of a scientist in the process of discovery qualifies as inquiry. Most instructors already use some inquiry methods and can learn to incorporate additional techniques quite easily.

General approaches to laboratory instruction can be categorized based on (1) the extent of instructor involvement and (2) the level of challenge students face. Terminology varies among authors; for clarity, the subsequent discussion will use the categories summarized in Table 1, which is adapted from Herron (1971).

Table 1

Features of each category of class exercise

Type of Exercise	Instructor Provides Problem	Instructor Provides Procedure	Solution or Outcome Is Known	Instructor's Involvement
Demonstration	Yes	Yes	Yes	Varies
Structured	Yes	Yes	Instructor only	High
Guided	Yes	No	No	Moderate
Collaborative	Yes	Shared	No	As peer
Open	Part or none	No	No	Minimal

Structured Inquiry. The instructor guides students through an investigation or project, asking them focused questions, giving them suggestions and ideas, and acting as a supervisor of students' work. In labs, the instructor provides a general procedure, but the expected outcome is unknown. This method is particularly suited for large-enrollment courses and for introducing groups of scientifically naive students to inquiry.

Guided Inquiry. The organization is the same as a structured inquiry. However, the procedure for conducting the investigation is developed by the students. Lab courses in which students design and conduct their own experiments usually fall into this category.

Collaborative Inquiry. Students and the instructor work side by side in an authentic investigation of a novel question. Neither party knows the precise outcome of the project. Procedures are developed in collaboration. This form of inquiry is best suited to advanced lab courses and is difficult to use in a nonlab setting.

Open Inquiry. Students investigate questions they themselves have formulated; use procedures of their own design; and conduct, modify, and report on their own experiments. The instructor facilitates this approach by providing physical resources, but for the most part students work on their own. This style of inquiry is, for all intents, the same work pattern that graduate students are expected to follow.

To illustrate the differences between types of inquiry, let's return to the scenarios at the beginning of this chapter. Scenario 1 clearly is a demonstration exercise (alternatively called a confirmation exercise). Students know they should find a linear correlation between temperature and enzyme activity for beta-galactosidase. As a stand-alone lab, this demonstration exercise has little pedagogical value. Suppose though that students are conducting a lengthy inquiry exercise that requires them to measure enzyme activity in extracts of *E. coli* grown under different metabolic stresses. If students cannot perform the basic assay accurately, it is a waste of reagents and their time to conduct the bacterial metabolic stress trials. So the basic assay might be presented as a mastery test; students cannot proceed with experiments until they achieve a predetermined level of competence. This creates a greater incentive to master the procedure quickly and perform it accurately.

In Scenario 1, students were given a worksheet on which to summarize their results. The worksheet can be amended to include a short informal assessment of students' thinking processes. The worksheet also could include one or more application scenarios. Here is a possible scenario:

Suppose your results for the enzyme assay show there is no enzyme activity in a sample collected from stressed bacteria. What are three reasonable explanations for why there is no activity? If you observe this outcome, how will you distinguish between these three possibilities?

This simple informal assessment gives the instructor insight into students' overall ability to interpret their results before they conduct the actual trials. If students cannot interpret their results properly, they may need additional instruction before proceeding.

Scenario 2 is inquiry-based, but what type of inquiry is it? The instructor asks several questions that lead students to their challenge for the day: Can cold slow down enzyme activity enough to prevent spoilage? Students are given tubes of enzyme and substrate and a one-page handout outlining the general steps for measuring enzyme activity. They work with their peers to devise the specific procedures they will follow. This scenario potentially could be classified as either structured or guided inquiry, because, in both types, the problem or challenge originates with the instructor, not the students. Moreover, the final outcome is (likely) known to the instructor but not the students. The major difference is how much procedural guidance the instructor provides and how involved he or she is with students' actual work. Since the instructor provided students with a basic protocol for the

enzyme assay, this scenario seems to fit most closely the description of a structured inquiry. However it can be argued that, since the instructor gave no further guidance beyond that, it fits the description of a guided inquiry instead.

In practice, it is not essential to know exactly what category of inquiry is being used. However, thinking about what type of inquiry is being employed can help instructors know how much written and oral guidance to give students. These categories can be useful for planning and discussing exercises under development or for communicating their approximate level of difficulty. Finally, the categories help guide developers in sequencing labs. Students with no prior experience with structured inquiry are going to struggle with guided or collaborative inquiry. They may succeed in meeting the challenges posed, but they are likely to be so demoralized by the experience that they actively resist subsequent inquiry-based efforts. It is vital to introduce students to inquiry in measured steps, rather than simply throwing them into it without considering their current abilities.

Going back to Scenario 2, how could it be modified to provide the more advanced types of inquiry-based instruction? In a true guided inquiry, the procedures should be devised almost entirely by the student. Rather than providing even a skeleton of a protocol, students would be required to find their beta-galactosidase enzyme assay protocol in the primary literature. Alternatively, students could be given just the enzyme assay, but have to devise their own method for extracting the active enzyme from the live *E. coli*. Both strategies leave an essential piece of the procedure up to the students to develop.

In general, collaborative and open inquiries are not practical in large enrollment labs. However, it is helpful to understand how they fit into the overall scheme. For a collaborative inquiry, a group of two to three students working with a faculty member might be challenged to adapt a standard beta-galactosidase assay to measure metabolic activity of marine archaeobacteria from thermal vents. In an open inquiry, one or two undergraduates who have learned about the beta-galactosidase assay in lecture may want to determine whether human lactose intolerance can be diagnosed by measuring beta-galactosidase enzyme levels in human saliva. They are provided space to work and access to reagents but mostly work alone to answer the question they have posed. Students who reach this stage are functioning essentially as independent investigators.

BENEFITS OF MIXING INQUIRY STYLES

It should be no surprise that teaching with inquiry requires the instructor to be more flexible and responsive to individual students' needs compared with didactic methods. This seems to be a fundamental obstacle to using inquiry in large-enrollment laboratory courses with multiple sections and instructors. However, in developing the units presented in this book, the author has learned two very important lessons about inquiry-based teaching. First, inquiry is not only possible

in such a setting; it actually makes it easier to manage and maintain consistency between instructors and sections. As later chapters will explain, developing a good inquiry-based course starts with establishing clear content and performance goals for each stage and for the course overall. These goals provide each instructor with a clear road map to the course and benchmarks for assessing how well students are meeting those goals. Second, mixing inquiry styles makes it possible to engage and challenge a large number of students across a broad range of ability levels.

To understand how mixed inquiry works, the reader should look at the organizational structure of one of the lab units. Every unit contains one or more exercises organized as structured inquiries. Most units also have an option for students to design and conduct their own experiments, that is, to engage in a guided inquiry. Unless otherwise noted, the units are designed so that students complete the structured inquiry as a class in one week, then work in smaller groups to design and conduct their own experiment during the following week.

