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Bioinformatics: The Retrieval and Analysis of DNA and Protein Sequences

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The exercise is designed for introductory students with only basic backgrounds in computer skills and molecular genetics. The objective of the laboratory exercise is 1) to demonstrate and reinforce major concepts and principles of DNA and protein sequences, 2) to teach the skills of using Web browsers and the availability of biological resources on the Internet, 3) to use a simple DNA analysis program, and 4) to recognize differences between prokaryotic and eukaryotic gene organization that will affect the use of such resources and software.

The concepts of the Internet, World Wide Web, and Web browsers are introduced by having students use a Web browser, NetScape, to search for information in the WWW and to access one of the many sequence databases, GenBank at NIH. The students are initially walked through a carefully scripted step-by-step procedure which includes images of the computer screen as they see it. During the first part of the exercise, students access a particular sequence and learn the format and related information of the sequence entries. Sequences are then downloaded or copied to a local machine for analysis using the Strider software.

For DNA sequences the analysis includes: searching for particular sequences, such as a start codon, and finding any ORFs (open reading frames), codon usage, and the anti-parallel, reverse and complementary sequences. The DNA sequences can be converted to RNA sequences and protein sequences to demonstrate their relationship. Using the software the students generate full restriction endonuclease (RE) maps, assess the number of cleavage sites from all or selected sets of a RE library, identify RE with unique or no sites and calculate the probability of sites in a random sequence.

The analysis of the corresponding proteins includes obtaining the molecular weight of the protein, the most frequent amino acid, the total number of occurrences of an amino acid and its percentage, comparing the most frequent codon and amino acid and explaining any discrepancies. From the primary sequence of the protein, the basic, acidic and hydrophobic regions are identified and their biological significance is discussed.

This format was developed to integrate the student's exposure to the Internet and analysis software with their knowledge of molecular biology at the introductory level. The use of the software allows several nuances of genes and prokaryotic vs. eukaryotic gene organization to be revealed while using the functions of the software. For example, the search for an ORF emphasizes the start and stop codons but also the importance of the reading frame, or when choosing a sequence for analysis the importance of introns in eukaryotic sequences is considered.

The laboratory write-up itself and the DNA analysis software are available from J. Lovett (lovett@uno.cc.geneseo.edu) in Macintosh compatible versions by e-mail or by sending a disk to Dr. Janice Lovett, Biology Department, SUNY College, 1 College Circle, Geneseo, NY 14454.

Human Nutrition: Using a Computer Program to Determine How Well You Eat.

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The science of nutrition deals with the amounts of proteins, carbohydrates, fats, vitamins, and minerals as well as the total number of calories of food energy a human (or other animal) must consume to stay alive and healthy. Nutrition scientists have suggested that a specific minimum amount of each nutrient be eaten each day. These dietary standards are set up by the Food and Nutrition Board of the National Academy of Sciences-National Research Council and are called Recommended Dietary Allowances or RDA's. The recommended allowances are designed for the healthy population of the United States and are revised periodically in order to include new research findings. Different RDA's have been assigned to specific age and gender groups. The most recent revision of the RDA's was in 1989.

In this laboratory, students compare their nutritional intake with the RDA's. In addition students calculate their energy needs and determine whether their diet meets these needs. The laboratory exercise runs two weeks. In the first lab session, nutrition is discussed and students learn to use a computer program to analyze the nutrients in a sample diet. There are many such commercial software programs available for both the Macintosh and Windows platforms. We have chosen The Diet Balancer Program from Nutridata Software. During the week between the first and second laboratory students are asked to keep a food and exercise diary, i.e., a list of everything they eat and drink and any dedicated exercise they may get, for three consecutive days.

There are several pitfalls in keeping a food diary. One of the most difficult aspects of keeping a food diary is determining the amount that is eaten. Students will have to make educated guesses much of the time. Another problem is in recording foods that are combination dishes. The best way is to mentally take the food apart and record the individual components. For example if students had a peanut butter and jelly sandwich on whole wheat bread they would record how much peanut butter and how much jelly were on the two slices of bread.

The second week the students bring their food and exercise diaries to class, analyze their nutrient and calorie intake and compare them with the RDA's and calculated energy requirements. Students may also pool their data in different ways to look at how different groups may consume foods and take in different nutrients and calories. For example, students might compare males and females, athletes versus non-athletes, or underclassmen versus upperclassmen.

Teaching Evolutionary Pathways with Imaginary Animals

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The best visual icon for teaching evolution is the tree diagram. The branching tree, starting with a single common ancestor, illustrates both the diversity of living things and their fundamental unity. The goal of this exercise is to better understand the meaning of evolutionary trees and the problems biologists have in constructing them. We use imaginary animals developed by Joseph H. Camin according to rules of relationship known only to him. The pictures of 29 “caminalcules” are copied and cut out so that each pair of students has 29 organisms to work with on separate small pieces of paper. Students are instructed to put the “caminalcules” into an evolutionary tree, assuming that each one represents a different species and that none are extinct. The work is done on a large sheet of paper so that the branches of the tree can be drawn in to connect all the organisms.

Students must discuss their choices with each other and the instructor and are encouraged to compare their trees to others in the class. The papers can be taped to the walls for class discussion. The exercise easily generates many questions because no one, not even the instructor, knows the right way to build the trees. Students see that in order to classify and relate the different organisms, they must make choices about which characteristics are most important. Not everyone chooses the same characters, so the trees are different. Students come to understand the uncertainty in deciding phylogenetic relationships with limited information, which leads to discussion about what more they would like to know about these creatures.

This exercise can easily be followed by a similar one using an assortment of real organisms. Put specimens around the room and give the students small pieces of paper with the names written on them to arrange in a tree. This is a good way to introduce a number of concepts in biological classification, including the dispute about the number of kingdoms.

