INQUIRY & INVESTIGATION

Small Fish, Big Questions: Inquiry Kits for Teaching Evolution

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Abstract

*Evolution education poses unique challenges because students can have preconceptions that bias their learning. Hands-on, inquiry approaches can help overcome preset beliefs held by students, but few such programs exist and teachers typically lack access to these resources. Experiential learning in the form of self-guided kits can allow evolution education programs to maximize their reach while still maintaining a high-quality resource. We created an inquiry-based kit that uses live Trinidadian guppies to teach evolution by natural selection using the VIST (**V**ariation, **I**nheritance, **S**election, **T**ime) framework. Our collaborative team included evolutionary biologists and education specialists, and we were able to combine expertise in evolution research and inquiry-based kit design in the development of this program. By constructing the kits with grant funds slated for broader impacts and maintaining them at our university's Education and Outreach Center, we made these kits freely available to local schools over the long term. Students and teachers have praised how clearly the kits teach evolution by natural selection, and we are excited to share this resource with readers of* The American Biology Teacher.

Key Words: *evolution; natural selection; sexual selection; artificial selection; kit-based teaching; guppy; live animals.*

○ Introduction

Evolutionary principles are pivotal to the understanding of biology, but students continue to perform poorly in their understanding of evolution (Rice et al., 2011). This may be due to pre-existing beliefs that inhibit acceptance of new or different information (Lord et al., 1979; Bloom & Weisberg, 2007). A better understanding of the nature of science and the scientific process can increase acceptance of evolution (Lombrozo et al., 2008), and previous work by some of the authors showed that an inquiry-based, authentic science approach, including engagement with live animals, is useful for increasing both knowledge and acceptance of evolution (Broder et al., 2018, this Issue). However, this previous program required a substantial time investment by the participating researchers and teachers, which prevented it from being implemented broadly. To decrease the effort involved and make the program more accessible, our current goal was to repackage this program into self-contained, self-guided kits (see also Broder & Kane, 2017).

Evolutionary principles are pivotal to the understanding of biology, but students continue to perform poorly in their understanding of evolution.

In the previous program, 7th grade students made observations of live fish, developed hypotheses and predictions, tested their hypotheses, and interpreted their data, just as a scientist does. Students engaged in experiments centered around the core concepts of Variation, Inheritance, Selection (sexual, natural, and artificial), and Time (VIST; www.evolution.berkeley.edu), and at the end of the program, students learned that these processes cause genetic changes over time, leading to evolution. This program was designed to address the Colorado 7th grade life science standards (Colorado Academic Standards, 2009; Appendix Table 3) but is also applicable to the Next Generation Science Standards (NGSS Lead States, 2013; Appendix Table 4). Students who participated in this program showed a 29.5 percent increase in knowledge and 10.7 percent increase in acceptance of evolution (Broder et al., 2018, this Issue), demonstrating that this approach is effective at teaching evolution. However, this approach has been difficult to implement on a large scale.

Typically, there is a tradeoff between the quantity of students reached and the quality of education (Duraisamy et al., 1997). Individually packaged, kit-based materials offer a solution by allowing high-quality programs to be broadly disseminated. Hands-on inquiry kits have been used by others as take-home assignments to engage elementary students (and their families) in science, where they were shown to be particularly effective for female students (Gennaro & Lawrenz, 1992). In our case, kits were advantageous

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because they allowed us to overcome the following challenges: (1) Programs using live animals are often prohibitive for K-12 teachers, despite live animals increasing engagement (Allen, 2004), which is important in teaching evolution (Dole & Sinatra, 1998; Nelson, 2008). We used resources and facilities at our university to house animals that are repeatedly checked out and used with kits. (2) University scientists tend to have limited resources to devote to outreach, but they can offer valuable expertise. Kits allow scientists to prioritize their time for development, ensuring a high-quality product where dissemination is not constrained by the researcher's schedule. (3) Teachers vary in both preparedness and how they teach evolution (Glaze & Goldston, 2015). Kits are prepared by scientists but are student-guided, and are reusable, giving teachers confidence that they are covering the subject accurately. By packaging this program as a kit, we were able to provide a high-quality activity that could be administered broadly.