The structured phase of each unit has been designed so that students discover basic content knowledge for themselves. To do so, students must apply certain process skills that the instructor is seeking to build. (For convenience, the primary and secondary learning goals, and the process skills being developed, are outlined in the Instructors' Notes for each unit.) Since each unit is designed to lead students fairly autonomously through the discovery process, differences in group facilitation skills of instructors is not a major issue in the structured phase. Thus the lab coordinator or faculty supervisor does not need to spend as much time ensuring that every instructor is conducting the lab in exactly the same way.

For the guided phase of each unit, student groups of two to four outline their experiment in advance on an experimental outline form (see Sample Form for Students' Experimental Outline in Appendix B) that they submit to their instructor for approval; experiments that have been proposed frequently are listed in the Instructors' Notes. Often small groups will devise experiments that are simple extensions of the procedures given in the first part of the unit (such as testing a higher temperature or a broader pH range than was used the first week). For many students—particularly nonmajors and students new to inquiry—this will be sufficiently challenging. As students gain confidence, they design more complex experiments that depart more from the procedures of the first week. Since small groups work independently to conduct experiments, each is free to design an experiment that interests and challenges its group members but still is within their ability to accomplish. The instructor can encourage students to push a little beyond their current abilities and knowledge each time they design and execute a new experiment. As students' skills improve, they frequently ask novel questions that are well outside the experience of the instructor. With a little creativity, though, it is still usually possible to accommodate their experimental designs.

Combining structured and guided phases offers other advantages. Small groups often evolve into informal learning teams. Individuals begin to share

knowledge (peer instruction) and test each other's thinking and understanding in much the same way that the structured inquiry is designed to do. More advanced students also serve as peer and near-peer role models for students whose thinking skills have not progressed as far yet. Instructors gain more time during lab to work with students individually or in small groups. There are fewer model systems that students must master each semester, so the instructor can conduct more in-depth explorations of key principles and help students refine their thinking-process skills. For lab coordinators, structured and guided inquiry reduce the number of model organisms and equipment that must be obtained and maintained. Time once spent managing materials becomes available to assist and coach novice instructors in inquiry-based instruction.

GOING BEYOND THE BASICS

This chapter provided only a very brief outline of the fundamental differences between inquiry and traditional didactic teaching and the key features of the major inquiry-based instructional styles. Those wanting to know more about the general theory and practice of inquiry should consult Bell, Smetna, and Binns (2005), Eick, Meadows, and Balkcom (2005), Gardiner (1994), Lawson (2002), and Mintzes and Leonard (2006). For a discussion of constructivism as it relates to teaching and learning, readers should consult Gardiner (1994) and Taylor, Gilmer, and Tobin (2002).

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UNIT 2

Mendelian Genetics

Student Pages*

BACKGROUND

In 1866, Gregor Mendel published a model that explained how seven characteristics are inherited in pea plants. His model established three laws: (1) the law of discrete inheritance, (2) the law of segregation, and (3) the law of independent assortment. According to Mendel, the seven physical features of pea plants are each controlled by two units of inheritance, which are passed from parental plants to offspring. One unit comes from each parent, and these units can be either dominant or recessive to each other. His law of segregation states that the two units of inheritance separate from one another when a pea plant produces offspring (seed). Each offspring inherits one of each parental plant's two possible units of inheritance. Finally, Mendel stated that the units of inheritance for the traits he studied assort independently. For example, a trait like seed shape passes to offspring independently of another trait like flower color. Using his simple rules, he could predict both what types of offspring would develop when he crossed any two pea plants and the relative numbers of each type. What made Mendel's rules so exciting to the scientific community was that they predicted inheritance patterns for many other eukaryotic organisms as well.

*Teacher Pages begin on page 123.

More than 30 years later, Walter Sutton finally described a physical mechanism that explained Mendel's observations. Sutton demonstrated that most cells are **diploid**, that is, they have two **homologous** chromosomes. Homologous chromosomes have the same overall shape and size, and genetic information is usually arranged in about the same order along the DNA strands. Sutton found that prior to mitosis (normal cell division), cells duplicate every chromosome. As part of mitosis, a copy of every chromosome passes to each of the two diploid daughter cells. The daughter cells still have one copy of each chromosome in the homologous pair. In preparation for reproduction, plant or animal cells undergo a distinctive form of cell division called **meiosis**. In the first stage of meiosis, the two homologous chromosomes separate, and only one goes into each of two daughter cells. The maternally and paternally derived member of a pair of homologous chromosomes can go randomly to either of the two daughter cells during meiosis. Sutton had uncovered a physical process that explained Mendel's law of segregation and independent assortment.

Chromosome Structure and Inheritance

Eukaryotic organisms have two types of chromosomes: (1) autosomes, which are inherited independently of the sex of the parent or offspring, and (2) sex chromosomes, which are inherited in a specific pattern from each parent and determine the sex of the offspring.

Genetic information is not scattered randomly along the strands of DNA that make up chromosomes. The DNA code for a specific enzyme, structural protein, or RNA is found at a discrete physical location on the strand, called a **locus** (plural is **loci**). Figure 2.1 shows the double-ended arrow pointing to a specific locus.