Pictures of the “caminalcules” and the results of research into their classification are available in the papers cited below. In fact, there are 48 “fossil” species, in addition to the 29 extant species, and their classification and phylogeny has been estimated with cladistic methods.

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A Multimedia-based Lab Manual: To Enrich, Improve and Expand Learning and Teaching in the Wet Lab

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We developed software, the “Interactive Lab Manual”, to support and expand existing wet lab experiments. The Lab Manual is composed of subunits, called modules, categorized as Experiments, Calculations and Techniques. Students can move unrestricted from one module to another, forming unique connections based on their individual intellectual needs and curiosity.

The Experiment Module allows students to carry out an experiment on the screen. Students are led through a protocol development, introduced to literature searching, encouraged to do the critical steps on the screen, and challenged to synthesized and construct knowledge by responding to the numerous food-for-thought questions.

The Technique Module develops theory and methodological principles behind a technique by intertwining theory and practice.

The Calculation Module allows students to practice those rusty skills one does not like to admit are deficient.

The course web is the typical entry point for a student. At one glance he/she gets an overview of the whole course and an understanding how techniques intertwine with experiments and calculations, and vice versa. Upon, selection of a module, students are transported to the module outline. The students must now decide how to move through the module: where to start, when to access additional information and what type of related information to call up. The outline suggest one possible, logical path through the material in the module, but it does not force the student to follow that track.

In addition to being a vehicle for presenting knowledge, the Interactive Lab Manual further engages students by posing questions. An Electronic Notebook built into the software allows students to record answers to these question. Very powerful is the built-in communication system between students and instructor via a user-transparent electronic mail session.

In summary, we designed software that creates a learning/teaching environment liberated from the constraints of the traditional instructor-centered way of teaching, and consequently learning. Rather than presenting information linearly like “pearls on a string”, knowledge is presented in an “onion-type” fashion with many different layers interconnected via a student-driven path. Topics are presented by using a variety of delivery tools: written words, still pictures, but -most importantly- audio, animation, and simulations.

How to Get Students to Stop Asking, “Is This Lab Going to Take the Whole Three Hours?: A Description of a Cell Biology Lab Curriculum Which Emphasizes Scientific Inquiry.

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In this presentation I described the transformation of our cell biology laboratory curriculum to make it more focused on experimentation and inquiry. The three most relevant features of this (ongoing) project are:

1. I have configured the course into modules so that some new labs can be introduced each year (and others retired) without necessarily altering course objectives substantially.
2. The lab program is in the process of shifting to the almost exclusive use of microbial eukaryotes. This approach has a lot of advantages.
3. The project was funded, in part, by a National Science Foundation Instrumentation and Laboratory Improvement (NSF-ILI) grant. The process of writing the grant helped me to make the course better than it otherwise would have been. I had not anticipated this outcome.

Course Design: The Cell Biology lab program is structured so that there are two class projects, several skills building experiments that all students perform, and each student group designs and implements an independent research project. Table 1 shows the outline of the schedule.

Table 1. Lab program.

I.	Introduction (1 week)
II.	Techniques and Experimentation (8 weeks) <ul style="list-style-type: none"> • Microscopy • Cell Culture • Cell Fractionation and Organelle Isolation • Quantitation of Enzyme Activity • Gel Electrophoresis
III.	Independent Projects (3-4 weeks)
IV.	Reports on Projects (1-2 weeks)



The “Techniques and Experimentation” component of the course consists of lab exercises and experiments that all students perform in order to build skills. Examples of some of these experiments include analysis of amoeboid movement, cytochemistry of red blood cells and various microbial eukaryotes, isolation study of mitochondria, and gel electrophoresis to study serum proteins. Another aspect of this “Techniques” section, is the implementation of two class projects. One is an examination of crown gall tumor formation and the other is study of cell growth in a variety of microbial eukaryotes.

Perhaps the most important part of the course is the Independent Projects. A team of three or four students asks or identifies a testable question, designs an experiment, does the experiment, and reports on their work both orally and in writing. Table 2 shows some examples of topics students have studied in their independent projects.

Table 2 Examples of independent projects.

- Isolation of Chloroplasts and Study of the Effect of Light Quality on the Rate of Photosynthesis
- Analysis of Red Blood Cell Membrane Permeability
- Effects of Lectins on Cell Recognition
- Gel Electrophoresis and Western Blot Analysis of Lactate Dehydrogenase in Different Types of Tissues
- Study of Growth and Feeding in *Tetrahymena*
- Study of Flagellar Regeneration in *Chlamydomonas*

Outcomes: There were several significant outcomes to doing the course as I have described it here. First, students gained many important laboratory skills the most critical one of which is the ability to design a good experiment. They got a lot of practice in experimental design and analysis. Second, the common goals of the class projects united the entire lab. While this outcome surprised me at first, I realized that it was inevitable since the students depended on everyone else in the class for these projects to work. Finally, students really enjoyed the independent projects. At first, they seemed nervous about the assignments, but very quickly, they became very excited about their experiments. The obvious and justifiable pride that the students displayed when they present their work was also very rewarding.

Use of Microbial Eukaryotes: There are several good reasons for the extensive use of microbial eukaryotes to teach cell biology laboratory. First, they are quite inexpensive, and easy to grow. No special facilities are needed. If an experiment fails or if a student wants to pursue further inquiry (!), more cells can be grown quickly. Second, you can avoid animal welfare issues without compromising rigor. For example, nuclei can be easily isolated from *Tetrahymena*, you don’t need a mouse. Finally, it is important for students to observe that not all cells are the same. Microbial eukaryotes are some of the most specialized (and interesting) eukaryotic cells that exist.

Writing an NSF Grant for Instrumentation: Here are four lessons that I learned in the process of preparing my grant:

1. You must demonstrate that funding will propel you to the “next step.” It is not adequate to improve what you are already doing.
2. While substance is important, presentation style is critical. I found it helpful to make tables containing specific information and examples of laboratories and to embed these tables in the text of the grant.
3. Plan on resubmission. While I am sure it happens, I don’t know anyone who received funding on the first try. I know a lot of people who got it on the second, or third try.
4. Talk to your NSF program officer. He or she will be very helpful.

