Abstract
Achieving a deep understanding of the many topics covered in middle school biology classes is difficult for many students. One way to help students learn these topics is through scenario-based learning, which enhances students’ performance. The scenario-based problem-solving module presented here, “The Strawberry Caper,” not only meets but connects ecological and genetic concepts that are required standards in middle school. Here, students are required to provide expert witness for a patent-infringement claim against an organic strawberry farmer by a large neighboring company. The students must think critically and formulate and test hypotheses to provide evidence for the case. Through phenotypic and genotypic analyses, the students are immersed in an inquiry-driven investigation that provides a real-world context for topics covered in the classroom. This, interspersed with integration of concepts, promotes understanding and application of these topics.

Key Words: Ecology; genetics; inquiry; phenotype; genotype; PCR; gel electrophoresis.

Middle school teachers face the challenge of covering a diverse array of topics in their biology or life sciences classes over the course of a single academic year. According to the Next Generation Science Standards (http://nextgenscience.org), middle school science classes must cover subject matters including cell biology (MS-LS1-1,2), plant anatomy and reproduction (MS-LS1-4), genetic and environmental influences on phenotype (MS-LS1-5), genes and heredity (MS-LS3-1), sexual and asexual reproduction (MS-LS3-2), and artificial selection (MS-LS4-5). Many teachers express that they are ill prepared to teach all the topics required, and that time constraints prevent them from delving into great detail for particular lessons. Because of this, students can be underprepared when they enter high school. Indeed, it has been widely reported that students in high school have difficulties understanding topics, such as Mendelian genetics, that ought to have been introduced or even mastered in middle school (Stewart, 1982, Guilfoile & Plum, 2000, Venville et al., 2005, Williams et al., 2012). It has been shown that scenario-based learning engages students and enhances their understanding of scientific processes (Haury, 1993). To that end, we have developed a 4-day, inquiry-driven curriculum module that blends the broad range of subject matters that must be covered in middle school to give students a thorough understanding.

The Scenario: The Strawberry Caper
To provide real-world context for the scenario, we enlisted an active research scientist (Dr. Tia-Lynn Ashman) whose research in strawberry genetics naturally overlaps with topics covered in the middle school curriculum. The scenario involves a fictitious organic farmer, Farmer John, who is accused of stealing a patented strawberry crop from a neighboring farm, a fictitious corporation we call “Elite Berries, Inc.” The students are enlisted by Dr. Ashman to help with her expert-witness testimony. Through a videotaped message, she tasks them with determining, through scientific investigation, whether the claims of Elite Berries are true. Over the course of the module, students perform phenotypic and genotypic analyses to gather data for the impending court case, in which Dr. Ashman will serve as an expert witness. It is on the basis of the students’ data that Dr. Ashman will give her recommendations to the court.

The students receive court documents and information about Farmer John and Elite Berries to use as they brainstorm how they will determine Farmer John’s guilt or innocence (for supplemental info, see http://biology.pitt.edu/k-12-outreach/classroom-support). It is natural to begin an inquiry process by using our senses to evaluate the world around us (Haury, 1993). Indeed, while brainstorming about how to determine whether Farmer John’s strawberries originated on the Elite Berries...
farm, the students often suggest that perhaps we can see if the straw-berries “look the same” – that is, are the strawberries from the two farms phenotypically identical to each other? While there are numerous phenotypes that we could measure, the students focus on length and mass as two quick, straightforward traits to observe. Each student receives a strawberry from Farmer John’s farm from which to collect data for comparison to what is known about Elite Berries (see http://biology.pitt.edu/k-12-outreach/classroom-support for supplemental info). The students use calipers to measure the length of the strawberries in millimeters, and a digital balance to quantify the mass in grams. Each student records his or her values, and the class data are collected in an Excel spreadsheet to display the results in the form of a histogram. The histogram shows that despite these strawberries being genetically identical to each other as a result of asexual reproduction on one farm, there is much variation in the phenotype (Figure 1). Upon discussion, this shows the students that in addition to genes, the environment is a determining factor in the ultimate phenotype of the organism. Because of this variation, phenotype is not the most reliable test to determine Farmer John’s guilt or innocence. The students realize that although we have not drawn conclusions as to the source of Farmer John’s strawberries, the data are still informative in showing that phenotype alone cannot be used as a definitive test of strawberry identity. As is true for many scientific processes, the initial approach must be reevaluated, and the class then decides that a genotypic analysis will be more informative than a phenotypic analysis.