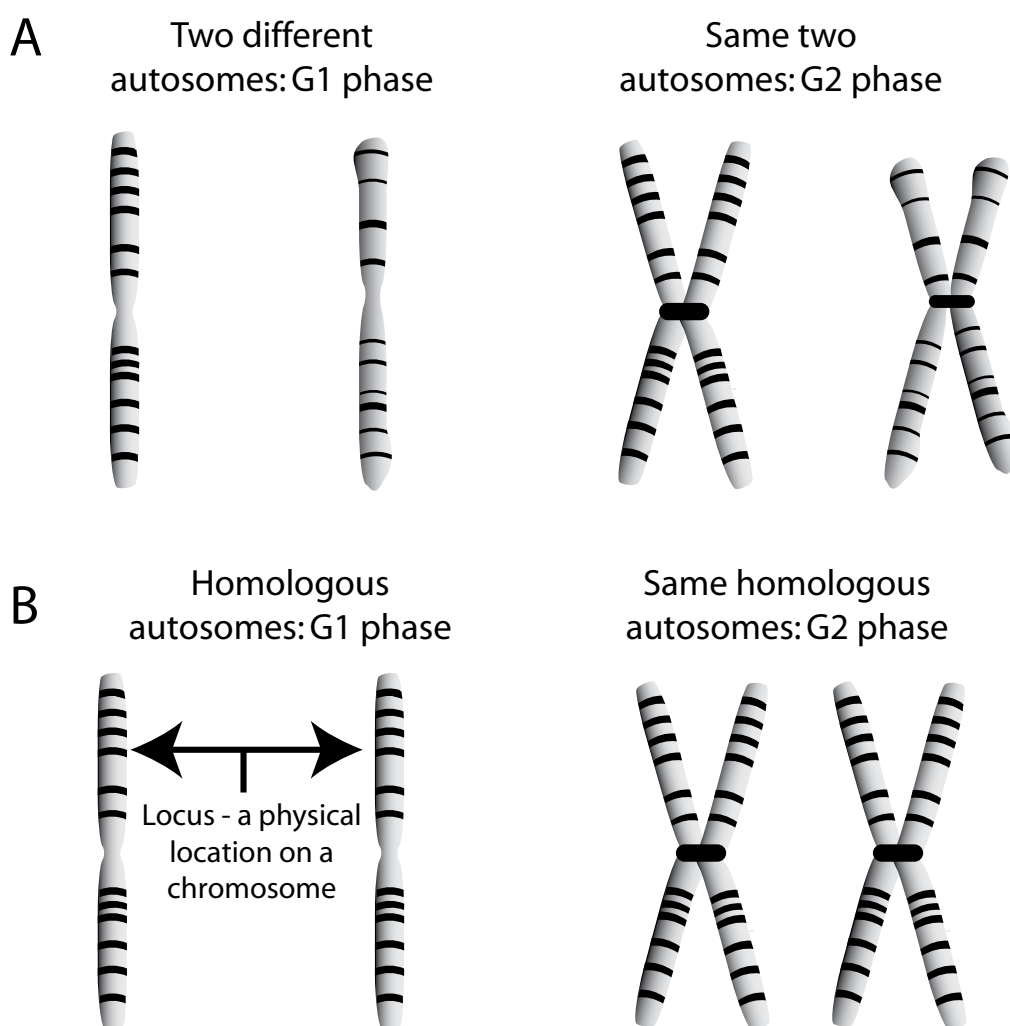
Figure 2.1

Basic genetic terminology

The left side of each panel shows the chromosomes before DNA replication in S-phase, the right side, after replication

Panel A shows two different, non-homologous chromosomes. They have different physical structure and banding patterns, and do not encode the same information.

Panel B shows two homologous chromosomes. They have the same general structure, and encode the same general information. The specific version of information (i.e., allele) at each position (locus) may be different, but the loci will be in the same positions on the two chromosomes.



In a normal homologous pair of autosomes, a particular locus will be at the same physical location on both of the chromosomes, regardless of which parent contributed the autosome. Yet the autosomes in a homologous pair may not be exactly alike. At any particular locus, there can be slightly different versions of the DNA code, called **alleles**. Each allele encodes the same general protein. However, the protein produced when each allele is translated may be slightly different.

Going back to the earlier example, imagine the locus shown in Figure 2.1 codes for the enzyme “greenase,” which catalyzes production of a green eye pigment. The most common allele (version) of that locus codes for a very active form of greenase. An organism that is homozygous for this most common allele makes lots of pigment and so has dark green eyes. A second, less common allele for that locus codes for a version of greenase that makes half as much pigment. As a result, an organism that is homozygous for the second allele probably will have light green instead of dark green eyes. A third allele may contain an early stop codon, so that no greenase is produced and no green eye pigment is made. The result: Organisms that are homozygous for this third allele have pale eyes that are not green at all.

Any given locus can have two, three, or dozens of alleles. However, an individual organism can only have two alleles for each locus: the one on the chromosome inherited from the female parent and the one inherited from the male parent. Which two alleles were inherited from the parents at that locus is what determines the eye color of the offspring.

Alleles Behave Differently on Sex Chromosomes

In diploid organisms, the sex chromosomes do not always form a homologous pair. If an organism has two copies of the X chromosome (which is the larger sex chromosome), it is usually a female. However, if it has one X chromosome and one smaller, Y chromosome, the organism is usually male. In some cases (like bees), there is no Y chromosome, and the male organisms carry just one X chromosome.

Like autosomes, X and Y sex chromosomes have loci that control certain functions, and there may be two or more alleles at each locus. However, the rules for expression of dominant/recessive phenotypes are different for sex chromosomes. Imagine there is a recessive allele for a locus on an X chromosome. A female organism (which has two X chromosomes) must inherit two copies of that recessive allele before it will express the trait. A male organism does not have a second X chromosome to compensate for any recessive alleles. Therefore, male organisms express nearly all recessive alleles they inherit that are on the X chromosome.

Goals for This Unit

Mendel, Sutton, and many others relied on experimental crosses between organisms to uncover the principles of inheritance. Today, experimental crosses still are an essential tool for genetics research. In the clinic, physicians and genetic counselors must understand the principles of inheritance before they can accurately advise patients who have a family history of genetic disease.

To begin this unit, you will complete two exercises that teach you how to handle and sort fruit flies (*Drosophila*) and how to recognize wild-type and mutant strains. Once you master these core skills you will be given two sets of vials containing separate stocks of wild-type and mutant strains of flies. The mutant strains are homozygous for one or more alleles and are true-breeding. However, not everyone in the lab will be assigned flies of the same genotype.

Over the next several weeks, you must accumulate sufficient data to answer these questions about your assigned mutant:

What is the mutant phenotype of my assigned strain of flies?

Is this mutant phenotype caused by a dominant or by a recessive gene? An autosomal or a sex-linked gene?

Is this mutant phenotype caused by one gene or by interactions among two or more genes?

If more than one gene is involved, does the inheritance pattern suggest they are on the same chromosome or on separate chromosomes?

Answering these questions will require you to perform several test crosses and tabulate the phenotypes of offspring through at least the F₂ generation. It is your responsibility to decide on crosses, set them up, and monitor their progress. To assist you, the General Procedures section on page 116 describes all the methods you will need to complete this project.

General Safety Precautions

Drosophila, their media, and the carbon dioxide tablets used for anesthesia are harmless. Escaped flies can become a nuisance if they are not kept under control. Make sure all vials and containers are plugged well to prevent escapes, especially before disposal. Destroy any vials of flies that you will not use again. To eliminate food sources for escapees, clean your workbench and the shared areas thoroughly each time you finish working with flies.

