Nature's Cutest Symbiosis: The Bobtail Squid

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Film Guide
Educator Materials

OVERVIEW

Nature's Cutest Symbiosis: The Bobtail Squid is one of 12 videos in the HHMI series "I Contain Multitudes," which explores the fascinating powers of the microbiome: the world of bacteria, fungi, and other microbes that live on and within larger life forms, including ourselves.

In this video, Ed Yong introduces viewers to a master of camouflage: the Hawaiian bobtail squid. It is a small predator native to the coastal waters surrounding the Hawaiian Islands. During the day, to avoid being eaten, it hides by burrowing into the sandy ocean floor, even attaching sand particles to itself. During the night, the bobtail squid leaves its hiding spot and forages in the water column. The squid has evolved to live in a symbiotic relationship with the bioluminescent bacteria species *Vibrio fischeri,* which serves to protect the squid from its predators and prevent it from being seen by its prey. The bacteria are housed in a special structure, called a light organ, located inside the body, and the light shines through the muscles and skin of the ventral side of the squid. At night, the bacteria glow to match the moonlight coming from above, preventing the squid from casting a shadow and camouflaging it well.

Scientists Margaret McFall-Ngai and Edward Ruby at the University of Hawaii have studied the bobtail squid for years. Among the many discoveries they have made, they found that immediately after hatching, the squid collect *Vibrio fischeri* from the ocean water, and that without the symbiotic bacteria, the juvenile precursor to the light organ does not mature into a functioning light organ.

KEY CONCEPTS

A. Symbiosis is a close, long-term interaction between organisms of at least two different species, often comprised of a large host and one or two species of microbe. For the host and/or microbes, one, both, or neither species may benefit from the relationship. When both host and associated microbes benefit, the relationship is called mutualism.
B. Microbes can play an important role in the development of a host organism.
C. The bobtail squid has evolved a number of camouflage strategies through the process of natural selection.
D. Model organisms are used to help scientists understand biological phenomena.

CURRICULUM CONNECTIONS

Standards	Curriculum Connections
NGSS (2013)	LS1.A, LS2.A, LS4.B, LS4.C
AP Biology (2015)	2.C.2, 2.D.1, 2.E.2, 2.E.3, 3.D.1, 3.D.2, 4.A.5, 4.B.3
AP Environmental Science (2013)	II.A
IB Biology (2016)	4.1, C.1
IB Environmental Systems and Societies (2017)	2.1
Vision and Change (2009)	CC2, CC5

PRIOR KNOWLEDGE

Students should

- have a basic understanding of natural selection and evolution;
- be familiar with the concept of food chains and food webs;
- know that bacteria are unicellular organisms, some of which are beneficial and others pathogenic;

- have a basic understanding that during development, cells differentiate to form specialized tissues and organs; and
- be familiar with bioluminescence.

Note: The video approaches these topics at a basic level, so it could serve as an introduction to these topics as well as a launching point for deeper learning.

PAUSE POINTS

The film may be viewed in its entirety or paused at specific points to review content with students. The table below lists suggested pause points, indicating the beginning and end times in minutes in the film.

	Begin	End	Content Description	Review Questions
1	0:00	1:26	• The bobtail squid has evolved a host of strategies to hide from predators, including covering itself with sand, changing color, ejecting ink, and glowing.	• Why is it important for the bobtail squid to be able to hide? • What are some of the strategies it uses to hide?
2	1:26	2:43	• The squid has a light organ that projects light downward when it swims. The light intensity matches that of the light from the moon and stars above. • This behavior, called counterillumination, camouflages the squid. • The squid cannot produce light on their own. *Vibrio fischeri*, housed in the squid's light organ, produce light through bioluminescence. • The squid and bacteria both benefit in this relationship.	• Why is it important that the light organ projects light on the ventral side of the squid and not on the dorsal side? • How does counterillumination protect the squid from predators? • How do the bacteria benefit from this relationship?
3	2:43	3:31	• Symbiosis is a long-term relationship between two different species. • The squid and its symbiont *Vibrio fischeri* are good study subjects because the interaction involves only one host species and one bacteria species.	• Explain how the relationship between *Vibrio fischeri* and the squid is an example of symbiosis. • What makes the bobtail squid and its symbiont good study subjects?
4	3:31	4:43	• Margaret McFall-Ngai and Edward Ruby discovered that the bobtail squid is born without a functioning light organ. • They conducted an experiment in which one set of squid were raised with their symbiotic bacteria and one without. Those without failed to mature the light organ. • *Vibrio* enter a structure in the juvenile squid and influence its development into the light organ.	• Explain the significance of the discovery that the light organ does not develop completely unless *Vibrio fischeri* are present. • How might bacterial and squid cells communicate?
5	4:43	7:44	• The juvenile light organ has appendages that draw in particles of bacterial size. A cocktail of chemicals generated by the squid selects for *Vibrio fischeri*. • Once in the juvenile light organ, bacteria receive signals to collect in a chamber and reproduce. Signals from the bacteria then help direct the maturation of the light organ. • The squid is able to regulate the amount of light emitted by the light organ.	• How does the squid select for only *Vibrio fischeri*? • Why was the discovery that *Vibrio fischeri* influences the development of the squid light organ significant? • Why is it important for the squid to be able to regulate the amount of light emitted by the light organ?