The students state that they would like to compare the DNA between Farmer John’s berries and Elite’s berries to show whether they are the same, but they are unsure how to make that comparison. Drawing upon their knowledge of cellular organization, they know that the DNA is in the nucleus of the cells that make up the strawberry, so the first thing they must do is extract DNA from the berries. The students follow a simple extraction protocol familiar to many middle and high school classrooms, which uses a buffer containing dish soap and salt to break down the cell membranes and unwind the DNA, respectively, and isopropanol to precipitate the DNA (for a detailed protocol, see Harrell et al., 2005). They can then spool the DNA out of the tube using a wooden skewer. This process connects what the students learn about how eukaryotic cells are organized to the process of extracting DNA from an organism. For example, they understand that there are barriers, made up of lipids, protecting the DNA; that the DNA is tightly compacted to fit inside the nucleus of the cell; and that each step of the protocol serves a purpose in obtaining the DNA.

Once the students have spooled their DNA, they quickly notice that each group’s DNA looks the same at the end of their sticks, despite having been extracted from phenotypically different berries. They must take this a step further and find a way to compare the genes within the DNA. The portion of the DNA we are interested in is called a “simple sequence repeat” (SSR). This is a region that varies between different strawberry varieties in the number of repeats, and each length variant constitutes an allele (Brunings et al., 2009). If one strawberry crop has an allele with a particular number of repeats, and another crop has more repeats, we will see the difference between the strawberries in the length of that region of DNA (Figure 2). This is quite similar to DNA forensic analysis commonly used in human DNA cases, and this type of analysis has been incorporated successfully in classrooms (Wagoner & Carlson, 2008).

![Figure 1. Example histograms of phenotypic data. The histograms of strawberry length (A) and mass (B) show that there is much phenotypic variation among genetically identical berries.](image)

![Figure 2. Schematic of simple sequence repeat (SSR) regions within a genome. The boxes indicate a repeated sequence. Here, we show two alleles: one with nine repeats and one with 11 repeats. PCR using primers flanking the SSR region (arrows) produces fragments of different length from the alleles.](image)
Elite Berries procured their patent by showing that their allele of a particular SSR is unique, so comparing Farmer John DNA to Elite DNA should definitively determine whether these strawberries are genetically identical. A control berry is also tested, which should not match the Elite berry.

To compare this region between berries, the students use polymerase chain reaction (PCR), which amplifies a specific region within the genome (for a detailed description of PCR, see Rosenzweig & Jejelowo, 2011). The students receive a PCR mix that contains the necessary components for PCR, such as buffer, nucleotides, primers specific to the SSR they are amplifying (Figure 2), and polymerase. Each group of two or three students is assigned a strawberry’s DNA to test – Farmer John, Elite, or control DNA. The students micropipette their assigned DNA and PCR mix in PCR tubes, and the PCR reactions are run overnight.

To visualize PCR products, gel electrophoresis is employed, which separates DNA on the basis of length. This is especially useful for our purposes because the SSR alleles that are being used to identify the strawberry cultivars vary in length. Therefore, the students can analyze a DNA fingerprint using gel electrophoresis, which has been utilized successfully in classrooms (Guilfoile & Plum, 1998, Phillips et al., 2008; Wagoner & Carlson, 2008). We use the Lonza FlashGel system in our analysis, which is ideal for the classroom. The FlashGels finish running in 10 minutes, so we can load and run a gel in one class period. Additionally, the gel is completely encased in plastic, and therefore it is safer than the standard ethidium bromide gels. Moreover, the gel cassette has an ultraviolet light and connects to a camera, allowing the class to watch the gel run in real time and to analyze the results together.

