OVERVIEW

Solving Crimes with the Necrobiome 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 lifeforms, including ourselves.

This particular video focuses on the microbes associated with decomposing corpses, or cadavers. Following death, the microbiome of a body changes dramatically in composition. The community of organisms associated with a cadaver, which includes these microbes, is known as the necrobiome. In the video, Jessica Metcalf, an associate professor at Colorado State University, describes how she tracks changes in the necrobiomes in, on, and around the decaying bodies of dead animals, including humans, over time. She also explains how the predictable sequence of microbial communities associated with a cadaver can serve as a "microbial clock" for estimating time since death, providing useful knowledge for crime scene investigators.

Additional information related to pedagogy and implementation can be found on this resource's webpage, including suggested audience, estimated time, and curriculum connections.

KEY CONCEPTS

- Living organisms host a complex community of microbes called a microbiome, which is usually regulated by the host's immune system.
- Decomposition is mediated by microbes in predictable stages of ecological succession, which correspond to measurable and consistent changes in the microbial community.
- Decomposition is fundamental for cycling energy and nutrients in ecosystems.

STUDENT LEARNING TARGETS

- Describe the process of decomposition and the role it plays in an ecosystem.
- Explain how the microbiome associated with a decomposing body can be used to estimate the time since death.
- Interpret evidence to support or refute a scientific argument.

PRIOR KNOWLEDGE

Students should have a basic understanding of:

- the role of microbes as decomposers
- how matter cycles between the living and nonliving parts of the environment
- what a microbiome is

PAUSE POINTS

The film may be viewed in its entirety or paused at specific points to give students an opportunity to ask questions, construct explanations, and make predictions. The table below lists suggested pause points, including the beginning and end times in minutes in the film.

Before starting the film, you may also ask students the following question: "When a person dies, what do you predict happens to the microbes that live on their skin and inside their body?"

	Begin	End	Content Description	Discussion Questions
1	0:00	1:26	• Death is a major event for microbes because the environment in, on, and around the body changes. • After death, microbes that play an important role in decomposition become abundant. • During decomposition, patterns of change in microbial communities are consistent from person to person.	• You might be surprised to discover that some scientists study the microbes in and on human bodies after death. Why does Jessica Metcalf think studying these microbes is important? • What are some changes in microbial communities that occur after death? Are these changes similar or different from person to person? Why do you think that is?
2	1:26	3:32	• A body goes through five basic stages after death: fresh, bloat, active decay, advanced decay, and dry remains. • Microbe communities change rapidly after death, in part because the immune system has shut down and is not regulating their spread. • Microbes in the gut, which were helping the body digest food, now digest the body. • Fluids rich in nutrients are released from body orifices. • The decomposition process releases gases that build up and cause the body to bloat and rupture. • After the body ruptures, microbes from soil, air, or insects can access the inside of the body. • The final stage, dry remains, is when all that remains of the body is a skeleton and dry tissue.	• Why are microbes able to colonize new areas of the body after death? • What causes the body to bloat and the skin to rupture? What are the microbes producing? • Describe what happens during the "active decay" phase. What happens to the flesh that disappears?
3	3:32	5:37	• Predictable changes occur in the microbial communities associated with a decomposing body. These changes are precise enough that scientists can determine when someone has died (time since death) based on the microbes found in and around their body. • When estimating time since death, scientists must take temperature into account because it greatly affects the rate of decomposition. • Scientists conduct research on decomposing human bodies at anthropological research facilities (sometimes referred to as body farms). Scientists sequence the DNA of microbes collected over time from skin and soil swabs.	• Explain how microbes collected from a dead body can act like a "clock" to determine time since death. • Why do scientists care about temperature when trying to determine time since death? • What are anthropological research facilities? • Why do scientists sequence microbial DNA from samples they collected from a cadaver? Why do they collect samples from the skin *and* the soil?
4	5:37	7:10	• Some microbes are involved in longer processes that occur during the "dry remains" phase. These microbes could be present for a long time after death. • Research on the necrobiome is important for crime scene investigations. Microbial data used to	• Why are crime scene investigators interested in the necrobiome? • Explain the comment "once a person dies, the recycling begins."

			determine time since death can help establish when a crime occurred. • Death of one organism is the beginning of new life for many microbes and other lifeforms. • Microbes are intricately involved in the processes of decomposition and nutrient cycling.	