UNIT EXERCISES

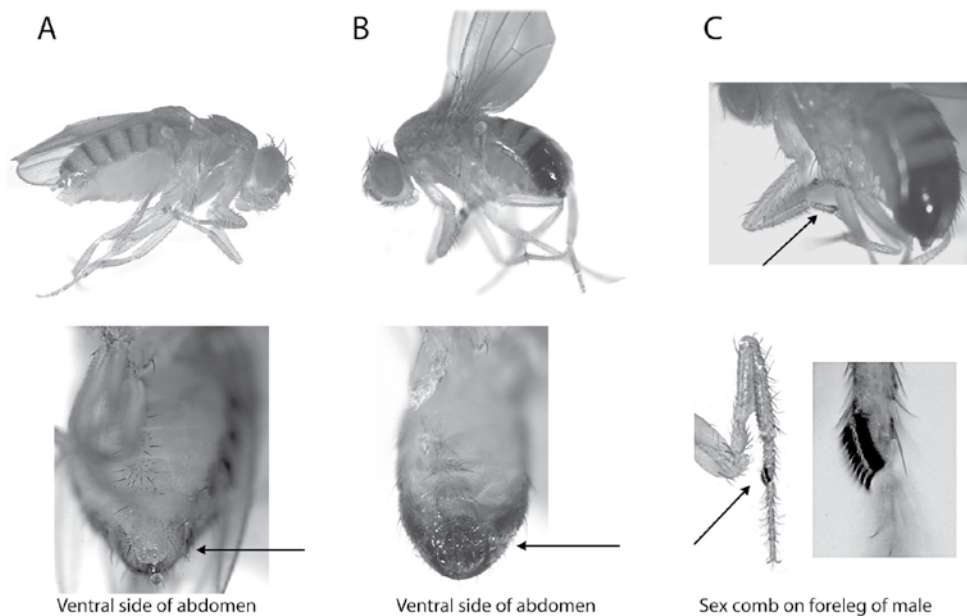
Exercise 1: Sorting Flies by Sex

Background

The major differences between male and female flies are shown in Figure 2.2.

Figure 2.2

Sexually dimorphic features used to distinguish male and female *Drosophila*



- Males have a dark sex comb on each of their front legs. This is the most reliable marker to use.
- On the posterior, ventral side of the abdomen the male has two claspers; females lack claspers.
- The male's abdomen tends to be more rounded and blunt with darker markings at the tip, especially on the ventral surface.

If you are not certain if you see sex combs, the other features should allow you to consistently separate males from females. If you are ever in doubt though, you should kill a fly of indeterminate sex and place it into the fly morgue, rather than risking adding a male to a vial of virgins you plan to use for test crosses.

Procedure

1. Take a vial of flies labeled “Practice: Wild Type” back to your desk. These flies are still awake, so you will need to anesthetize them. Instructions are given in General Procedures (p. 116) and your instructor will demonstrate this procedure for you.
2. Empty the vial of flies onto a white index card attached to a cold block, then place the block on the stage of a dissecting microscope equipped with an overhead light. Using a soft brush, gently push and turn flies on the card.
3. Look at several flies until you are certain that you can identify each of the three dimorphic features previously listed. Sort out six flies that you are certain are male and six more that are female. Then ask your instructor to see whether you have sorted them accurately. If you made a mistake, go back and double-check the features on each one.
4. After you have successfully sexed your first group of 12 flies, sort the remainder of the flies in the practice vial into three groups: males, females, and undetermined. Record your counts in Table 2.1.

Table 2.1

Sex ratios of sorted flies

	# Males	# Females	# Undetermined
Your count			
Partner’s count			

5. Have your partner double-check your sorted flies. If your partner doesn’t agree with you the way you have sorted the flies, ask your instructor to check your work.
6. When you are finished, return all of the wild-type flies to the practice vial so others may use them.

Exercise 2: Identifying Mutant Phenotypes**Procedure**

1. Take one of the vials labeled “Practice: Mutants” back to your bench. This vial contains flies with several different mutant phenotypes. Anesthetize them as before.
2. Using the microscope, look for differences between the mutants and wild-type flies in any of the following features: eye shape, eye color, body color, wing size or shape, or body bristles.

3. Each time you think you have identified a distinct mutant phenotype, give it a descriptive name and write it down in Table 2.2.
4. As you work, you will find other flies with the same mutant phenotype. Sort your flies into phenotypes, then tally up the number and sex for each phenotype you find, and write them down in Table 2.2.
5. When you are finished, have your instructor double-check that you have sorted the flies correctly.

Table 2.2

Summary of the fly mutations in your mixed stock

Description of Mutant Phenotype	# Males	# Females	# Undetermined

Exercise 3: Determining the Inheritance Patterns of a *Drosophila* Mutant

Procedure

1. Your instructor will give you four vials of flies.
 - Two vials are marked “+” and contain normal, wild-type flies. You can assume that their genotype is “+/+” at every locus.
 - Two vials will be marked “Mutant” and have some other designation letter or number. All the flies in your mutant vials should have the same phenotype; flies with different phenotypes were not mixed.
2. Identify the mutant phenotype of your assigned flies. Choose a descriptive term for any mutation you see (e.g., eyeless or purple-striped).

- Record which mutant you were assigned and your description of it in your lab notebook. Check with the instructor to confirm that you have correctly identified its mutant phenotype.

From here on, you must care for your own flies. Written procedures for setting up cultures, anesthetizing flies, collecting virgins, and other procedures are provided. However, more specific instructions are not possible. Your instructor can guide you, but it is your responsibility to decide which test crosses you need to identify the inheritance pattern of the mutation(s) in your flies.

Hints for Planning Out Test Crosses

Your instructor will show you how to use a Punnet square and the known genotypes of two parents to predict the genotypes (and phenotype ratios) of their offspring. If you need additional assistance, there is a tutorial at the end of the General Procedures section.

In a test cross, virgin flies of an unknown genotype are mated with flies of a known genotype. The first and second generation of offspring are counted and sorted, and the phenotype ratios calculated. Given the known genotype of one parent and phenotype ratios observed in the offspring, you can determine the genotype of the unknown parent.

- Start by drawing a Punnet square for one possible cross and inheritance pattern (e.g., flies that are homozygous for a single autosomal recessive mutant crossed to wild-type flies). Calculate and record the expected normal and mutant phenotype ratios for the F1 and F2 generations.
- Do the same for other possible patterns of inheritance.
- Based on your Punnet squares, determine which crosses you can use to differentiate between various patterns of inheritance. Record them in your notebook, along with your rationale. If you will need to perform more than one test cross, explain why.
- When you have determined which test crosses allow you to distinguish the various inheritance patterns, you and your partner should set up those test crosses using your assigned mutant flies. Set them up as soon as possible.
 - Determine the number of males and virgin females you will need of your wild-type and assigned mutant flies.
 - Look for pupae in your vials, and try to estimate when you can begin to collect virgins (it should be within five to seven days).
 - Once flies begin to emerge, you will need to come to the lab every six to eight hours. Make up a schedule of the times outside of the normal lab meetings when you or your partner will come in to collect virgins.