As with the previous program, our kit-based approach uses self-guided, hands-on observations and experiments with live animals to teach evolution by natural selection. Differences from the previous program include a shortened duration from 5 hours to 1.5 hours, resulting in abbreviated activities. Additionally, some activities were modified to encourage a range of skills to be utilized or to help facilitate interpretation. Students also utilize science notebooks in which they are prompted to draw, make tables and graphs, make hypotheses, answer questions, and reflect on what they observe. Below, we describe specific details of the kit activities so that they can be replicated by teachers who are interested in using this program in their own classrooms.

○ Materials List

Each kit is packaged using a hard-sided plastic case that contains materials that a pair of students shares (Table 1). Most materials are freely available on a website maintained by Colorado State Univeristy, http://www.cns-eoc.colostate.edu/smallfish.html. Items that are not included here should be available at the school or can be purchased from online retailers. The teacher maintains three holding tanks for the three populations of guppies and distributes guppies individually to the students.

○ Guppies as a Model System

The activities in this kit utilize live Trinididian guppies (*Poecilia reticulata*) from populations adapted to different environments (Figure 1). However, we note that the general approach described below could be modified to use any live-animal or plant system that has at least two populations adapted to different environments, including situations where one of the populations has been artificially selected or domesticated (as described in Broder et al., 2018, this Issue). We chose guppies as a model system for these activities because kit activities could be designed around current research questions.

Table 1. Supplies necessary for a single kit.

Quantity	Item	Availability
1	Computer or tablet	Available in the school
1	Hard-sided plastic case	Purchased (ex. Pelican 1150 case)
2	Science notebooks	Purchased
2	Nesting observation tanks	From template on website
2	Tank lids	Purchased (ex. crochet mesh)
1	Instruction booklet	PDF on website
1	Guppy color scale	JPEG on website
2	Guppy stencils (3D printed)	From template on website
2	Mechanical pencils	Purchased
2	Magnifying glasses	Purchased
1	Clear 15-cm metric ruler	Purchased
1	Colored pencils, 12-pack	Purchased
1	Predator silhouette	JPEG on website
1	Four-sided gaming die	Purchased
2	Sticky note pads (2 colors)	Purchased
1	USB memory stick	Purchased
1	Predator encounter video	Videos and labeled still images available for download on website
1	Spray bottle of ethanol	Purchased
As necessary	Paper towels	Purchased

Guppies are found throughout the Caribbean in freshwater streams. On the island of Trinidad, they have diverged multiple times into two phenotypes, driven by differences in predation pressure (Figure 1). Guppies can be found in large downstream rivers with many other fish including predators (High predation). Guppies have also colonized sections of streams above natural waterfall barriers that exclude major predators. In these low-predation environments, factors such as female choice are more important than predation for shaping traits. The differences in these environments have resulted in evolved differences in traits such as life history, morphology, and behavior (Magurran, 2005; Table 2). Guppies are also a useful system because a third domestic population has been artificially selected for exaggerated traits (Figure 1), and a comparative population can be acquired from local pet shops. Below we describe the activities associated with the kit, focusing primarily on natural high- and low-predation guppies, but also including domestic guppies in discussion.