Selected Laboratory Experiments for Elementary Education Major or Other Non Biology Majors

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Selected laboratory exercises were presented from a course specifically designed for Elementary Education majors. Many students who have chosen primary school teaching as a career goal lack confidence in science. The primary focus of the laboratories is to provide students with the opportunity to increase their science process skills. Providing a forum for the exchange of ideas is a common thread running throughout the course.

One of the labs we do is an experiment which tests what effect a certain factor has on oral bacteria. The primary focus of this exercise is experimental design. Students are provided with sterile Luria broth agar plates and sterile Q-tips. An assortment of oral hygiene products such as different types of mouthwash, sugar and sugarless gum, toothpaste and dental floss are provided. Working in groups of four, students generate their own question and design their experiment. As they work out the details of this very simple project, they have to make many decisions. Questions such as, how do we standardize the sampling, how often do we sample, and how do we assess our results, are just a few examples.

Once each group has designed their experiment, they present their project proposal to the rest of the lab group. Based upon feedback from the class, the individual groups then revise their designs and set up their experiments. After completing their project each group gives a brief presentation to the lab. Included in this summary is a section on how they would modify their experimental design and what would be their next question.

Other exercises, designed to foster creative thinking and integrate science into other areas, include designing a board game of the human circulatory system, and writing imaginary interviews between a prokaryote and a eukaryote cell. Additional information is available upon request.

A Qualitative and Quantitative Approach to Investigating Cellular DNA Repair Mechanisms

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Light and dark repair are two common cellular-DNA-repair mechanisms available in most cells. Wild type and mutant (dark-repair deficient) *E. coli* cells are exposed to UV light resulting in pyrimidine dimer formation. After the cells are irradiated, samples are either kept in the dark or exposed to blacklight which induces the light repair system. Through the various combinations of *E. coli* strains and repair mechanisms, students create inactivation curves for irradiated *E. coli* with no repair, light repair only, dark repair only, and both light and dark repair. In graphical form students have a qualitative measurement of the contribution by each of the DNA-repair mechanisms. Quantitative results are obtained by calculating inactivation rates from each of the four inactivation curves. From the inactivation rates, photoreactivable and host-cell reactivable sectors are determined. The sector values provide a measurement of the fraction of lethal damage repaired by light and dark repair, respectively. Comparable yeast strains exist for this procedure and the experimental design also allows for an inquiry based approach.

Why Should I Wear Sunscreen? Ultraviolet Radiation, DNA, And Mutations

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Ultraviolet radiation is part of the electromagnetic spectrum that comes to the earth from the sun. DNA absorbs ultraviolet radiation and forms thymine-thymine dimers. If these errors are not repaired, the DNA is altered and no longer codes for the original message. In humans, this damage can result in uncontrolled growth or skin cancer. In bacterial cells, which reproduce asexually, these mutations are passed onto the daughter cells. If the amount of radiation is sufficient, the cells will not reproduce.

This can be observed in the laboratory by exposing plated cultures of *Serratia marcescens* (available from American Type Culture Collection) to a source of ultraviolet radiation. Each group is given a liquid culture of the bacteria in nutrient media. They are instructed in aseptic techniques. Students use a sterilized swab to streak the nutrient agar plates. (Culture tubes and petri plates with the nutrient agar are available from Baxter/Scientific Products.) Plastic wrap is used as a support for various solar protectants. Each group covers half of the culture with a solar protectant and allows the other half to be exposed to the ultraviolet source. We use the ultraviolet light in a hood as our source. A hand-held source or the sun should work equally well. The exposure time will vary with the source. You should test times from 1 to 30 minutes. Exposed plates are allowed to incubate at room temperature for 24 hours. If students cannot examine plates at this time, they may be kept in the refrigerator for one week. The students determine relative amounts of bacterial growth based on the numbers of colonies. They relate the growth to the exposure to ultraviolet radiation.

This experiment is easy to do. It can be done using a very small amount of time in 2 or 3 consecutive lab sessions. It gives students a good chance to design an experiment and evaluate the results. If you have sufficient materials, it will take very little time to allow students to redesign and re-implement their experiment.

Using the Scientific Method to Study Optical Orientation in Blowfly Larvae

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This introductory lab in beginning biology courses for majors or nonmajors illustrates scientific investigation more realistically than do many textbook treatments of the “scientific method” and many “exercise” type labs. It helps students appreciate that scientific investigation not only involves reasoned conclusions based upon relevant and valid evidence, but also that it is iterative, cooperative, and tentative.

In the first activity, each lab group simulates the scientific process by playing a simplified version of the card game New Eleusis in which a group of “scientists” seeks to determine a “law of nature” (the rule governing a sequence of playing cards) through a series of “experiments” (Gardner, 1977). One lab group member acts as “Mother Nature” and the others act as “scientists.” To set up the game, “Mother Nature” devises a relatively simple “secret law” which will govern the sequence of cards which can successfully be played by the “scientists” and writes it on a sheet of paper out of view of the “scientists.” For example, the law could be “each card is the same color but a different suit from the previous card.” “Mother Nature” selects two cards which obey the written sequencing law and places them face up on the table next to each other. These two cards serve as the “initial observation” made by the “scientists.”