Keep Up With Your Notebook Entries

Your notebook is a running record of everything you do for lab. It should contain every bit of the data you collect. When your instructor looks through your notebook, there should be entries for every time you came to the lab to work. Your notebook should contain all of your daily observations, a copy of any data your partner(s) collected, detailed descriptions and rationale for all experimental crosses you do, the Punnet squares you used to calculate genotype and phenotype ratios, the raw numbers from when you count flies, and other data you collect. In short, based on what you write, the instructor should be able to reconstruct every last thing you did, why you did it, and what the outcome was.

General Procedures

Routine Care

Once you receive your vials of flies, you must maintain your own working stocks. Working stocks are essential; if your first test crosses fail, you can start again using your working stocks.

1. Every week, transfer 10–15 flies of each type to new vials with fresh food. You do not even need to anesthetize flies to transfer them to new vials.
2. Keep older working stocks until adults start emerging from newer working stock vials, then dispose of the older stocks.

Anesthetizing Flies

1. Transfer flies to be anesthetized into an empty plastic vial. It requires some skill to transfer flies between vials, then plug both vials. Your instructor will demonstrate the proper technique. Some flies may escape initially, but you will become better at doing this with practice.
2. Place the vial of flies to be anesthetized into a vial holder or tube rack.
3. Get a cold pack from the freezer. Secure a white index card onto the cold pack with rubber bands.
4. Take an anesthetizer, and add 10 mL of water to the vial. Place the vial next to your vial of flies in the holder.
5. Carefully slide the end of the anesthetizer tube between the foam plug and the wall of the vial containing your flies. Do not remove the foam plug!
6. When you are ready to anesthetize flies, add half of one CO₂ tablet to the water in the anesthetizer vial. Immediately plug it with the stopper, so the gas produced is directed into the vial of flies. Do not allow liquid to bubble up and push through the tubing into the vial of flies; they will drown.
7. As the CO₂ is being administered, gently tap the vial of flies on the counter so they drop to the bottom of the vial. As soon as flies are immobile, pour them onto the white index card attached to the cold pack.

8. Place a paper towel on the viewing stage of a stereomicroscope to catch condensation. Place the cold pack on top, then use a benchtop lamp to illuminate the flies from above.
9. Once chilled, you can work with the flies for up to one hour. Use a soft brush to move them around.
10. If you need to keep flies after observing or sorting them, carefully brush them from the card into a fresh vial containing food. Immediately plug the vial, and lay it on its side to keep the flies from getting stuck to the food. You can stand the vials upright when the flies revive after one to two minutes. Dump unneeded flies into the “fly morgue” on your bench.

Preparing Food Vials

1. Determine the number of vials you need for the day. Do not make up a large number of extra vials of media. Without flies present, they become overrun with bacteria.
2. Label each plastic vial with your initials, lab section, date, and contents.
3. Working at the sink, put 10 mL of dehydrated fly media in the bottom of each tube. Add 10 mL of spring water or distilled water to each vial, and immediately swirl it gently to mix the media and water.
4. Once the media solidifies (less than five minutes), add one grain of dry yeast to each vial. **WARNING:** If you add too much yeast to a vial, your flies can die of carbon dioxide poisoning.
5. Place a plug in the top of each tube, with about half of the plug sticking out of the vial.
6. Clean up all media or yeast you spilled and sanitize the counter with ethanol.

Disposing of Used Fly Vials

Flies are grown in inexpensive plastic shell vials. The vials cannot be adequately cleaned for reuse, so do not try to recycle them.

1. To destroy a culture, push the foam plug down so that it is flush with the top of the vial. **DO NOT** push plugs all the way down into the food!
2. Place vials in the box marked “Discarded Vials.” They will be frozen to kill the flies prior to disposal.

Controlling Bacteria

If there are not enough larvae present, bacteria can overrun the media. Heavy infections usually kill the flies. To control bacterial growth:

1. Do **NOT** open any fly vials you find containing a large amount of bacteria, which appear as a pale to tan slimy film on the surface of the food. Push the stopper down in the vial and place the vial into the waste box for disposal.

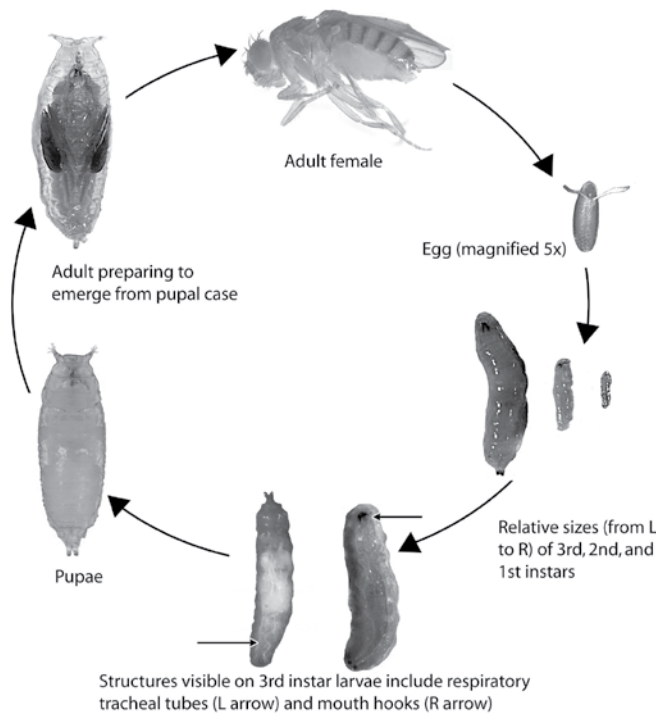
2. Do not keep old stocks around. Once you are finished with a cross or a stock, put it in the waste box.
3. When you are making up vials of fly food, make sure to use clean vials and stoppers. Do not reuse vials and stoppers.
4. Do not put more than one or two grains of yeast into a vial of media. Excess yeast provides nutrients that encourage bacterial growth.
5. If a vial has only a small amount of bacteria, it can be treated with antibiotics. Using a disposable transfer pipet, add three drops of 100x antibiotic solution to the surface of the culture media. After three days, add another three drops. It is not necessary to unplug a vial to add antibiotics. Simply slide the transfer pipet between the vial wall and foam plug.
6. If the bacteria continue to spread, do not try to rescue the flies. Instead, destroy the vial and reset the cross or stock.

Collecting Female Virgin Flies

The general life cycle of fruit flies is shown in Figure 2.3.

Figure 2.3

Life cycle of *Drosophila*



Female flies mate for the first time 10–12 hours after emerging from their pupal cases. They mate with more than one male, and store sperm for fertilization later. The only way you can ensure that you know which male they mate with is to use virgin females for test crosses.