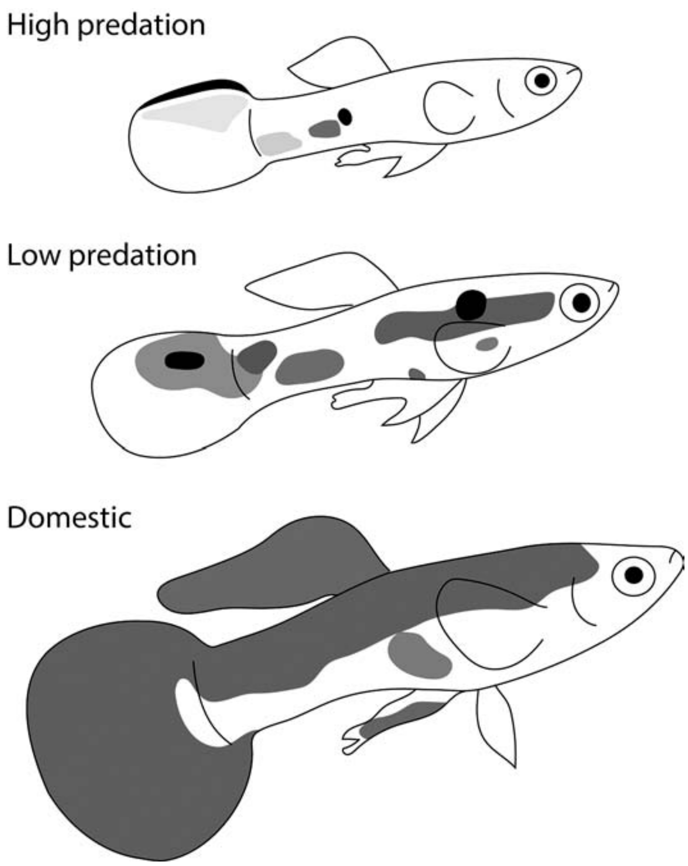

Figure 1. Drawings representing the physical differences between guppy populations, including size, shape, and color patterns (type and amount of color).

○ Activities

Introduction Video

Students begin by watching a 6-minute video to introduce them to the guppies and the researchers who study them (available on the website). The video introduces natural habitats on the island of Trinidad, shows researchers working in the field and laboratory, and discusses the research questions they will be pursuing. The introduction places the kit activities within a broader scientific context, rather than an isolated learning activity. It includes interviews with scientists who represent different academic career stages, genders, and ethnicities to put a human face on scientific research. The video concludes by outlining guppies as a model system for studying adaptation to different environments. Inclusion of the video introduces students to the scientists even when the scientists cannot be present.

Activity 1: Variation

Learning outcomes: Observe that variation exists within and among populations; collect, plot, and interpret data. Teachers maintain holding tanks with guppies in the classroom. Pairs of students obtain a guppy from each natural population and carry them in smaller observation tanks back to their desks. Domestic guppies are maintained in a holding tank but are excluded from some activities in the interest of time. Only males are used in these activities because they vary in coloration. The identity of each population is hidden from students so they can deduce population of origin as they work through the activities. Instead, populations are labeled as 1, 2, and 3.

Each partner chooses one of the two guppies at their desk, draws an outline of the corresponding guppy using the stencil, and records their observations of color pattern and fish size (using a ruler) in their notebooks. Color pattern is a complex trait that includes the number, position, and hue of multiple color elements. Students estimate an overall color score using a scale derived from the authors' research. Scores for the natural populations are recorded on a histogram template on the board using color-coded sticky notes to represent each fish (Figure 2a) that reveals differences within and between populations. Students qualitatively compare these results to observations of

Table 2. Differences in traits between natural populations of guppies.

Trait	High predation	Low predation
Life history	Many small babies, shorter life span	Few large babies, longer life span
Mating strategy	More sneaky forced copulations	Conspicuous male mating displays
Body shape	Small body size, more streamlined	Large body size, bulky shape
Color	Dull males	Brightly colored males
Response to predators	Faster escape speeds	Slower escape speeds
Other behaviors	Shoaling, stay in the shallows	Swim throughout the water column