The gel analysis is the culmination of the genetic analysis. The students determine whether or not Farmer John’s strawberries are genetically identical to those owned by Elite Berries. We have two scenarios from which the cooperating teacher chooses, according to the students’ background and problem-solving abilities (Figure 3). First, the Farmer John DNA is different from that of Elite Berries, which shows that Farmer John’s cultivar is genetically distinct and, thus, that Farmer John is innocent. The second scenario is not as cut-and-dried. Farmer John’s and Elite’s berries have the same DNA fingerprint, indicating that these strawberries may have come from the same source. The students’ immediate reaction is to find Farmer John guilty, but does this definitively prove that Farmer John stole strawberries from the Elite Berries farm? Not necessarily, and the students must discuss how Elite Berries DNA might end up on Farmer John’s farm. Perhaps plantlets grew from runners that extended from one farm to another. Seeds could have been dispersed on Farmer John’s farm after an animal ate strawberries from the Elite Berries farm. Mutations could have arisen in one population, leading to genetically identical crops. The students brainstorm these and many other possibilities. This discussion of gene flow, or the transfer of genes from one population to another, shows the students how Farmer John could still be found innocent, even though the genetic evidence is not in his favor.

This scenario addresses several key concepts and topic areas covered in middle school. Over the 4 days, students learn about ecology topics such as plant morphology, reproduction, and artificial selection. They also spend much time linking genotype to phenotype. Though students may have been exposed to these topics in class, this module provides practical application of those terms and concepts. The students take the concepts a step further by discussing ways in which phenotype is influenced by the environment in addition to an organism’s genetic code. By the end of the module, the students will have learned and used sophisticated techniques commonly used in research labs and, in doing so, become problem-solving scientists who apply the concepts they learn in class to a real-world problem.

Strawberries are a perfect model organism for this type of analysis. The module covers both ecological and genetic topics that middle school teachers must address. In doing so, the students perform simple explorations of variation via measuring, weighing, graphing, and DNA extraction of store-bought strawberries. While strawberries are commonly used in classes to demonstrate DNA extraction, students take it a step further here by comparing the genetic makeup of different varieties of strawberries from which DNA has been isolated in the Ashman lab. The premise is that these cultivars are from the competing farms, and the students must analyze the genotypic data and formulate a conclusion as to Farmer John’s guilt or innocence. These types of genetic analyses have been successfully used in high school and college classrooms in forensic analyses and DNA fingerprinting (Guilfoile & Plum, 1998; Palladino & Cosentino, 2001, Reed, 2001; Kurowksi & Reiss, 2007; Wagoner & Carlson, 2008). But the Strawberry Caper is more age-appropriate for middle school and links a common fruit with current research as well as with patent-infringement court cases that are often seen on the nightly news. This thought-driven module sharpens students’ problem-solving and critical-analysis skills while integrating many of the topics that are required in middle school life science.

**Methods**

**Background Preparations**

To prepare the students to brainstorm possible outcomes of the court case, they are taught the basics of strawberry biology. We created a 10-minute video of Dr. Ashman describing strawberry flower anatomy, sexual and asexual reproduction, and artificial selection for cultivar.

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**Figure 3.** Sample gels of the two possible outcomes of the genetic analysis. In scenario 1, Farmer John’s (FJ) berries are genetically distinct from those of Elite Berries (E), as shown by different-sized PCR fragments. In scenario 2, FJ and E are genetically identical because of their identical banding on the gel. Control berries (C) are also added to each gel to show that strawberries from a distant farm will not share genetic identity with E.
development (for supplemental info, see http://biology.pitt.edu/k-12-outreach/classroom-support). This video serves two purposes: first, it delivers the necessary background information to the students from an expert, and it also introduces Dr. Ashman as a character in the scenario. This engages the students and puts a face to the name in the scenario. It has been shown that when middle school students watch videos of scientists, their interest in STEM is increased (Wyss et al., 2012). Thus, the students see Dr. Ashman as a real scientist and may be able to envision themselves in that role.

Once they learn the basic strawberry biology, they are introduced to the scenario through legal documents that their teacher provides (see http://biology.pitt.edu/k-12-outreach/classroom-support). These include a news article about Farmer John’s “Grower of the Year” award, a cease-and-desist letter from Elite Berries’ lawyers to Farmer John, a letter from Farmer John’s lawyers to Dr. Ashman asking her to be an expert witness in the court case, a map of the farms, and promotional material for Elite Berries. As a class, they go through the documents and brainstorm how they will test whether Farmer John has stolen Elite Berries’ crops. They must complete this brainstorming prior to the start of the module so that they are ready on day 1 to begin experiments.