You obtain virgin female flies by removing all of the adult flies from a culture, waiting six to eight hours, then collecting and separating the newly emerged females from the males. To collect virgins for crosses, you will need one or more vials of flies that are 9–11 days old. Look for dark pupae on the sides of the vial; these are flies getting ready to emerge.

It can take several days to collect enough virgins for crosses, and you will need to return to the lab every six hours or so. Divide the workload equally between you and your partner, decide in advance who is collecting when, and exchange e-mail addresses and telephone numbers, in case you must get in touch quickly.

1. Remove all adults from the vials from which you plan to collect virgins. If live flies are stuck to the food, push them down into the food with a probe or pencil.
2. Return the vials to the incubator for six to eight hours.
3. After six to eight hours (*no longer*), collect all the newly emerged adult flies. Anesthetize them with carbon dioxide, and separate the sexes into two different vials. The females will be virgins that can be used for crosses. The males can be used in an appropriate cross or discarded in the fly morgue.
4. If you did not obtain enough virgins during the first collection, put the stock vials back in the incubator. Return every six to eight hours; each time, collect and sort the adults by sex.

Setting Up a Test Cross Vial

Usually you will need two to three vials for each test cross to produce sufficient offspring for counting. If you do not have enough offspring, your phenotype ratios may be skewed.

1. Collect five to seven female flies (use virgins if necessary), and three to five males of the appropriate phenotype. These will be the parental, or P1, generation. Label the vial with the genotypes and sexes of the parents, your initials, and the date.
2. Add the flies, and plug the vial. When the flies wake up, place the vial in your rack in the incubator.
3. After four to five days, place the entire vial on its side under a stereomicroscope. Focus on the food near the wall of the vial. You should see small larvae crawling around within the food.
4. Once larvae are present, remove the P1 parents from the vial. Transfer them to a fresh vial of food (you do not need to anesthetize them), and label it carefully. This is a backup vial for the P1 mating. After four to five

days in the second vial, larvae should be present in the backup vial too. At this point, discard the adult flies in the morgue.

5. Twelve to fourteen days after you first added adults, the first generation of offspring (the F1 generation) will begin emerging from pupal cases along the side of the vial. Anesthetize them and separate them by phenotypes under the stereomicroscope. Record the phenotypes and sexes of all the flies. Keep counting flies in the F1 generation until you have scored 100–200 flies.

Frequently you will need to cross siblings from the F1 generation to produce an F2 generation. This is known as an **inter-se cross** (brother-sister mating). For inter-se crosses, the females need not be virgins.

6. After sorting, and while they are still anesthetized, transfer five to seven female and three to five male F1 flies to a fresh, labeled vial of food. Some of the females will already have mated and will begin laying eggs shortly after being transferred to the new vial.
7. After four to five days, transfer the F1 flies to a fresh vial of food, to make a backup of the F1 cross. After another four to five days, remove the adults and discard them.
8. About 12–14 days after you added the F1 adults, F2 progeny will begin to emerge from pupal cases. Anesthetize them, separate the flies by sex and phenotypes, and count them. Record the phenotypes and numbers in your lab notebook.
9. Continue to sort and count emerging F2 flies for several more days. You should count as many flies as you can (ideally, 200 or more). Do not count vials beyond six days, because early F3 flies may begin to emerge that will confound your results. If necessary, count the adults that emerge in your backup vials.
10. Once you have counted your F2 flies, dispose of them in the fly morgue. Do not put flies that have already been counted back into their vial.
11. Use a chi-square test to determine whether your observed results are significantly different from the expected phenotype ratios for a particular pattern of inheritance.

Using Punnet Squares to Determine Expected Genotype and Phenotype Ratios

To determine whether the mutation(s) in your unknown flies follow a particular inheritance pattern, you first must know what the potential patterns could be. You should already know that when wild-type flies and flies with a recessive mutation are crossed, the mutation may disappear from the F1 generation. If F1 siblings are mated though, the mutation usually reappears in some of their offspring. The precise ratio of normal and mutant phenotypes can tell you if the mutation is dominant or recessive, sex-linked or autosomal, single or double, or some other type of inheritance pattern.

For example, wild-type *Drosophila* have brick red eyes and flat wings that extend beyond the tip of the abdomen. There is a recessive mutation in a locus on Chromosome #2 called *apterous*; homozygous recessive flies never develop wings. Another recessive mutation called *eyeless* is found on Chromosome #4; homozygous mutants never develop compound eyes.

Suppose an apterous fly was crossed with an eyeless fly and, after two weeks, their F1 offspring were crossed. What would be the predicted outcome? Using a Punnet square (see Table 2.3 and Table 2.4), the crosses break down as follows:

First cross: EEaa (normal eye, apterous) × eeAA (eyeless, normal wing)

Table 2.3

Punnet square for the first cross

		Potential Alleles From <i>Apterous</i>	
		Ea	Ea
Potential alleles from <i>Eyeless</i>	eA	EeAa	EeAa
	eA	EeAa	EeAa

F1 inter-se cross: EaAa (normal eye and wing) × EeAa

Table 2.4

Punnet square for the F1 inter-se cross

		Potential Alleles From First Parent			
		EA	Ea	eA	ea
Potential alleles from second parent	EA	EEAA	EEAa	EeAA	EeAa
	Ea	EEAa	EEaa	EeAa	Eeaa
	eA	EeAA	EeAa	eeAA	eeAa
	ea	EeAa	Eeaa	eeAa	eeaa

Now assuming that the alleles E and A are fully dominant to the recessive e and a alleles, there are 16 flies with nine possible genotypes, that lead to four possible phenotypes (Table 2.5, p. 122).

Table 2.5

Genotypes and phenotypes of the F2 generation

Genotype	# Flies w/ Genotype	Description of Phenotype	# Flies w/ Phenotype
EEAA	1	Normal eyes, normal wings	9
EEAa	2		
EeAA	2		
EeAa	4		
EEaa	1	Normal eyes, wings absent	3
Eeaa	2		
eeAA	1	Eyes absent, wings normal	3
eeAa	2		
eeaa	1	Both eyes and wings absent	1

The expected **phenotypic ratio** would be 9:3:3:1. This means that, on average, for every 16 flies in the F2 generation there should be 9 normal flies, 3 flies without eyes, 3 flies without wings, and 1 fly with both eyes and wings missing.

You can use phenotype ratios to work backwards as well. For example, if you count flies in a test cross and find there are four phenotypes that occur in a 9:3:3:1 ratio, it is very likely that the phenotypes are the result of two mutations on two different autosomes. If you generate Punnett squares for each of the other possible mutations (such as autosomal, sex-linked, and so on) that can occur, you can calculate phenotype ratios for each of them as well. If you subsequently see a particular ratio of phenotypes in a test cross, you will know the most likely mutation(s) to have caused that phenotype ratio.