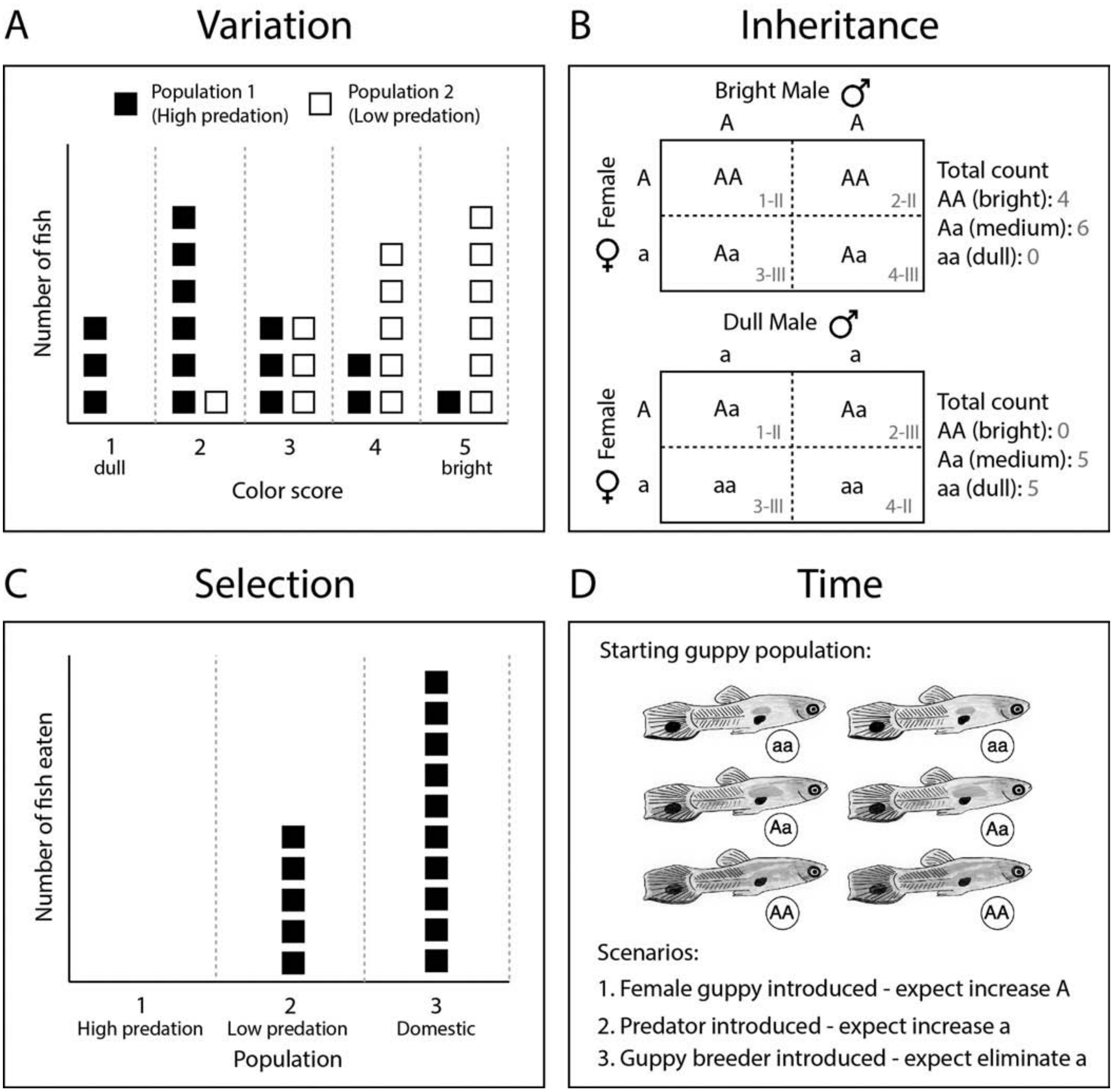

Figure 2. Students collect data during each section of activities to help them understand the key points about evolution. **(A)** Variation exists within and among populations. Students quantify color patterns from dull (1) to bright (5) and pool class data. **(B)** Much of this variation is inherited. Punnett squares are used to illustrate inheritance of alleles, and students roll a die to simulate possible offspring from each male-female combination. **(C)** Not all individuals are equally likely to survive and pass on their genes in a given environment, and this depends on their traits. Selection by predators is illustrated by pooled class data of survival from predator encounter videos. **(D)** Over time these processes result in changes in allele frequencies in populations. A thought exercise illustrates how allele frequencies can change in different scenarios.

domestic guppies in the holding tank, and answer questions about variation in their notebooks.

