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Termites and the Scientific Method

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Termites can be used as tools in the introduction of the scientific method. This exercise allows the student to simply and easily follow each step of the scientific method without the need for encumbering equipment and procedures. Each group of students is supplied with ballpoint pens of various colors and brands, paper, two or three termites, and a small brush to control strays. They are also given a handout which reviews the scientific method and guides them through each step. The only instructions given are to begin observation of termite behavior as the termites move about the paper, on which the students are encouraged to draw lines.

Once observations have been made and recorded, students are asked to develop one hypothesis based on these observations, and devise an experiment with one variable. Once designed, the experiments are run and data collected. Each group interprets their data and "publishes" it either orally or as a laboratory report.

There are many hypotheses that students might generate. There is no single "right" answer. The one proposed most often is that the termites follow a certain color line. If they test this hypothesis, they will often be able to support it with their data. However, for most students there will be one overlooked variable. It is *not* a color that the termites follow, but a pheromone-like chemical in the ink formulation of pens manufactured by Papermate but *not* pens manufactured by Bic. Upon "publication," this overlooked variable will become obvious in much the same way that insight is gained and information is added to the body of knowledge in the scientific community.

Becker, G., and R. Mannesman. 1968. "Behavior of termites towards some scent trail laying-substances." Zeitschrift fur Angewandte Entomologie, 62(4):399–436. [German]

Karson, P. J. L., and H. Humel. 1968. Extraction und biologische auswertung, des spurpheromons der termite *Zootermopsis nevadensis*. Journal of Insect Physiology, 14:1763–1771.

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Discussion-Oriented Exercises on Two Hot Topics: Global Warming and Tropical Deforestation

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These exercises focus on two issues of global importance: global warming (the "greenhouse effect") and tropical deforestation. We hope that by doing these exercises and through class discussions, the students will develop an understanding of the biological phenomena related to each issue. They should then, as world citizens, be able to form intelligent, fact-based opinions as these issues evolve and new information becomes available.

Climatic data for the past century suggest that the earth's atmosphere is warming and that this is due to the build up of heat-retaining gases, such as carbon dioxide. These gases are by-products of industry and are released through the use of fossil fuels. While the burning of fossil fuels adds increasing amounts of carbon dioxide to the atmosphere, the fast pace of deforestation in the tropics is decreasing the removal of CO_2 by plants during photosynthesis. As a result, the CO_2 concentration is rising. Carbon dioxide, and other atmospheric gases, retain much of the longer-wavelength energy that is radiated from the earth and this increases the temperature of the atmosphere. This is much like heat retention within a greenhouse.

The exercise concerning the "greenhouse effect" asks the students to predict biological responses to a given change in climate. They first consider the responses of a variety of species to changes in temperature and precipitation. Next, they evaluate the confounding effect of migration rates, soil preferences, and other parameters that determine species habits.

Tropical deforestation is another global issue. This topic provides an avenue for the discussion of many subjects, all of which are at least tangential to biology. These include political science, economics, sociology, medicine and public health, agriculture, and biodiversity. The exercise is intended to foster an understanding of the causes and consequences of tropical deforestation and to illustrate the complexity and multidisciplinary nature of land management decisions.

Prior to each exercise we recommend showing a series of slides or video to provide background information and to explain the basis of each problem.

Why Do Mendel's Peas Wrinkle?

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Students have difficulty imagining how the genotype, operating at the primary level of organization to produce a polypeptide, can effect higher order phenotypic expression. One reason for the difficulty is that hierarchial complexity of cell, tissue, and organ system interaction is skirted when a genotype is correlated to a complex trait (as, for example, *R* = round, *r* = wrinkled). This exercise explores the biological basis for a classic genetic trait, round versus wrinkled peas, by investigating the multiple (pleiotropic) effects that the gene product, starch branching enzyme, has on metabolism, shape of the starch grain, and osmotic potential.