The bulk of the game consists of the lab group members then seeking to discover the secret law. The “scientist” on “Mother Nature’s” left proposes out loud to the other “scientists” an hypothesis to explain this “initial observation” (i.e., tries to guess what the secret law is). That “scientist” then selects a card of his or her choosing from the deck and gives it to “Mother Nature.” This card is thus the scientist’s “experiment” or “further observation” to test his hypothesis. Discussion among the “scientists” is encouraged during the choosing of any hypotheses or “experiments.” If the card given to “Mother Nature” correctly follows the secret sequence law, then “Mother Nature” places it to the right of the last correct sequence card. If it does not follow the rule, then “Mother Nature” places it beneath the last card played. Play then proceeds to the next “scientist” around the table who either agrees with the previous hypothesis or else modifies it. This “scientist” then performs an “experiment” of his own. Figure 1 shows an example of a card layout generated during a game using a very simple rule. At any stage of the game, the scientists as a group may ask “Mother Nature” if their current hypothesis is correct. If the hypothesis is correct (i.e., it essentially restates the secret rule), then the game is over and the scientists win. Conversely, if the hypothesis is not correct, then the game is over and “Mother Nature” wins.

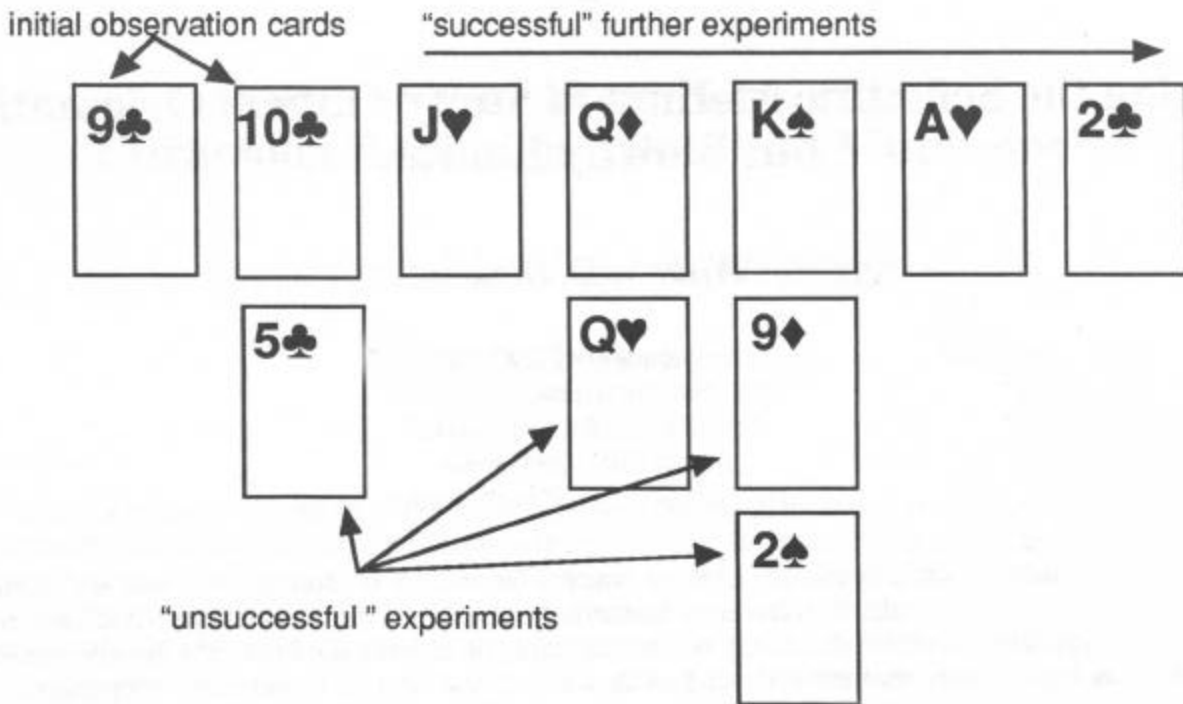


Figure 1. Possible sequence of play when the law is “Each card is one value higher than the previous one.”

The game (and “real” science) requires cooperation among the players (experimental results, ideas and new hypotheses are shared), iteration (a single observation or experiment is usually not enough to convince one of the validity of an hypothesis), revision of hypotheses in the face of new evidence (you must modify your hypothesis until it explains all of the relevant data), and the use of “negative experiments” (sometimes playing a card which your hypothesis predicts will be “unsuccessful” is a good additional way to test the hypothesis). Conversely, the main way in which the game is not like real scientific investigation is that “Mother Nature” is not there to tell you if your hypothesis is right or wrong - the “real” scientist never knows absolutely for sure.

The second activity applies the lessons learned above in the analysis of the phototaxis of blowfly (*Sarcophaga bullata*) larvae (Carolina Biological Supply Company, Burlington, NC) in response to simultaneous challenges with lights of different colors. About an hour before lab, select active larvae which show consistent and strong positive phototaxis toward an incandescent light source. The general experimental protocol the students perform in lab consists of arranging a piece of black construction paper and two adjustable lamps with 60 watt light bulbs on the benchtop as indicated in Figure 2. Secure a color filter (colored cellophane or plastic sheet) over the front of each lamp and adjust the distance between the center of the black paper and each lamp (typically from 5 to 20 cm) so that the light intensity at the larval position will be the same for each color as determined by a light meter. The filters will typically be of two different colors. With both lights turned off, place the larva on the black paper and wait a few seconds for it to right itself and start crawling approximately in a straight line. Rotate and position the paper so that the crawling larva is in the direction indicated in Figure 2 and it is at the correct distances from the two lamps. When the larva is crawling in the desired spot, simultaneously turn on both lamps. Observe the behavior of the larva without further movement of the black paper. When it is clear which direction it is finally moving,

simultaneously turn off both lamps. Record the larva's final position as color one, color two, or straight. Repeat this for five trials.

