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# "Chemical defenses can leave a bad taste in your mouth"

A common misconception held by many advanced high school biology students and college level undergraduates is that "plants are weak and can't defend themselves against herbivory." It is true that plants are sessile and are unable to escape attack by predators leading some students to consider them defenseless. If these students were asked for examples of how plants might defend themselves against consumers, some might say plants produce thorns (cactus) or a tough, waxy exterior (like aloe). However, many plants that have no obvious physical defense and yet are high in nutrient value are still not consumed. Why might this be?

Many students fail to realize that plants have evolved chemical defenses against attack by herbivores. Much of the emphasis in introductory ecology courses on adaptations focus on the behavioral or morphological features that improve an organism's ability to survive and reproduce in a particular environment, with little or no mention of physiological or biochemical adaptations. We now know that some compounds produced by plants help them survive the ecological battles that often exist among organisms. Amazingly, these chemicals can do much more than deter potential predators; they can ward off pathogens, keep living space free from competing organisms, and even reduce the impact of exposure to environmental stresses, such as high levels of solar UV radiation. These chemicals can have an enormous impact on the outcome of interactions between species and, thus, also on the structure and function of communities and ecosystems.

**Background**

Terrestrial ecologists have long known that toxins and other compounds commonly produced by diverse plant species play a large role in the dynamics of terrestrial communities; but until about 25 years ago, little was known about chemically-mediated interactions in the marine environment. As chemists began making discoveries of novel chemistry in marine plants and animals, they found that the most chemically-prolific marine species tended to be seaweeds and soft-bodied, slow-moving/sessile, invertebrates that inhabit coral reefs. Given the great abundance of grazers and predators on coral reefs, as well as the intense level of competition for limited bottom space, the chemists frequently speculated that the compounds they were isolating acted to deter predators and competitors. Several of these chemists began collaborating with marine ecologists to test these hypotheses through rigorous field and lab experimentation. These collaborations played a critical role in establishing the foundations of marine chemical ecology.

Objectives: This activity is intended to introduce students to the field of marine chemical ecology; develop an understanding of chemicals as agents to mediate interactions among marine organisms; and explore an experiment scientists use to assess chemical defenses.

**Key concept: In marine communities many sessile, benthic animals and plants are chemically defended against predators. Marine ecologists can use controlled feeding assays to determine if a marine organism is chemically defended.**

**Invitation Stage:**
Ask the following questions to the students in a group setting (or Thought Swap activity)

1) How do you think algae and soft-bodied sessile invertebrates living in habitats where predatory fishes are abundant avoid being eaten?
2) How do you think natural products help marine organisms survive in their environment?
3) Some organisms are so toxic, that they could kill many predators in a single dose. Why do you think some prey need to be so toxic?
4) How do you think scientists determine the ecological roles of natural products produced by marine organisms? What kinds of experiments would you do to see if an animal were chemically defended?

**Exploration Stage:**
Students will explore an activity that will help them understand how chemical ecologists design and conduct experiments in the field. Review the basic concepts of scientific experimentation.

> A scientific experiment is one that addresses a hypothesis, and uses appropriate experimental methodologies to test the hypothesis. These experiments typically have “controls” to distinguish “treatment” effects, and sufficient levels of replication.

We are going to design an experiment to test if a seaweed is chemically defended. We are going to assay predator deterrence. What kind of control should we use? How is a control different from the treatment? Why would we not want to use only one fish in the feeding assay, and just use this fish over and over again?

Tell the students that you have just isolated a new compound from an encrusting seaweed on a coral reef. You would like to know if the compound has any defensive functions against fish predation. Luckily, you have a classroom filled with herbivorous reef fish (i.e. students) willing to participate in your experiment. Tell the students that this feeding assay activity mirrors the experimental methods used by marine ecologists to examine the effect of a chemical isolated from marine plants or animals on the feeding preferences of predators. Students will be offered equal opportunities to eat each of two cookies: a “control” cookie that tastes good and a “treatment” cookie that is identical to the first one except that the compound (salt) is added to the dough. (The excess salt should make the treatment cookie distasteful to most students and thereby act as an analogue for a toxic or noxious-tasting compound produced by a marine organism.

Experiment setup:

1. Prepare palatable “Control” and unpalatable “Treatment” cookies using your favorite cookie recipe. Unpalatable “Treatment” cookies will use the same recipe, except add 1 teaspoon of salt. Each “treatment” cookie contains less salt than a hamburger from a fast food restaurant. (Could use onion powder instead of salt)
2. The data from your feeding assay are easily analyzed statistically by Fisher’s exact test. Have your computer on and running a statistics program ready to input data during the feeding assay.