BACKGROUND

The necrobiome, the community of organisms associated with a decomposing cadaver, is a microcosm for studying ecological community interactions and energy transfer. During decomposition, the environment of the body changes rapidly and interacts with the surrounding environment (for example, the soil, if the body was buried). Changes in abiotic factors, such as pH and temperature, as well as biotic factors, such as available nutrients and body composition, will influence the presence of scavengers and microbial species as decomposition progresses. Because the process of decomposition recycles nutrients essential for life, it is not an end but a beginning for a variety of organisms.

At the onset of death, the heart stops beating, and the body becomes depleted of internal oxygen. Enzymes in the body start to destroy cells and tissues in a process called autolysis, or self-digestion. Without immune cells to keep them in check, bacteria of the microbiome grow rapidly and begin to digest the body. There is a marked shift from aerobic species, which use oxygen in cellular respiration, to anaerobic ones, which do not. Anaerobic digestion results in gaseous byproducts, such as methane, hydrogen sulfide, and ammonia. These gases accumulate within the body, causing the body to bloat and then break open, or rupture. Rupture marks a major event in the decomposition process because it allows other microbes, insects, and scavengers to access the body more easily. As decomposition progresses, predictable changes in the microbial communities, as well as in the scavenger and insect communities, occur.

Because changes in the microbes in and around a dead body are measurable and consistent from person to person, scientists have been able to build a "microbial clock" that can be used to determine the time since death, or postmortem interval. Scientists conduct research on human cadavers at anthropological research facilities (also referred to as "body farms"). They collect samples from cadavers and the soil around them and sequence the DNA of microbes in these samples to determine which types of microbes are present at various time points after death. When a body is found, for example, at the scene of a crime, scientists can estimate the time of death by comparing the microbes found on this body to the microbial clock. Establishing time of death is critical in criminal investigations. It can be used to establish when a murder occurred, place suspects at the scene, and check alibis. The microbial clock technique is still in the research phase, and scientists are working with justice practitioners to bring the new technology into the justice system (e.g., make it admissible as evidence).

DISCUSSION POINTS

- The human body is home to a variety of microbes adapted to live in that environment. When a person dies, the environment changes, and different species of microbes may become dominant in the new environment. You may want to review the diversity of microbes and types of microbial metabolic strategies using the interactive module *Winogradsky Column: Microbial Ecology in a Bottle*.
- Many students learn about decomposition when studying ecology and food chains. Set the stage for students by reminding them of the essential role of decomposers in ecosystem function. After students view the *Solving Crimes with the Necrobiome* video, discuss with them how decomposition of a body serves to cycle nutrients in the ecosystem. This video provides an opportunity for reviewing the concept that matter is never created or destroyed, just transformed.

- The changes in the microbial community during decomposition are an example of ecological succession. Similar to how grasses are the first plants to grow back after a forest fire — followed by ferns, shrubs, and eventually trees — new species of microbes colonize a dead body in a predictable sequence.
- Students are probably familiar with the fact that crime scene investigators look for DNA evidence left behind by those involved. Make sure they understand that the DNA that is being sequenced in this context is from *microbes* present on or around the body. Identifying these microbes via DNA sequencing allows investigators to estimate *when* the crime occurred (time of death).
- Metcalf points out that investigators also consider temperature when determining time of death. Ask students to clarify why that is important. This is a good opportunity to talk about the role of temperature in chemical reactions, such as decomposition.
- Ask students how microbial analysis could be used to help solve crimes or test alibis. For example, investigators may use evidence from microbial DNA to determine when a murder occurred, then ask suspects to describe their whereabouts during this time period (and verify their statements using other evidence, such as security camera footage). It's important to emphasize that investigators should use multiple lines of evidence to support their claims of how and when a crime occurred, and that establishing a time of death may involve analyzing other environmental conditions beyond the microbial species present and temperature.
- Discuss with students how embalming prevents the natural decomposition process and the ecological implications of embalming. It is important to point out that embalming isn't universally practiced and that many religions and cultures have burial practices that do not involve embalming. Students may be interested in researching alternative burial methods.