The *RR* seeds contain two forms of starch branching enzyme, one that activates early in seed formation and the other late. In wrinkled seeds, *rr*, only the late acting form is present. Without the starch branching enzyme in early development, sugar precursors are converted to straight chain polysaccharides (amylose) rather than branched polysaccharides (amylopectin) by the enzyme starch synthase. Fewer branches means fewer sites to bond sugars to growing polysaccharides and, therefore, an accumulation of sugar molecules. This, in turn, increases osmotic pressure, increases water accumulation as the pea seed grows, and causes greater water loss when the pea dehydrates by the completion of development. A shriveled (wrinkled) seed results. Bhattacharyya et al. (1990) cloned the gene and discovered the *r* allele contains an 0.8 kb insertion of a transposable element not found in the *R* allele. The insertion alters the protein sequence.

Students are given dehydrated peas of three types (Early Alaska [EA], Thomas Laxton [TL], and Little Marvel [LM], available from Carolina Biological Supply Co.) and asked to determine which are round [EA] and which are wrinkled [TL, LM]. Several peas of each strain, hydrated in water overnight, are ground with mortar and pestle containing 10 ml of water. Wet mounts reveal that EA produces kidney bean-shaped starch grains; those of the other two strains resemble a sand dollar and are often fragmented. Extracts are centrifuged at 2500 rpm and the supernatant dropped onto agar plates prepared with glucose-1-phosphate (5 g G-1-p, 20 g agar, 1000 ml water). After 30 minutes, the plate is flooded with IKI and observed for starch production (positive for LM, TL) that reflects enhanced starch phosphorylase activity. The latter, which is not coded by the *r* gene, is likely an indirect metabolic effect of starch branching enzyme. Finally, students compare the wet and dry weight of peas to determine the percent difference in water content (highest for LM, TL).

Bhattacharyya, M.D., A.M. Smith, T.H. Noel Ellis, C. Hedley, and C. Martin. 1990. The wrinkled-seed character of pea described by Mendel is caused by a transposon-like insertion in a gene encoding starch-branching enzyme. Cell, 60:115–122.

Use of a "DNA Cookbook" to Demonstrate Transcription and Protein Synthesis

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Students often have trouble visualizing the processes of transcription and translation that lead to the formation of proteins. As such, a simulation using the analogy of a cookbook containing instructions for making lemonade was developed. This simulation was designed to help students see the relationship between DNA in the nucleus containing the code (the cookbook), mRNA (a note card) which carries the code in a slightly different language from the nucleus to the ribosome, and tRNA (a cup) which functions to hold a specific amino acid (ingredient) and to properly line up the amino acids by matching anticodons (on the cup) to codons (on the note card). The protein product is represented by the mixture of all of the ingredients (the drink). By changing a base pair in the DNA, the student can see the effect of mutations. A change in the DNA results in a change in the mRNA which then requires different tRNAs (cups) containing different amino acids (ingredients) to contribute to the product (drink). What started out as a tasty drink becomes unpalatable, or a different color, or has a different taste. As in nature, most "mutations" are worse than the original.

There are unlimited possibilities as to the products that can be made. Depending on one's creativity, many different combinations are possible. In the simulation presented, coding from a 12-base pair piece of DNA resulted in a four amino acid product: lemonade.

Electrophysiology of Frog Sensory Receptors

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This introductory neurophysiology laboratory examines the innervation patterns of frog cutaneous sensory nerves, and the sensory receptor properties of graded response and adaptation. Sensory receptors depolarize when stimulated by a specific stimulus, producing a generator potential. The generator potential is transformed into an action potential on the sensory neuron axon, and is transmitted to the spinal cord and cerebral sensory cortex. Sensory receptors demonstrate the property of graded response. The stronger the stimulus, the greater the number of generator potentials produced by the receptor, and sent as action potentials to the sensory cortex. The increased number of action potentials are interpreted by the sensory cortex as a stronger stimulus. Sensory receptors also demonstrate the property of adaptation. When a stimulus is applied to a sensory receptor continuously over a period of time, the receptor produces fewer generator potentials as time progresses. The reduced number of action potentials received by the sensory cortex are interpreted as a weakening stimulation.

The innervation patterns of cutaneous sensory nerves are examined by exposing cutaneous sensory nerves innervating the frog's dorsal surface. A flap is produced by making three incisions, with the longitudinal portion of the flap lateral to the dermal plica. Elevating the skin flap exposes the cutaneous sensory nerves extending from the body wall to the skin flap midline. A pair of recording electrodes connected to an oscilloscope are positioned to contact the exposed cutaneous sensory nerve. As the cutaneous touch receptors are stimulated by rubbing a glass rod on the skin, the action potential produced along the cutaneous sensory nerve is traced on the oscilloscope screen. Areas of innervation for each sensory nerve are determined and mapped on a diagram of the frog's dorsal surface.