Conducting the feeding assay:

3. Give each student one control and one treatment cookie and then ask them to taste each one. Then tell the students to eat the ones they like.
    a. When conducting feeding assays, scientists are not "grossed out" when a predator spits out a test food. However, this is not appropriate behavior in a class room, so pass out napkins in case a student needs to discretely empty their mouth. Suppressing the desire to spit out a bad cookie could skew the results of the feeding assay and needs to be avoided.
4. For each student, record whether or not he or she ate each type of cookie. If more than 25% of a cookie is left uneaten, score this as a rejection because some predators require a few bites to decide that a "prey" is inedible.
    a. Collect and discard any uneaten cookies.
5. Then give the students an Oreo cookie and tell them it is, in fact, an Oreo cookie, fresh out of the bag, and to eat it if they care to do so –but they do not have to eat it or even taste it. After giving the students a few minutes to eat the Oreo cookie, record for each student whether or not they ate it. The Oreo serves as a "satiation control" to ensure that the students were hungry and thus had motivation to sample and eat the control and treatment cookies during the actual test.

Analysis of the feeding assay:

6. You have to assume that your students like Oreo cookies. This is important because you will disregard data from the control/treatment feeding experiment for each student that DID NOT completely eat the Oreo cookie because you have to assume they were full during the experiment and thus not sufficiently motivated to eat either the treatment or the control cookie.
7. Write the data on the chalk board or overhead. Ask the students to score a cookie type as rejected if more than 25% of it was not eaten, and to disregard data from students that rejected the Oreo cookie.
8. Have your computer set up to do the Fisher's Exact test to see if your compound successfully deterred feeding

**Concept Stage**

(In conjunction with the concept stage, have slides highlighting key concepts)

Group discussion of the results:

- Ask the students to define a rejection

Define a rejection: often fishes will attack and then spit out a food item several times before deciding to reject it. If a significant part of a food pellet is not eaten, although plainly visible to the fish, this is scored as a rejection. It is possible that students who ate a bad-tasting cookie are just not interested in eating anything else. This could also affect the use of the Oreo as a control.

- Ask students who did not eat the Oreo cookie whether their choice not to eat it was the result of them not being hungry or whether it was the result of them being disgusted by the bad-tasting cookie and thus, not being interested in eating any more.

- Ask the students, “Did they trust you that this was a real Oreo? Could one bad-tasting cookie affect interest in all other cookies? (Introduce concept of learned aversion). What might happen if the instructor used a piece of candy rather than a cookie as the control?

- Give a presentation on different kinds of chemical defenses with examples in coral reef ecology.
    - Sponge and symbiotic host chemistry (bacteria, fungi, cyanobacteria) – antipredatory, antifouling, allelopathic
    - Soft corals and the chemistry produced by their zooxanthellae – antipredatory, UV protectants
    - Marine isopod protected from predation by toxic cyanobacteria - antipredatory
    - Obligate bacteria that lives on crustacean eggs that produces a fungicide – antifungal
    - Nudibranchs sequester defenses from prey – antipredatory
    - Lion fish and trunkfish are toxic
    - Ask students for other examples they may have read about

- Give examples of how natural products have directly helped mankind
    - Anticancer compounds from bryozoan
    - Anti-inflammatory compounds from gorgonians

**Extending the lesson further:**
It is interesting to note that if often takes higher doses of a chemical to deter feeding as the nutritional quality of the food increases (i.e. protein content increases), because nitrogen is often a limiting nutrient for most consumers.

Tell the students each treatment cookie contained less salt “deterrent compound” than a hamburger from a fast food restaurant. Obviously, the amount of salt needed to make a cookie distasteful is far less than what most people crave in their hamburger.

> Have students discuss why too much salt in a cookie tastes bad, while you wouldn’t notice it in a hamburger.

Also, increased toxicity of some organisms may have evolved because of interaction with their predators. As predators have evolved resistance to dietary compounds, prey species have to either increase the amount of toxin, or evolve new toxins to remain protected. (Introduce the concept of a chemical arms race or coevolutionary arms race)

Also describe how benign species can evolve to resemble a noxious or dangerous species and gain protection because the visual predator has learned to avoid the coloration pattern. (Introduce the concept of Batesian mimicry)

(FOR COS CLASS) – 20 min presentation
Because of the short time frame, I will introduce the questions that I would have asked for the thought swap and give fictionalized student answers to the questions. I will focus most of my time on the exploration phase, describing the assay and asking the students

questions about experimental design.  We will complete the assay and I will do a short presentation of topics I would cover in the Concept Introduction portion of the class.