BACKGROUND

The relationship between the bobtail squid and *Vibrio fischeri* illustrates how beneficial bacteria can influence animal health. It also confirms that bacteria are able to communicate with animal cells and influence the development of the tissues with which they associate.

At night, the bobtail squid hunts in shallow water, where its body can be silhouetted against a star or moonlit water surface and/or cast a shadow over the ocean bottom. Either way, it could be easily detected by predator or prey lurking below. The squid would then be in trouble—either eaten by the predator or unable to catch its prey, which would have fled.

But bobtail squids have a way to remain hidden. They have evolved a light organ that can generate a faint glow to cancel out the shadow and camouflage them. This phenomenon is called counterillumination. However, squid cells do not generate light. The light comes from symbiotic bacteria of a specific species—*Vibrio fischeri*—that can generate light via the process of bioluminescence.

The adult light organ is located inside the squid's mantle cavity (Fig-1A). Its structure and function are akin to a "backward eye": instead of light going into the organ, it comes out. The organ's tissues ensure that the light can only be projected out of the ventral side. The dorsal covering of the central core containing the luminous bacteria has reflective molecules that redirect any light traveling toward the dorsal side. Light that makes it past the reflector in the wrong direction is absorbed by the surrounding ink sac (Fig. 1C).

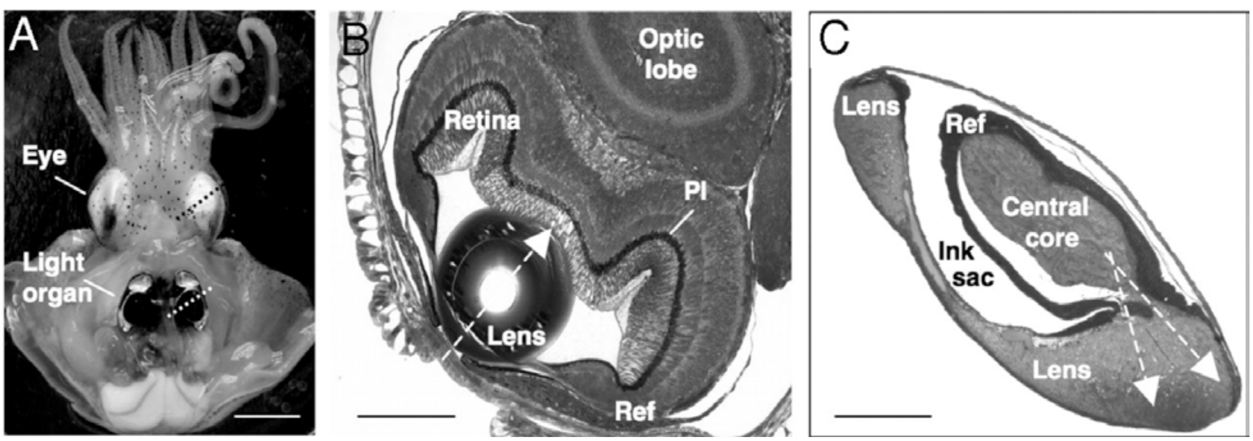

Figure 1. Anatomy of the eye and light organ of bobtail squid. (A) Location of the light organ and the eye in an adult. The animal has been dissected to reveal the light organ in the center of the mantle cavity (scale bar 1 cm). (B) Cross section of the eye, cut along the black dotted line in A. The lens focuses light (dashed line, arrow) onto the retina; the pigmented layer (Pl) and reflector (Ref) collect stray light (scale bar 2 mm). (C) Cross section of the light organ along the white dotted line in A. The "lens" acts as a diffusion filter to pass light from the bioluminescent symbiont in the central core to the outside (dashed line, arrow). The reflector (Ref) reflects light that tries to escape dorsally, and the ink in the ink sac absorbs stray light (scale bar 2 mm). From Tong *et al*., 2009.