Activity 2: Inheritance

Learning outcomes: Understand that variation can be inherited through genes; use probability theory to make predictions about offspring traits. To explore one mechanism affecting color pattern in male guppies, students consider the role of female choice and how this choice interacts with inheritance to determine offspring coloration, via completion of Punnett squares. A basic understanding of Punnett squares is sufficient for this activity, and templates are provided in the instruction booklet. Although the genetics of guppy coloration are complex, we teach a simplified example of incomplete dominance using genotypes for bright (*AA*), intermediate (*Aa*), and dull (*aa*) males. Students create Punnett squares using an intermediate female (*Aa*) paired with each male (Figure 2b). To simulate ten possible offspring of each of these pairs, students roll a four-sided tetrahedral gaming die ten times, where each number corresponds to a box ("choice") in the Punnett square (Figure 2b). They tally the number of times they get a particular allele combination (e.g., *AA*) and trait value (e.g., bright). After doing this for each female-male pair of guppies, students answer questions in their notebook about which males produce the brightest offspring and how a female's choice of mate can affect the outcome since females prefer bright males (Figure 2b).

Activity 3: Selection

Learning objectives: Recognize that not all individuals survive to pass on their traits and that this is a nonrandom process; use behavioral observations to make predictions; collect, plot, and interpret data. To explore a second mechanism of selection that can influence inheritance, students consider the role of predation. Students begin by observing differences in guppy antipredator behavior among the three populations using a silhouette of a cichlid predator placed next to each tank (including the domestic guppy holding tank) to simulate an approaching threat. Students are asked to consider what they have learned about differences in size, color, and antipredator behavior to make hypotheses and predictions about which of the three populations is most likely to be attacked by a predator. Students will likely hypothesize that predators will attack the large and colorful domestic fish, and domestic fish will have poor escape behaviors.

To test their hypotheses, each student pair watches a different pre-recorded video of an encounter between a cichlid predator and three male guppies, one from each of the three populations. Students record which male was eaten as well as any antipredator behavior they observed during the video. Still images with males labeled by population number are provided to assist with identification because the fish move quickly and it can be difficult to track individuals. Students tally the fish that were depredated on the board to observe variation in survival across all fifteen videos (Figure 2c; key provided on the website). In their notebooks, students make conclusions on whether their hypotheses were supported or not based on observed antipredator behaviors and predation rates for each population. Because color, body size, and behavior co-vary in these populations, it is not possible for students to tease apart the specific factors influencing predation in this experiment, but this activity allows them to test a simple hypothesis. At the conclusion of this activity, students are asked to use what they learned about the roles of sexual and natural selection on color patterns to identify which environment each population of fish came from and provide their reasoning in their notebooks.

Activity 4: Time

Learning objective: Discover that variation, inheritance, and selection will lead to changes in allele frequencies in a population over time. To explore how variation, inheritance, and selection interact across generations to lead to evolution, students consider the role of time. Instead of directly observing evolutionary changes across generations, they learn about the process of evolution by natural selection using the different guppy populations as contemporary outcomes of this process. Students are given a thought exercise in which a hypothetical population of guppies is exposed to three scenarios: (1) sexual selection, in which female guppies are choosy about the coloration of males they mate with; (2) natural selection, in which a fisherman releases a predatory fish in a habitat that previously lacked predators; and (3) artificial selection, in which a business owner chooses guppies to breed and sell in pet stores. Students are asked to calculate the ratio of *A* and *a* alleles in the starting population (6:6), and asked which of the males in the Punnett square will be most likely to pass on its genes in each scenario. Finally, students determine how the ratio of alleles changes in response to the new selection pressure (i.e., increase or decrease one of the alleles; Figure 2d). Teachers can use this discussion as an opportunity to examine the common misconception that the dominant allele (*A*) always becomes more common, since this would not be expected in the presence of a predator.

Synthesis

The activities conclude with a synthesis section that describes the processes students learned about in the activities, including variation, inheritance, selection (sexual, natural, and artificial), and time. The term "evolution" is introduced to the students for the first time, using the formal definition of "a change in allele frequencies in a population over time." This section is accompanied by a class discussion of the activities and results, which is led by a teacher. Students then return their guppies to the appropriate holding tanks, sterilize their observation tanks with ethanol, and return their kits.