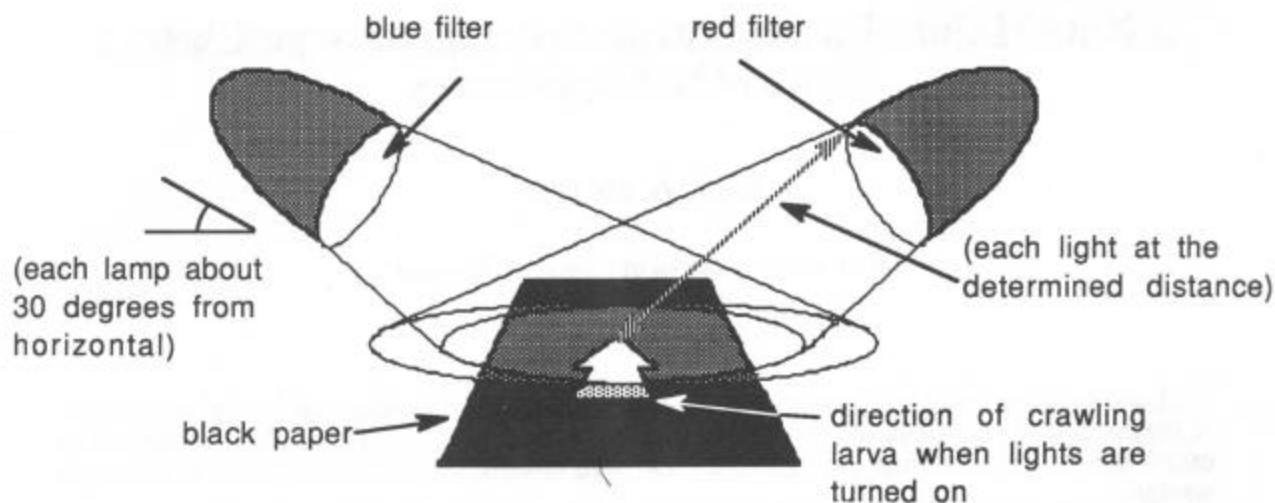


Figure 2. Experimental setup for testing the phototactic response of larvae to different colors of light.

The entire class makes an initial observation using a red and a blue light source, and the findings of all the lab groups are shared. The results are usually very much in favor of the larvae ending up on the “blue” side. Then each lab group forms an hypothesis involving either positive phototaxis toward blue or negative phototaxis away from red, and chooses an experiment from a list of possibilities to test their hypothesis. After sharing the class’s results of these further experiments, it should be clear to all the lab groups that a positive phototactic response is being observed. Each lab group then modifies its initial hypothesis and, through further experiments consisting of trials using pairs of various other filter colors of their choosing, tests and further revises its hypothesis until it is consistent with all the data collected by the group.

Pooling the results of the different lab groups at the end of the lab period leads to a more comprehensive hypothesis. The pooled results can be easily summarized by counting the number of preferences each color showed in comparison to others. For example, if pooled class results showed that blue was consistently preferred in choice trials against red, yellow, violet, and no light, but was not preferred in trials against green, then blue would rank four in a “relative preference ranking.” In typical sets of trials in which the larvae are challenged with each possible combination of five filters (and no light at all), the “preferred” choices lead to a self-consistent ranking of the colors as to degree of positive phototaxis (most “preferred” color equals a five), with a maximum preference being shown in the green region of the visible spectrum. The students should not be told of such results until the end of lab. Pooling the various class results to put together this “bigger picture” shows the benefit of sharing data and of communicating results to other scientists in the field.

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Non-Majors Environmental Biology: Large Class Hands-On Experiences

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These experiences are designed to give non-major biology students some 'laboratory' experiences in environmental biology (a lecture-only course). Classes normally consist of 300 students. The exercises listed are all carried out in a lecture hall with minimal supplies, during a 50 minute class session.

The students, working in groups of ten, carry out an environmental survey of campus, which is designed to promote an awareness of environmental issues related to everyday life. Each group collects data concerning traffic flow around campus, campus recycling programs (in the computer laboratories, in the student center food court, and in the residence halls), and participation in the university car pooling program. Each group of students submits a short report containing the collected data and the conclusions reached by the group.

A chocolate chip mining activity, designed by Kutscher (1991), promotes an awareness of the financial and ecological costs of regulated versus unregulated strip mining. Working in groups, the students are given two minutes to 'mine' as many chocolate chips as they can from a chocolate chip cookie. After the two minutes have elapsed, the students count the number of chips mined. The students then attempt to reassemble the fragmented cookie. The activity is repeated using a second cookie but this time the students are required to mine the chips while preserving the overall appearance of the cookie. The students compare the results of the two mining episodes, noting the number of chips mined and the final appearance of the cookies. Using this activity as a model, the students draw comparisons between regulated and unregulated strip mining practices.

The students use Bottle Biology Methodology (National Association of Biology Teachers, 1994) to determine the effects of household liquids on water quality. Artificial ponds are constructed from 2-liter plastic bottles. Pollutants (household liquids) are added to the water in the experimental ponds. Duckweed plants are added to each pond. After one week the students compare the appearance of the duckweed in a control pond (no pollutants added) to the duckweed in the experimental ponds. Students determine the relative toxicity of each of the household liquids tested.

All of these activities promote student-directed learning in the classroom and have increased student interest and participation in this environmental biology course.

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Lights, Bean Plants, Action: Starch Printing, a Student Exercise

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Photosynthetic production of carbohydrates and starch is dependent on light intensity. The primary leaves of a young bean plant which has been stored in the dark for 24 hours are depleted in starch; these leaves will rapidly produce starch when provided with light and sufficient CO₂. Because light intensity greatly affects the rate of photosynthesis, the amount of starch produced will depend on the availability of light. If a photographic negative or a stencil is placed over the leaf, starch production will be the greatest where the light reaching the leaf is most intense (Reiss, 1994). The duration of the light exposure can be greatly reduced by placing a piece of black felt soaked in a bicarbonate solution on the back of the leaf. The leaf is then removed, placed in boiling water for a few seconds, then placed in 95% ethyl alcohol to remove chlorophyll. Finally, the leaf is placed in an iodine solution, which will stain the newly produced starch, giving a positive image of the photograph. This exercise is fun and easy to do. It would be appropriate at the high school level, but is entertaining for the college student as well. The amount of detail present in the final starch prints always surprises the students and encourages them to consider the importance of available light for photosynthesis.