Vibrio fischeri constitute approximately 0.01% of the bacteria found in ocean water. Newly hatched squid do not contain any *Vibrio fischeri*. But within two to three hours of birth, the squid selectively collects enough bacteria to colonize the developing light organ. Without these bacteria, the light organ does not develop properly. Chemical messages between the bacteria and squid cells are thought to facilitate the process by which *Vibrio fischeri* selectively colonize the juvenile light organ and induce subsequent organ development.

The bacteria, protected and nourished within the light organ, rapidly increase in number. At night, they provide the light needed for counterillumination. By dawn, the bacteria have multiplied to an unsustainable level, and the squid ejects about 95% of them. During the day, as the squid hides buried in the sand, the remaining bacteria reproduce, and by night, the population has increased once again to the density required to produce the light needed for counterillumination. This cycle repeats every day throughout the life of the squid. Each light organ contains between 10^7 and 10^9 bacteria, depending on the size of the animal.

Vibrio fischeri only produce light (or bioluminesce) when they multiply to a high cell density, at which point all the bacteria bioluminesce together. The phenomenon of producing a signal or behavior in response to population density is called quorum sensing and is based on chemical communication among individual bacteria living together in a colony. Bacteria make and release small molecules called autoinducers, which act as a chemical signal to regulate the expression of genes involved in bioluminescence. At low population density, the autoinducers mostly float away and have no effect. At high population density, more autoinducers reach bacteria and the chemical signal is received. Thus, the concentration of autoinducers acts as an estimate of population density. Once the autoinducer level is sufficiently high, it triggers gene expression in bacteria throughout the entire colony and they start producing light. In addition to light production, different bacterial species use quorum sensing to regulate a variety of physiological processes, including symbiosis, virulence, and antibiotic production. Disrupting quorum sensing is a potential alternative approach to developing antibiotics. Bacteria are less likely to become resistant to a drug that disrupts bacterial communication because it is not lethal and the selective pressure to develop resistance to it is not as strong.

DISCUSSION POINTS

- In the video *Nature's Cutest Symbiosis: The Bobtail Squid*, the juvenile light organ is described as having appendages that contain cilia that draw in water and bacteria. Ask your students "Why are these appendages not needed later in the life of the squid?"
- There are many species of bioluminescent bacteria. Most of them are symbionts. The bioluminescence benefits the host animals in a variety of ways, by helping them avoid predators through counterillumination, attract prey, communicate to members of the same species, and navigate in dark environments.
- In many mutualistic relationships, the two species obtain nutrients from one another. Bioluminescent symbiosis between bacteria such as *Vibrio fischeri* and the bobtail squid is unusual in that the host does not receive any nutrients from the bacteria. Ask your students "Why would this relationship be considered mutualistic?"
- The structural components of the light organ are remarkably similar to those of the eye and include iris, lens, and reflectors. These components are used to modulate the amount of light that is emitted by the organ so that it dynamically matches the changing moonlight and starlight shining on the animal, and ensure that the light is projected ventrally and not in any undesired direction. Researchers have discovered that symbiotic bacteria can induce the expression of a series of genes in the developing light organ that are also expressed in the developing eye. Investigation of the squid light organ provides scientists an opportunity to examine the effects of bacteria on the development of ocular structures. Ask your students "How might this information aid in the study of diseases of the human eye?"
- Camouflage experts in the United States Air Force have studied the symbiotic relationship between the bobtail squid and *Vibrio fischeri*. How might a better understanding of the light organ and its reflective qualities help in the design of better aircraft camouflage?
- Researchers at UC Irvine have used the reflective molecules of the light organ (the reflectin protein) to design and produce infrared camouflage clothing, so that a soldier or other individual wearing this clothing would be camouflaged from an infrared scope. Reflectin has also been found to be highly conductive, and scientists are

researching how to fabricate transistors using reflectins. Ask your students "Can you think of other uses of biomolecules as structural, textile, or electrical building materials?"