○ Kit Reception

To date, the kits have been used with middle school classes, high school students, members of the public (ranging from elementary school to adulthood), and teachers participating in professional development (total: 9 events, 224 participants). All participants were focused during the activities, suggesting active engagement, and completed the kits in the allotted 1.5 hours. A formal assessment of the kits has not yet been performed. However, given the high level of interest, we have chosen to disseminate these materials more rapidly by presenting preliminary measures below. Overall, results support the utility of the kits in teaching evolution.

Student Feedback

To gauge student opinion of the kits and to help make corrections, we asked the first participating class of 25 middle school students to submit feedback on aspects of the kits they did or did not like. This sample is not representative but was able to provide valuable feedback from the students' perspectives. Responses were open-ended and not all students responded, but certain phrases appeared often. For example, eleven students mentioned live animals as a strength. Thirteen students praised the kit design (e.g., the lesson plan, fun activities, level of organization, visual aids, and clear explanations), the ideas presented (e.g., the role of genetics and the environment), and specific activities (e.g., the introduction video, the predator-prey videos, the use of stencils, and the silhouette activity). Seven students appreciated that the kits were self-guided. Student suggestions for improvement were primarily in terms of clarification of wording in the instruction booklet and reducing time constraints, and all suggestions have been incorporated into the current version of the kit.

Teacher Feedback

A group of nationally distributed teachers who were participating in a National Association of Biology Teachers professional development workshop at Colorado State University completed the kit activities. These teachers had not implemented the kits in their classrooms, but did participate in the activities as though they were students as part of this event. They were aware that they would be asked to reflect on the kits at the end of the event and were not compensated for participation or for their interest in the kits in their own classrooms. Eleven teachers responded to open-ended questions about how the kits compared to other approaches they have used to teach evolution, techniques from the kit that they found particularly effective, and strengths or weaknesses of the kits.

As with the previous survey with middle school students, the teachers were positive about the kits and the associated activities. Alternative techniques used by the teachers included lectures, hands-on games, a skull collection, and even a virtual guppy lab, but teachers agreed that the kits were more engaging than other approaches. Suggested areas of improvement were minor and included adding more discussion, adding activities (particularly for the "Time" section), and shortening the duration of activities (particularly the guppy color activity in the "Variation" section). Some of these changes could easily be taken into account by a teacher during the activities. For example, a teacher could encourage students to move ahead if they are taking too much time and could have students pause to further discuss an idea as a class. Overall, these results indicated strong support of the use of these kits by teachers.

○ Summary

Through a collaboration with diverse scientists and education experts, we created a resource that teaches evolution by natural selection through engaging, inquiry activities with live animals. These kits also encourage students to participate in the scientific process, including making scientific illustrations, observing animal behavior, making predictions, collecting and analyzing data, drawing conclusions, and justifying their conclusions by writing open-ended responses in their science notebooks. One goal for these kits is that, by making this resource freely available, we can provide a valuable learning opportunity for schools that serve populations that are low income and/or underrepresented in science, technology, engineering, and math disciplines. We are confident that students with preconceptions will come away with a better understanding of the science behind evolutionary theory, and we are excited to make these ideas and approaches available to readers of *The American Biology Teacher*.

○ Acknowledgments

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Appendix Table 3. The kit activities were developed to be applicable to the 7th grade Colorado science standards (Colorado Academic Standards, 2009). Specifically, students learn concept 1: "Individual organisms with certain traits are more likely than others to survive and have offspring in a specific environment." To demonstrate mastery of this concept, students should be able to perform the tasks listed as evidence outcomes. Descriptions are given of how our kits address each outcome.