Graded response is examined by stimulating the proprioceptors in the frog's toe joint with varying weights. The peroneus communis nerve is located and positioned over recording electrodes. The tendon of the interosseous muscle on the fourth digit bone is isolated and connected to a thread. Weights ranging from 0.5 to 10 g are added to the thread to stimulate the proprioceptors and produce sensory nerve action potentials along the peroneus communis nerve. The weight applied and the number of the action potentials observed during the first 2 seconds are recorded, plotted using an *X*-*Y* graph, and statistically analyzed for correlation.

Adaptation is examined by stimulating the proprioceptors of the frog's toe joint with weight continuously over a period of time. The number of action potentials observed in 2 seconds (at 30-second intervals) and the time interval are recorded. The data are plotted using an *X*-*Y* graph, and statistically analyzed for correlation.

Course of Infection of *Trypanosoma lewisi* in the Rat

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Trypanosoma lewisi infection in the rat provides an excellent model for investigating immunological mechanisms for undergraduates. Trypanosome infections are easily established by inoculating rats intraperitoneally with a sample of infected blood. Trypanosomes are available in infected rats from Carolina Biological Supply Co. Following inoculation the trypanosomes are found in the bloodstream and are readily observed and quantified by examination of blood samples. They are large flagellated protozoa (15–25 µ), and details of structure are clearly seen with an oil-immersion objective.

Trypanosoma lewisi is referred to as a non-pathogenic trypanosome, because rats acquire immunity and survive the infection. The course of the infection was described many years ago by Taliaferro (1932). The trypanosome numbers in the blood increase to a peak about 1 week post-inoculation. The numbers remain stationary for a brief period, and then there is a decrease to imperceptible levels. Taliaferro (1932) called the factor which inhibits trypanosome reproduction during the stationary phase ablastin. It appears now that ablastin is an IgG antibody which coats the trypanosome surface. The decrease in trypanosome numbers is thought to be due to a lytic event which follows the appearance of an IgM antibody specific for the IgG coating the trypanosome surface (Clarkson and Mellow, 1981).

Various factors are known to influence the course of this infection. Some of these can be easily investigated in an undergraduate laboratory and can provide additional insight into the immune response. For example, both size of the inoculum and age of the rat affect the outcome. In addition, previous exposure to trypanosomes as well as administration of immune plasma alter the course of the infection.

Aside from the overall value of introducing students to the immune response with an animal model, where they can actually participate in the inoculation process and follow the course of infection by examining blood samples, experience with the following procedures is also gained: (1) animal care and handling; (2) injection and blood collection; (3) blood smear preparation and staining, blood dilution, and hemacytometer cell counting; (4) trypanosome counting, measuring with a calibrated ocular micrometer, and drawing with a camera lucida from stained blood smears; and (5) data collection and semi-logarithmic graph preparation (showing trypanosome numbers on the logarithmic axis and days post-inoculation on the arithmetic axis), and statistical analysis. Statistical analysis of trypanosome measurements illustrates the influence of the rat on trypanosome reproduction.

I was first introduced to the *T. lewisi*-rat model by the late Leslie A. Stauber at Rutgers University in 1961.

Clarkson, A. B., and G. H. Mellow. 1981. Rheumatoid factor-like immunoglobulin M protects previously uninfected rat pups and dams from *Trypanosoma lewisi*. Science, 214:186–188.

Taliaferro, W. H. 1932. Trypanocidal and reproduction-inhibiting antibodies to *Trypanosoma lewisi* in rats and rabbits. American Journal of Hygiene, 16:32–84.

Microsurgery on Protozoa Using Cactus Spines

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Gary Schoenwolf has published a method for preparation of cactus-spine microsurgical needles for use in surgical manipulation of chick and frog embryos (Schoenwolf and Watterson, 1989, *Laboratory Studies of Chick, Pig, and Frog Embryos*, Macmillan). Here we describe an application of cactus-spine needles in the teaching laboratory for study of cell reorganization and regeneration of the giant protozoan, *Spirostomum*, following microsurgical bisection.