- *Vibrio fischeri* only produce light when they multiply to high cell density, at which point all the bacteria bioluminesce together. The phenomenon of producing a signal or behavior in response to population density is called quorum sensing and is based on chemical communication among bacteria living together in a colony. Because many pathogenic bacteria rely on quorum sensing to infect their hosts, finding ways to disrupt quorum sensing could provide an alternative to antibiotics to fight infectious disease. Ask your students "What advantages would such a strategy have over the use of antibiotics? Why do some researchers refer to this strategy as 'silencing the mob'?"

STUDENT HANDOUT

The student handout is available as a separate file. It is designed as a learning assessment that probes students' understanding of the key concepts addressed in the film and can be used to assess students' prior knowledge before watching the film or to guide students as they watch the film. We encourage you to choose the use that best fits your learning objectives and your students' needs. Moreover, because the vocabulary and concepts are complex, we encourage you to modify the handout as needed (e.g., reducing the number of questions, explaining complicated vocabulary for English learner students).

ANSWER KEY

1. [Key Concept C] The bioluminescent glow of the light organ helps the bobtail squid
   a. camouflage itself on the sandy ocean floor.
   b. signal and attract mates.
   c. hide from predators and prey.
   d. illuminate the ocean floor so it can locate prey.

2. [Key Concept C] The squid light organ has structural and functional similarities to an eye, including the ability to detect light. Explain why it is important to the squid's survival to be able to detect light as well as emit it.

The squid must be able to determine how bright the moonlight coming in from above is so that the amount of light emitted by the light organ matches it.

3. [Key Concept A] Mutualism is defined as a close, prolonged association of two or more species that is of benefit to all participants. Explain how the relationship between the bobtail squid and *Vibrio fischeri* is an example of mutualism. Defend your claim with evidence.

It is a close and prolonged relationship. Once Vibrio *bacteria enter the light organ, a population of* Vibrio *will be there for the lifetime of the squid. Both species benefit from this relationship. The bacteria housed in the light organ are protected and nourished. Counterillumination provided by the glowing bacteria benefits the squid. It makes the squid "invisible" to both predators and prey.*

4. [Key Concept B] If no *Vibrio fischeri* are present in the environment, what is most likely to happen during the development of the bobtail squid?
   a. The squid does not mature into an adult.
   b. The light organ develops completely but does not glow.
   c. The light organ does not develop completely.
   d. The squid selects different prey as a source of food.

5. [Key Concept D] The bobtail squid and *Vibrio fischeri* serve as model organisms for studying cellular communication. Provide at least two examples of communication either between squid and bacteria or within bacteria from the film.

Without the ability to communicate with **Vibrio fischeri*, the bobtail squid would not be able to select for this species of bacteria and direct it to the appropriate location in the light organ. In turn, the bacteria communicate with the cells of the light organ to signal its maturation. Another example of cellular communication would be quorum sensing within the bacterial population. The squid and bacteria provide an attractive system for studying cellular communication because in the light organ, only one species of bacteria interacts with the host.***

6. [Key Concepts B and D] Describe the experiment that proved the presence of *Vibrio fischeri* is required for the maturation of the light organ.

Scientists raised two sets of bobtail squid. **Vibrio fischeri** ***were present in the environment of one set of squid and absent from the environment of the other. Both sets of squid were healthy. But those raised in the absence of the bacteria did not form mature light organs. Those with the bacteria present in their environment did form mature light organs.***

7. [Extension Question: Not covered in film] Within the light organ, bacteria are protected and nourished, and rapidly increase in number. At night, they provide the light needed for counterillumination. But by dawn, as the squid prepares to hide in the sand for the day, it ejects about 95% of the bacteria from the light organ.
   a) [Key Concept C] Write a possible explanation for why eliminating 95% of the bacteria present in the light organ is necessary.

   Students answers will vary. The correct explanation is that if bacteria are not ejected, populations would continue to grow and there would be too many bacteria for the squid to support.

   b) [Key Concept B] Once the light organ is colonized, the squid no longer takes in *Vibrio fischeri* or other bacteria from the environment. Explain how a sufficiently large population of bacteria can be maintained in the light organ to produce light.

   Vibrio ***have a rapid rate of reproduction. During the day, they multiply rapidly to a population density high enough to trigger quorum sensing and light production.***

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