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DNA: From Lab to Courtroom

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The laboratory exercise on DNA fingerprinting was specifically developed for inclusion in a two part laboratory sequence for nonscience majors. This exercise is placed in the lab sequence to correspond with the end of the lecture discussions on molecular genetics. The data used in this exercise are derived from actual FBI crime cases which occurred in Georgia between 1989 and 1993. Dr. Wyatt Anderson, Department of Genetics at the University of Georgia, assisted in the development of this exercise. As a technical consultant to the State of Georgia crime laboratory, and a frequent expert witness on forensic use of DNA fingerprinting, Dr. Anderson felt that the inclusion of this exercise in the nonscience major sequence would provide the students with a basic understanding of the DNA fingerprinting process and create technically literate potential jurors.

Four objectives are outlined for students at the beginning of the exercise:

- Observe chromatin bands corresponding to “genes” in *Drosophila* giant salivary chromosomes.
- Recognize types of DNA: **unique DNA** and **repetitive DNA**.
- Learn how DNA is chemically cleaved and how the resultant fragments are separated by electrophoresis.
- Understand how DNA technology is used to identify individuals.

Students observe prepared slides of *Drosophila* salivary polytene chromosomes. A chromosome map is provided for visual reference to assist the students in locating specific chromosome regions corresponding to known *Drosophila* mutations. A discussion of restriction endonucleases, RFLP analysis, and the Southern Blot technique follows. Students view a 10 minute video clip on electrophoresis and observe a gel loaded with multiple tracking dyes of differing colors and watch molecular weights separating.

Three sets of autoradiograms are provided to the students. Each case is of increasing complexity paralleling the development of forensic DNA technology from 1989 through 1993. Each set of films is associated with a crime case description. The first case is worked by the teaching assistants with the class as an example. Three different probes are used and the band shifting phenomenon is demonstrated and discussed.

The second and third cases are assigned to different student groups. The students divide themselves into prosecution and defense teams and evaluate each set of films from their legal position. The students summarize their results in a “mock” trial. Students must calculate the total probability of a match for the suspect and the evidence. Students are required to discuss the technical problems associated with the forensic application of DNA technology including: the small numbers of certain ethnic groups in the FBI data base used to generate the probabilities, problems associated with the use of the DXY probes, the effects of poor sample preservation, and forensic laboratory quality control.

NOTE: 30 mm negatives of the autoradiograms were distributed at the Boston University meeting for cases 2 and 3. Additional copies of the crime films are available upon request from the author. Please allow

316 Mini Workshops

sufficient time for the author to obtain the original films from the FBI lab and have the autoradiograms copied (4-6 weeks).

Species Identification and Systematics

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An important objective for biologists is to determine the phylogenetic relationships among species. This exercise is designed to introduce introductory biology students to the study of systematics. First, students learn how to use a dichotomous key to identify tree cones and fruits. The key to 30 cones and fruits is written so students can easily identify at least 10 specimens in less than one hour. The cones and fruits were chosen to be sufficiently sturdy to endure use by students for many years, and they are substantially different from each other to allow easy use of alternative choices in the key. The language in the key does not require a background in botany.

Second, students use characteristics of the cones and fruits to design a phylogram. A phylogram reveals which species descended from other species or, alternatively, which species share immediate common ancestors. The construction of a phylogram is based on a fundamental assumption: when two species share similar characteristics, the characteristics are acquired from a similar characteristic in a common ancestor. If the assumption is correct, then it is possible to state that there is a phylogenetic relationship between the two species. Similar characteristics in two species that are inherited from a common ancestor are homologous. Students determine the numbers of homologous characteristics in common in five tree species and construct a phylogram to organize the species on the basis of their evolutionary relationships.

Species Identification. Using a Dichotomous Key to Identify Tree Species

Use the dichotomous key to identify ten cones and fruits.

KEY TO TREE CONES AND FRUITS

- | | | |
|----|--|--|
| 1. | Cone with overlapping scales | 2 |
| 1. | Fruit, not a cone. If cone-like lacking overlapping scales | 6 |
| 2. | Cone scales more or less thickened (pines) | 3 |
| 2. | Cone scales almost paper thin (spruces and hemlocks) | 5 |
| 3. | Cones at least 4" long | White pine <i>Pinus strobus</i> |
| 3. | Cones less than 4" long | 4 |
| 4. | Cones unsymmetrical (lopsided), with stout prickles | Table mtn pine <i>Pinus pungens</i> |
| 4. | Cones with slender prickles | Virginia or scrub pine <i>Pinus virginiana</i> |
| 5. | Cones less than 1" long | Eastern hemlock <i>Tsuga canadensis</i> |
| 5. | Cones 1" long or more | Red spruce <i>Picea rubens</i> |
| 6. | Fruit shaped like a bean pod | 7 |
| 6. | Fruit not shaped like a bean pod | 8 |
| 7. | Pod about 2" to 3" in length, very straight | Eastern redbud <i>Cersis canadensis</i> |
| 7. | Pod 8" or more in length | Honeylocust <i>Gleditsia triacanthos</i> |
| 8. | Fruit with a thin wing | 9 |
| 8. | Fruit not winged | 15 |