In addition to strawberry background knowledge, the students may also practice micropipetting skills prior to performing the experiments in the module. This extends their math skills in lessons of scale by having them manipulate microvolumes, as is done in most labs. This skill comes in handy during the module, when the students are working on their genetic analysis, which requires the use of small volumes of reagents. Together with the strawberry background and court-case information, the students are now prepared to start gathering their data.

**Recommended Daily Breakdown of Module & Equipment Needed**

**Day 1:** Phenotypic analysis
- Strawberries
- Calipers
- Balance
- Excel spreadsheet or graph paper to collect class data and make a histogram

**Day 2:** DNA extraction
- Strawberries
- Plastic knife
- Baggie
- Extraction buffer
- Funnel
- Cheesecloth
- Scissors
- Plastic tube (e.g., 15-mL conical tube)
- Isopropanol
- Wooden skewer

**Day 3:** PCR*
- Purified strawberry DNA (Farmer John, Elite, and Control samples)
- PCR mix (contains SSR-specific primers, buffer, dNTPs, and Taq polymerase)
- PCR tubes
- Markers
- Micropipettors (to measure 3 μL and 20 μL) with tips
- Waste cup for used tips
- PCR machine

**Day 4:** Gel electrophoresis*
- PCR samples prepared on day 3
- FlashGel system (Lonza) or standard gel electrophoresis system
- Micropipettors with tips
- Waste cup for used tips

*If teachers do not have access to, or do not feel comfortable, using these molecular techniques, they can show a sample gel (Figure 3) after describing the theory behind these steps in the genetic analysis. That way, they have data to analyze and can still determine an outcome to the court case.

**Conclusions**

The Strawberry Caper module addresses major topics that must be covered in middle school and does so by incorporating these topics in an exciting courtroom-based scenario with sophisticated hands-on experiments. In this module, students learn plant morphology and reproduction, how and why farmers use artificial selection, the link between phenotype and genotype, and gene flow. Indeed, postmodule assessments show a significant improvement of the students’ content knowledge in these areas over premodule assessments (P < 0.001, n = 1220 students).

The curriculum was designed and implemented in 40-minute class periods. However, there is much flexibility within the curriculum for teachers to expand on the content or tailor it to their classes. The module offers teachers many avenues for expansion of the 4-day module to suit their classroom needs and context. For example, one school had a mock trial in which students presented their findings as part of the court proceedings. Another school developed cross-curricular activities to incorporate the Strawberry Caper into the other subject areas at that grade level. For instance, the math classes used geometry to determine which plots of land would be most economical for farmers, the language arts classes developed promotional materials for organic versus nonorganic farms, and the social studies classes researched the history of important genetic discoveries and created a timeline of these hallmarks. The integration of this module within their biology classes and across other curricula ties many concepts together and makes for a more engaging and lasting experience for the students.

Overall, the Strawberry Caper can provide teachers with content and experiments that are typically lacking at the middle school level. This module addresses topics that are difficult for students to grasp, such as the connection between genotype and phenotype. At the conclusion of the module, they will understand how genes are transferred among organisms, and that phenotype is influenced by not only genes but also the environment. It also allows the students to work through the problem in an engaging inquiry-based curriculum. Since they must brainstorm each step, they use their critical-thinking skills and experience firsthand how scientists work through a problem. This sets them up for higher-level thinking in their middle school classes and prepares them for high school.
Acknowledgments

We thank graduate student Matt Koski for help with concept development; middle and high school teachers Jen Cramer, Mimi Loeffler, Dawn Mostowy, and Stacey Falk for help with curriculum development; and the 20 cooperating teachers who have already integrated The Strawberry Caper into their classrooms. We also thank Tom Harper for video directing, filming, and editing. Funding for this project has been provided by Grable Foundation grant 103R20, National Science Foundation grant DEB 1020523, Howard Hughes Medical Institute Undergraduate Sciences Education grant 52006957, and the University of Pittsburgh.

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