Select a cactus plant that has a variety of spine sizes. Use a fine forceps to remove a number of spines of various sizes for mounting. You will learn by experience what types and sizes of spines work best for you.

Ordinary wooden applicator sticks (e.g., Fisher Scientific, catalog #01-340) make good handles for the spines. Split and slightly sharpen the end of the stick with a single-edge razor blade. Use clear nail polish to glue a spine in place in the split end of each stick. (We use Revlon Super Nails Natural Wonder.) An angle of approximately 45° of spine to stick is a good one for starters. Cactus-spine needles can be sterilized by dipping them in alcohol and letting them air dry, but not flame sterilized!

Spirostomum can be observed and manipulated in spring water in a depression slide. Note especially the prominent contractile vacuole which is clearly visible near the posterior end of the cell. The presence of a contractile vacuole in each of the two cells within 2 or 3 hours following bisection will serve as an easily observable sign of cell reorganization and regeneration.

A *Spirostomum* cell can be cut in half by laying a cactus-spine needle across the cell and pressing down. Sometimes a moving cell can be cut by one quick stroke, but often the organism contracts and avoids being cut. A more reliable technique involves gently touching the organism and then quickly cutting across it during the momentary pause after it has stopped moving and contracted strongly in response to the initial touch.

The two cut cell halves can be left in the depression slide if it is placed in a covered petri plate lined with a folded wet paper towel to retard evaporation. Repeated examinations over the next several hours will allow observation of cell reorganization and regeneration.

We have done only a little testing of cactus-spine needles in other microsurgical procedures, but generally we think that cactus-spine microsurgical needles substitute quite well for drawn glass needles or needles made of sharpened tungsten wire. Some cactus-spine needles seem a bit too flexible for certain operations, but because of the ease with which they can be constructed, we foresee a bright future in the teaching laboratory for “Nature's microsurgical needles.”

A Chemistry Primer for General Biology: Using the Macintosh™ to Present Text, Graphics, and Animations in a Lecture Setting

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General Biology 1009 at the University of Minnesota—Twin Cities is required for students majoring in biology and many related fields. It can also be used to fulfil a science laboratory distribution requirement for students in fields outside the natural sciences. The course focuses at the molecular and cell levels early in the quarter. Because many of our students are inadequately prepared in basic chemistry, the course becomes a frustrating experience for some of them. A poor understanding of chemistry early in the course can hinder later learning and produce negative attitudes towards biology as a whole.

To assist students who lack adequate preparation in chemistry, we have developed a series of three presentations covering basic chemical concepts (e.g., atoms, elements, molecules, and bonding; energy and chemical reactions; and, macromolecules in biology). The presentations are offered outside the regularly scheduled lecture periods during the first week of each quarter and are open to all students in the course. Attendance is voluntary.

Our goal is to help students better understand the basics of chemistry, and to bridge the gap between real phenomena and the abstract representations so often used in science teaching. To make the presentations visually interesting and concrete we utilize the graphic and animation capabilities of the Macintosh computer and software. The presentations are in a lecture format and are accompanied by computer graphics organized in HyperCard™ or SuperCard™. The graphics are projected directly from the Macintosh and present text, illustrations, or animations. Both black and white, and color versions of the presentations have been developed. We use a Mac SE/30, Mac Data Display™ LCD panel, and overhead projector for the black and white version, and a Mac IIci and color video projector for the color version. Students also receive a lecture outline containing text and illustrations to help them follow along with the presentations.

Student reactions to the presentations have been very positive. Of the students attending the sessions during the academic year 1989–90, 84% thought that the computer-assisted instruction aided their understanding of the material.

This work has been made possible by a grant from Project MinneMac at the University of Minnesota. Tim Sundell of the Faculty Resources Center has provided invaluable advice and technical support during this project.

Laboratory Equipment List Spreadsheet

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A student-generated Lotus-123 spreadsheet which outlines laboratory equipment lists for an introductory biology course is used at St. Lawrence University. Useful facilities included: (1) macros to print designated lists without redefining print ranges; (2) an imbedded recalculation of solution quantities and equipment needs based on number of students input; and, (3) an easy keystroke command to move from the Table of Contents to any desired list of laboratory equipment.