9. Fruit paired (double), the two parts united at the base (maples)..... 10
9. Fruit single, not in pairs 13
10. Fruit red or reddish brown Red maple *Acer rubrum*
10. Fruit green or yellow..... 11
11. Fruit wings forming an angle greater than 90°, nearly straight from end to endNorway maple *Acer platanoides*
11. Fruit wings forming an angle less than 90° 12
12. Fruit V-shaped Boxelder *Acer negundo*
12. Fruit U-shaped Sugar maple *Acer saccharum*
13. Wing encircling the seed cavity..... American elm *Ulmus americana*
13. Wing terminal (at end of seed cavity)..... 14
14. Seed cavity 4-angled in cross section Tulip poplar or yellow poplar *Liriodendron tulipifera*
14. Seed cavity flat in cross section.....Green ash *Fraxinus pennsylvanica*
15. Fruit made of many small units packed tightly together or borne in a loose cluster 16
15. Fruit solitary 19
16. Fruit cone-shaped.....Magnolia *Magnolia grandiflora*
16. Fruit not cone-shaped..... 17
17. Fruits borne in a cluster, bright red when fresh or dark red or black when dry Dogwood *Cornus florida*
17. Fruit round, golf ball size or smaller 18
18. Fruit round and hard with sharp projectionsSweetgum *Liquidambar styraciflua*
18. Fruit round, lacking sharp projectionsSycamore or plane tree *Platanus occidentalis*
19. Fruit an acorn (oaks) 20
19. Fruit not an acorn 25
20. Acorn about 1/2" long Willow oak *Quercus phellos*
20. Acorn longer than 1/2" 21
21. Cup conspicuously fringed at its edge Bur oak *Quercus macrocarpa*
21. Cup not fringed 22
22. Cup deep, almost covering nut Overcup oak *Quercus lyrata*
22. Cup shallow 23
23. Nut tawny, cup usually elongate at base, cup scales long and relatively thinBlackjack oak *Quercus marilandica*
23. Nut brown, cup usually round at the base, scales warty in appearance 24
24. Nut narrow and oblong, thin spike at the tip..... White oak *Quercus alba*
24. Nut wide, thick spike at the tip Red oak *Quercus rubra*
25. Nut shiny dark brown with one light spot..... Buckeye *Aeculus glabra*
25. Nut not shiny dark brown with one light spot..... 26
26. Husk covering nut without seams Black walnut *Juglans nigra*
26. Husk covering nut splits along definite seams 27
27. Husk prickly..... 28
27. Husk not prickly..... 29
28. Nut rounded in cross section, more than 2" in diameter, spine of husk branched, needle sharp American chestnut *Castanea dentata*
28. Nut triangular in cross section, less than 1" long, spines of husk weak, not branched American beech *Fagus grandifolia*
29. Husk winged at seams Bitternut hickory *Carya cordiformis*
29. Husk not winged, either smooth or slightly ridged along seams Pignut hickory *Carya glabra*

Systematics. Construction of a Phylogram

To construct a phylogram the systematist first observes the structural differences and similarities that exist among organisms. It should be noted that such an analysis is based on a fundamental assumption: when two species are found to share similar characteristics it is assumed that these similar characteristics were acquired from a similar characteristic in a common ancestor. If this assumption is correct, then it is possible to state that there is a phylogenetic relationship between these two species. Similar characteristics in two species that are inherited from a common ancestor are spoken of as being homologous. A systematist determines the organisms with the largest number of homologous characteristics in common and then constructs a phylogram to organize the species into larger groupings. Suppose you have to construct a phylogram to represent the ancestor descendent relationships of five vertebrate animals found in the surrounding area. First, you list characteristics that indicate structural similarities and differences that exist for these organisms. Next, you indicate with a '+' if the organism possesses the characteristic and a '0' if it does not possess the characteristic.

Vertebrate Animals

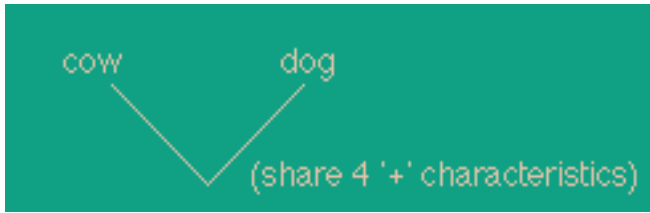
Characteristic	Bass	Lizard	Duck	Cow	Dog
Gives birth to living young	0	0	0	+	+
Walking legs present	0	+	+	+	+
Body with hair	0	0	0	+	+
Warm-blooded	0	0	+	+	+

Compare each organism to one another and indicate the number of shared '+' characteristics between each pair.

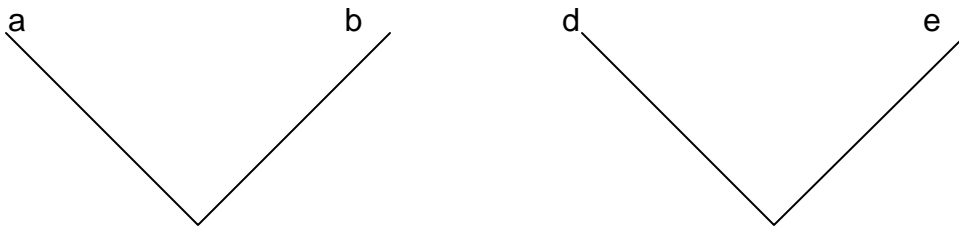
Total number of shared '+' characteristics

bass, lizard = 0	lizard, duck = 1	duck, cow = 2	cow, dog = 4
bass, duck = 0	lizard, cow = 1	duck, dog = 2	
bass, cow = 0	lizard, dog = 1		
bass, duck = 0			

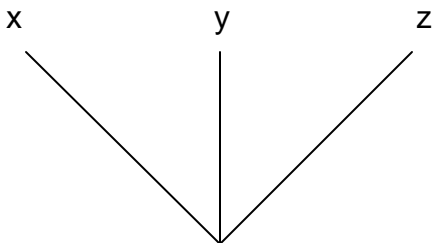
A phylogram is constructed by joining first the most similar pair(s) which, in our example turns out to be the cow and the dog because they share 4 '+' characteristics. This pair is connected by a bifurcating branch:



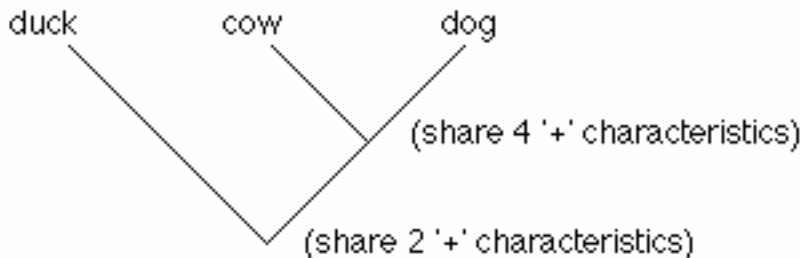
Note that if two pairs of organisms shared the same number of characteristics, for example, hypothetical organisms a, b sharing 4 '+' characteristics and hypothetical organisms d, e sharing 4 '+' characteristics, they would have been simultaneously joined as follows:



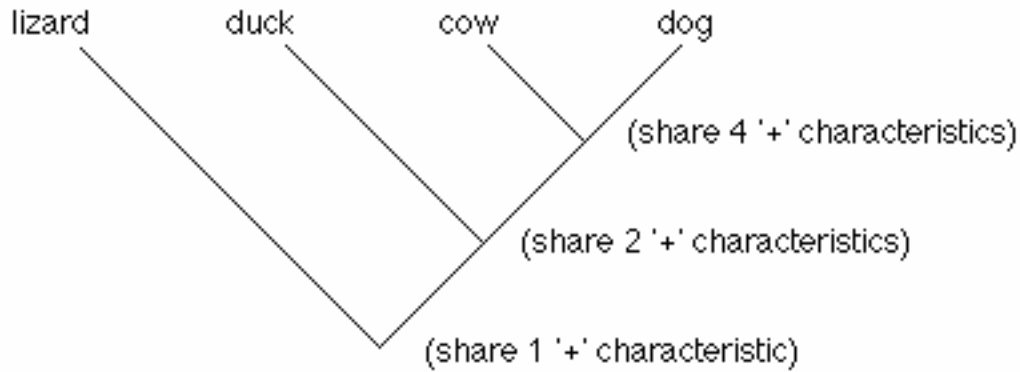
If three organisms x, y, and z are equally similar among themselves, for example, if x, y share 3 '+' characteristics and x, z share 3 '+' characteristics and y, z share 3 '+' characteristics, then the three are joined by a trifurcating branch:



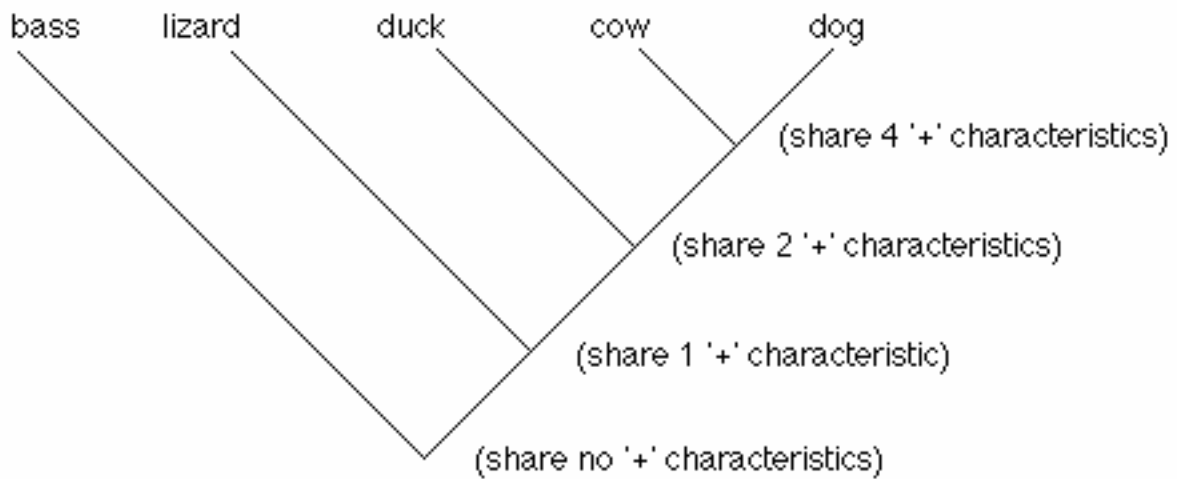
In the next step you compare the unconnected organisms to each other and to the (cow, dog) branch. You measure the number of shared '+' characteristics between an unconnected organism to an established branch such as the (cow, dog) by finding the largest number of shared '+' characteristics between the unconnected organisms and either the cow or the dog. In this step you find that the most similar pair(s) is the duck, (cow, dog) sharing 2 '+' characteristics. You now connect the duck to the (cow, dog) branch by a new bifurcating branch.



Repeat the above step until all organisms are connected by branches. Thus, in the next step you find that the most similar pair is the lizard (duck (cow, dog)) branch sharing 1 '+' characteristic. The lizard is then connected to the (duck (cow, dog)) branch by a new bifurcating branch.



The final step connects the last remaining unconnected organism, the bass, to the (lizard (duck (cow, dog))) branch to form the completed phylogram.



Constructing a phylogram with a large number of organisms or a large number of characters can be quite laborious. Systematists, therefore, rely on computers whenever they can to shorten the effort involved in computation. In the remaining part of this exercise you will construct a phylogram of five tree cones and fruits.

1. Examine five fruits and cones carefully. Enter distinguishing traits in the box under “Characteristic”.
2. Fill in the box by putting a “+” showing that the characteristic is present or a “0” showing that the characteristic is absent.

Tree species

Characteristic	Species 1	Species 2	Species 3	Species 4	Species 5

3. Compare the five tree species to one another and indicate the number of '+' characteristics between each pair.

Total number of shared '+' characteristics

1, 2 =	2, 3 =	3, 4 =	4, 5 =
1, 3 =	2, 4 =	3, 5 =	
1, 4 =	2, 5 =		
1, 5 =			

4. Using the procedure described above, construct a phylogram for the five tree species.