Colorado standards—evidence outcomes	Guppy kits
A. Develop, communicate, and justify an evidence-based explanation for why a given organism with specific traits will or will not survive to have offspring in a given environment.	Students use their data on the traits of individual guppies from different populations to make arguments for why those individuals will or will not survive and reproduce in environments with different selective pressures.
B. Analyze and interpret data about specific adaptations to provide evidence and develop claims about differential survival and reproductive success.	Students collect, analyze, and interpret data about coloration and antipredator behavior of guppies, and relate that to survival and reproduction in different environments.
C. Use information and communication technology tools to gather information from credible sources, analyze findings, and draw conclusions to create and justify an evidence-based scientific explanation.	Students pool and analyze class data and use the information to make conclusions about how guppy populations are adapted to specific environments. They justify their scientific explanations using evidence gathered through the activities.
D. Use computer simulations to model differential survival and reproductive success associated with specific traits in a given environment.	Students complete thought exercises in which they predict how color traits and associated alleles change in a population over time depending on the environment.

Appendix Table 4. The Next Generation Science Standards have been adopted in many states, and represent a broad standard for science education. We address how our program also applies to the Next Generation Science Standards (2013). For middle school, one of the topics is "natural selection and adaptations," which include several performance expectations. Our program targets MS-LS4-4 (Construct an explanation based on evidence that describes how genetic variations of traits in a population increase some individuals' probability of surviving and reproducing in a specific environment) and MS-LS4-6 (Use mathematical representations to support explanations of how natural selection may lead to increases and decreases of specific traits in populations over time).

Next Generation Science Standards (2013)	Guppy kits
Science and engineering practices: • Use mathematical representations to support scientific conclusions and design solutions. (MS-LS4-6) • Construct an explanation that includes qualitative or quantitative relationships between variables that describe phenomena. (MS-LS4-4) • Analyze and interpret data to determine similarities and differences in findings. (MS-LS4-1) • Apply scientific ideas to construct an explanation for real-world phenomena, examples, or events. (MS-LS4- 2) • Connections to Nature of Science: Science knowledge is based upon logical and conceptual connections between evidence and explanations.	Students make and interpret graphs to draw conclusions about quantitative relationships between the environment and color pattern variation. Students compare their findings, both within their own two guppies, as well as between guppies throughout the class by assessing the outcome of class data that is presented on the board. Students also use Punnett squares to determine probability of outcomes and help explain how variation is inherited. Students learn about the nature of science by building their own evidence based on their data as well as class data. Different forms of evidence are collected throughout the activities and are combined at the end to explain variation between populations.

Appendix Table 4. Continued

Next Generation Science Standards (2013)	Guppy kits
Disciplinary core ideas: A. Natural selection: Natural selection leads to the predominance of certain traits in a population, and the suppression of others. (MS-LS4-4) B. Adaptation: Adaptation by natural selection acting over generations is one important process by which species change over time in response to changes in environmental conditions. Traits that support successful survival and reproduction in the new environment become more common; those that do not become less common. Thus, the distribution of traits in a population changes. (MS-LS4-6)	Students compare and contrast the effects of multiple selective forces to determine the role of each in defining patterns of variation. Specifically, the contrast between female choice and predation highlights alternative selection on trait values. Students assess how these factors affect the frequency of traits across generations by completing an exercise assessing how the ratio of alleles changes in response to a new environmental pressure. They use the information and evidence they gained from the other activities to make an informed prediction. They also think about how these processes generated the distribution of color patterns observed in their populations.
Crosscutting concepts: • Patterns can be used to identify cause and effect relationships. (MS-LS4-2) • Graphs, charts, and images can be used to identify patterns in data. (MS-LS4-1), (MS-LS4-3) • Phenomena may have more than one cause, and some cause and effect relationships in systems can only be described using probability. (MS-LS4- 4), (MS-LS4-6) • Connections to nature of science: Science assumes that objects and events in natural systems occur in consistent patterns that are understandable through measurement and observation. (MS-LS4-1), (MS-LS4-2)	Students plot their data in graphs and tables and use these to identify patterns in color variation, inheritance, and survival against predation. These patterns are then used to identify cause and effect relationships between a selection pressure and trait values. Students learn that there are many types of selection and that different selection pressures may result in similar or different outcomes. They also learn that predicting inheritance is based on probability and that the actual outcome of a mating event may be different. Students learn about the nature of science by making their own observations, such as quantifying color score, measuring their fish, and describing antipredator behavior, and they use these observations to support or refute their hypotheses.