Frequently the observed phenotype ratios in a test cross do not exactly match the expected phenotype ratios. For a detailed explanation of how to perform a chi-square analysis that compares observed and expected phenotype ratios, consult a statistics textbook or online source.

UNIT 2

Mendelian Genetics

Teacher Pages

INSTRUCTORS' NOTES

Background and Key Concepts

In a typical Mendelian genetics lab, students cross flies or other organisms of known genotypes, score phenotypes of the offspring, and determine if their results are significantly different from expected phenotype ratios. For this unit, the traditional dihybrid cross lab has been reformatted into an inquiry-based exercise. Students receive two vials of wild-type (Canton S or Oregon R) flies and two vials of mutant flies, but they are *not* told the genotype of assigned mutants. During the first week they must identify the mutation(s) present by comparing the adults in the two vials. Subsequently students must cross mutant flies to wild-type flies and, based on phenotype ratios in the F1 and F2 generations, determine the pattern of inheritance and most likely genotype of the original mutants. Students decide for themselves what crosses must be done to uncover the pattern of inheritance.

Other skills that students learn during this unit are

- *Drosophila* culture and care,
- how to sort flies by sex and identify mutant phenotypes, and
- how to use Punnet squares to predict genotype and phenotype ratios.

For unknowns, there are four different strains of white-eyed flies. Eye color in *Drosophila* depends on two pigments—one bright orange and the other dark brown. In wild-type flies, these two pigments are present in about equal concentrations, making the eyes brick red. There also is a central point where ommatidia are much darker than the surrounding ones. A mutation that inactivates any enzyme in

the brown pigment pathway causes flies to have bright orange or scarlet eyes. Usually they also lack the dark central spot, a feature that helps in distinguishing older orange-eyed flies from young wild-type flies. Conversely, a mutation that inactivates any enzyme in the orange pigment pathway causes the eyes to be dark brown.

A white-eyed phenotype can occur for several reasons. There is an X-linked, single allele mutation that inactivates the ABC transporter that carries both pigments to their final destination in the ommatidia; as a result, the eyes are white. White-eyed flies also may be homozygous recessive for inactivating autosomal mutations in *both* pigment paths. Other fly strains have an autosomal mutation in one pigment pathway and the X-linked mutation in the ABC transporter. When students cross their particular strain of white-eyed mutants to wild-type flies, different ratios of orange-, brown-, white-, and brick red-eyed progeny will emerge in the F1 and F2 generations. Based on the phenotypic ratios, students can deduce the pattern of inheritance and, from this, the most likely genotype of their original parental strain of white-eyed mutant flies.

General Teaching Strategy and Common Problems

The author's program uses this unit as a self-paced, half-semester lab project in a genetics course for sophomore majors. The open format works best because collecting virgins, transferring adults, setting crosses, and backing up stocks invariably needs to be done at times other than when lab meetings are normally scheduled. At the first lab regular meeting students complete Exercises 1 and 2, then are given their stock vials of white-eyed mutant and wild-type flies. Subsequently students work mostly on their own. They are responsible for completing Exercise 3 and maintaining their stocks. Students are required to come to lab weekly for 30 minutes so the instructor can check their progress and notebooks. Students who are on track to solve the problem may leave or work independently, while those who are not making progress spend additional time with the instructor to get back on track. Alternatively, the students and instructor can use part of the lab meeting time to solve and discuss genetics word problems.

All of the eye color mutants can be identified using the same two crosses. In the parental generation, students should mate wild-type males to virgin mutant females, and mutant males to wild-type virgin females. If students score the phenotypes of the F1 progeny, then cross siblings to each other, they should have sufficient information to determine the inheritance patterns for any mutant strain. However, the instructor should NOT tell students what crosses to do. The ultimate success of this unit depends on the students selecting the required crosses themselves.

Students respond in two very different ways to this unit. One group will claim that "we did this in high school," decide the phenotype is the result of a single, sex-linked mutation, and plan their crosses accordingly. When these students count offspring, there will be two entirely new phenotypes, which is inconsistent with

their starting idea that the phenotype was the result of a single mutant locus. Use their confusion as an opportunity to explain how a single phenotype can result from many alternative mechanisms. Their task is to determine which of all these options is actually operational in *their* mutant line.

The unknowns overwhelm other students because they think there are endless possibilities. Tell students to assume for a moment that eye color is due to a single autosomal mutation. Ask them, What phenotypic ratios would you expect in the F1 generation if you crossed wild-type and homozygous mutant white-eyed flies (i.e., a monohybrid cross)? Now what about in the F2 generation? Once they can answer these basic questions, ask them to repeat the thought process for dihybrid autosomal crosses, then sex-linked monohybrid crosses. Most students soon realize there are a limited number of discrete possibilities.

Recommended Prelab Skills

Students should be able to generate a Punnet square for a monohybrid cross. They will learn to generate Punnet squares for dihybrid and X-linked crosses as the unit progresses. It is useful if students have a basic working knowledge of a dissecting microscope, but the skill can be learned quickly if not done previously.

Assessment

If pre- and postlab quizzes are used as part of formal assessment, both the prelab and postlab quiz should focus on problem-solving skills. Since students work primarily on their own, class participation is difficult to evaluate directly in this unit. Fortunately, students should be keeping notebooks, which provide an indirect measure of the relative effort put in by the students in each pair. If both students put forth the same amount of effort, their data will be evenly distributed or the same between notebooks; if one student is not participating, most of the data analyses will be found in only one student's notebook.

On postlab quizzes, students should be able to use basic genetic terminology correctly. To test this, they could be asked to differentiate between an allele and a locus or genotype and phenotype. Students should be able to calculate phenotypic ratios from raw counts, and know which ratios to expect from monohybrid, dihybrid, and other crosses. If the instructor includes chi-square analyses as part of the unit, students should be able to calculate and interpret the statistic.

Students' lab reports (if assigned) should state which type of inheritance pattern (monohybrid, dihybrid, sex-linked) their particular eye color mutation follows; the lab reports should also contain both summary data and a detailed explanation of how the data led the student to that conclusion.

Safety and Housekeeping

Old vials of flies should be plugged tightly, collected, and frozen overnight to kill the flies before disposal in general trash.

Other Tips

- The same mutant strains may be used more than once within a single lab section, as long as they are given different name or number designations.
- This unit describes how to anesthetize *Drosophila* using carbon dioxide plus cold. An alternative anesthesia method is FlyNap (triethylamine), used as described in Unit 8: Animal Hormones. Both work well, but each has disadvantages. Flies wake up more quickly from carbon dioxide/cold, and may escape, while FlyNap is effective for longer periods of time; however, stale material can sterilize or kill flies. Ultimately the choice comes down to instructor preference.
- Students may try to use other groups' phenotypic ratios to explain their own results. Remind them that each group may be working with a different strain or mutation. Students who worked with the X-linked white-eyed mutant in high school may assume their flies have the same genotype again. Remind them that different genotypes can lead to the same phenotype.
- This project requires considerable out-of-class work by students. Do not schedule a second inquiry unit so it runs concurrently with this one.

Supplemental References

These references explain the biochemical pathways underlying eye color in *Drosophila*.

- Ferre, J., F. J. Silva, M. D. Real, and J. L. Mensua. 1986. Pigment pattern in mutants affecting the biosynthesis of pteridines and xanthommatin in *Drosophila melanogaster*. *Biochemical Genetics* 24: 545–567.
- Lloyd, V., M. Ramaswami, and H. Kramer. 1998. Not just pretty eyes: *Drosophila* eye-colour mutations and lysosomal delivery. *Trends in Cell Biology* 8: 257–259.
- Mackenzie, S. M., M. R. Brooker, T. R. Gill, G. B. Cox, A. J. Howells, and G. D. Ewart. 1999. Mutations in the white gene of *Drosophila melanogaster* affecting ABC transporters that determine eye colouration. *Biochimica et Biophysica Acta* 1419: 173–185.

PREPARATORY NOTES

Quantities listed are for a lab section of 20 students working in pairs.

Week 1: Shared Materials

- 12 vials of live, wild-type flies, labeled “Practice: Wild Type”
- 12 vials of live mutant flies (equal mix of white, brown, orange, and wild-type eye colors), labeled “Practice: Mutants”
- 22 stock vials of flies labeled “Wild Type,” seven to eight days old
- 22 stock vials of flies labeled “Mutant #N,” seven to eight days old
- White or clear labeling tape
- Seltzer tablets (1 box of 36 tablets)
- 100x penicillin-streptomycin solution (store 10 mL aliquots in refrigerator)
- Plastic-coated ice blocks (used for shipping; store in refrigerator freezer)

Week 1: Materials at Each Work Station

- Resealable box or bag of dry food
- 2 measuring scoops (10 mL)
- 25 plastic shell vials (2–3 cm diameter)
- 25 foam plugs for shell vials
- Microtube with 0.2 mL of granulated dry bread yeast
- Soft paintbrush
- Permanent marker
- Dissecting microscope
- White index cards
- Anesthesia apparatus
- Fly morgue (jar one-quarter full of mineral oil, with a funnel taped in the top)

Weeks 2 to 7: Shared Materials (keep stocked)

- Shell vials
- Foam plugs
- Fly culture media (powder)
- White or clear labeling tape
- Seltzer tablets
- Penicillin-streptomycin solution (stored in 10 mL aliquots in refrigerator)
- Ice blocks (stored in refrigerator freezer)

Sources of Materials

Flies can be purchased from the Bloomington Stock Center or an education supplier. It is more economical to order stock vials to arrive eight weeks in advance of the lab and amplify them locally than to purchase all the required vials. Although not essential, it is best to maintain stocks locally between semesters, as some strains are only intermittently available. The mutant strains, eye color, and chromosomal locations are summarized in Tables 2.6 and 2.7.

Table 2.6

Features of single mutant fly lines

Strain	Location of Mutation on Chromosome	Eye Color
Wild type	NA	Brick red
Vermilion	Chr. 1 (X)	Orange
White	Chr. 1 (X)	White
Brown	Chr. 2	Brown
Cinnabar	Chr. 2	Orange
Scarlet	Chr. 3	Orange
Sepia	Chr. 3	Brown to black

Table 2.7

Features of double mutant fly lines

Strain	Mutation	Chromosomes	Eye Color	Comments
Mutant #1	Vermilion X Brown	Chr. 1, Chr. 2	White to pale apricot	Can be confused with orange single mutant.
Mutant #2	White X Sepia	Chr. 1, Chr. 3	White	
Mutant #3	Brown X Scarlet	Chr. 2, Chr. 3	White	
Mutant #4	Cinnabar X Brown	Chr. 2, Chr. 2	White	Loci are far enough apart to assort independently.
Mutant #5 (optional)	Vermilion X Sepia	Chr. 1, Chr. 3	Light at eclosion; darken w/ age	Can be hard to score if students do not check flies when young.

Instructors are encouraged to experiment with other strains carrying mutations that affect body color or eye, wing, or bristle morphology.

Solutions, Reagents, Equipment

100x Penicillin-Streptomycin Solution

Purchase premixed antibiotic solution designed for tissue culture; do *not* use formulations that contain glutamine or anti-fungal agents (amphotericin or ketoconazole) Break the solution into 10 mL aliquots and store refrigerated.

Students should regularly inspect stock and cross vials using a dissecting microscope. They should see numerous larvae feeding near the surface of the media. The fly larvae churn the surface of the medium enough to limit bacterial growth. When there are not enough larvae, a slimy tan or pale yellow scum forms on the surface of the medium. Severe infections will kill the entire vial of flies.

The best strategy is prevention. If only one or two adults are available to set up a cross or new vial, students should add more of the same types as soon as possible to increase the number of eggs and larvae. In the early stages of an infection, cultures can be salvaged by adding antibiotics. As soon as bacterial slime appears, prepare a new vial of media, then add three drops (~150 μ L) of 100x antibiotic solution directly to the media. Transfer adults from the contaminated vial to the new vial. Treat the old vial with another three drops of antibiotic solution to try and save existing larvae. Treat both tubes again two days later. Note that the antibiotic treatment can delay eclosion up to two days.

Anesthesia Apparatus

Obtain a one-hole black rubber stopper that fits the brand of shell vials used. Wet the narrow end of a 1 or 2 mL disposable polystyrene serological pipet and insert it in the hole of the stopper so that the narrow end of the pipet projects 1 in. beyond the larger, outer side of the stopper. Cut off the pipet flush with the inner face of the stopper. Attach an 8 in. piece of plastic aquarium air tubing to the narrow end of the pipet. Add 10 mL of water to the shell vial, and mark point of the meniscus with a permanent marker.

Wine Traps

Fill empty wine or beer bottles one-quarter full of red wine. Add a pinch of dry yeast. Place a funnel into the top of the bottle, and tape the funnel into place. Flies that escape are attracted by the smell of the wine and yeast. They will fly or crawl down the funnel, and drown. Three to four traps are sufficient for one large lab for an entire semester. At the end of the semester, flush dead flies and wine down the sink with copious water.

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