STUDENT HANDOUT

The "Student Handout" is designed as a learning assessment that probes students' understanding of the key concepts addressed in the film. It can be used before the film to assess students' prior knowledge or during the film to guide students as they watch. You should choose the use that best fits your learning objectives and your students' needs. You may modify the handout as needed (e.g., reducing the number of questions, adding explanations of vocabulary for English learner students, etc.).

ANSWER KEY

1. Death is a major event not only for the person who dies but also for the microbes that inhabit their body. Which of the following statements best describes why?
   a. All the microbes that were living inside the person's body die when the person dies.
   b. ***Microbial populations grow and spread to different areas of the body after the person dies.***

2. Microbes in the body have fewer resources after the person dies, which increases competition. Which of the following statements about cadavers (dead bodies) is most likely to be true?
   a. ***Microbial communities in and around a cadaver change over time.***
   b. Samples taken from different cadavers always contain the same microbes.

3. The microbial community found in a cadaver is very similar to the one found in a living body. After death, gases build up inside the body and cause the skin to rupture (break open). Rupture is a significant event because microbes from outside the body can now access the inside. Which of the following can be a source of the microbes that enter the body after it ruptures? Select all that apply.
   a. ***skin***
   b. ***air***
   c. ***soil***
   d. ***insects***

4. Describe how the scientists in the video are using microbes to create a tool to estimate the time since death.
   Different specific microbial communities are consistently associated with different stages of body decomposition. Scientists can sample the body and surrounding soil at multiple time intervals after death and identify the microbes in the samples. Based on the types and abundances of microbes they find, they can then determine how long the person has been dead.

5. The law of conservation of energy states that the amount of energy in an isolated system stays constant. In other words, energy cannot be created or destroyed; it can only change form. Explain how the decomposition of a cadaver demonstrates this law.
   An organism consumes energy in the form of food and may transform that energy into fuel for activity or heat. It can also store the energy in its body. When the organism dies, the energy stored in its body is not destroyed. Instead, through the process of decomposition, it is converted into forms that fuel other life processes. For example, the cadaver becomes food for scavengers and microbes. These organisms also break down the cadaver into nutrients that are transferred into the surrounding environment, where plants and other organisms can use them.

6. Examine Figure 1, which shows the five stages of decomposition. In the table below, assign the letters of these descriptions to the stages in Figure 1.

Stage	1 (First)	2	3	4	5 (Last)
Name	Fresh	Bloat	Active Decay	Advanced Decay	Dry Remains
Description	*b*	*d*	*e*	*a*	*c*

A completed version of Figure 1 with additional information is shown on the following page.

LIFE AFTER DEATH
THE STAGES OF DECOMPOSITION

FRESH

The body's environment changes drastically after it shuts down. The heart stops beating, depleting the body of internal oxygen, cells begin to die, and a stable temperature is no longer maintained. With no immune system regulating microbes that normally live inside the body, their populations start to shift and grow in the changing environment. Flies deposit eggs in the body's openings and orifices, which will develop into maggots.

1

BLOAT

The depletion of oxygen inside the body results in the flourishing of anaerobic bacteria. Carbohydrates, fats, and proteins of the body are metabolized by these microbes, producing byproducts such as hydrogen sulfide, methane, and ammonia. The accumulation of gases results in strong odors and extreme bloating of the body. Maggots start feeding on the body tissue.

2

ACTIVE DECAY

Eventually, the cadaver reaches a point of rupture: massive fluid loss occurs through the body orifices, releasing an abundance of nutrients like nitrogen and phosphorus into the soil. This localized area of organically rich matter is called the cadaver decomposition island (CDI). The dissolution of barriers promotes microorganism growth, and breaks in the skin allow air and additional microorganisms, insects, and scavengers to gain access.

3

ADVANCED DECAY

Fungi flourish, and decomposition byproducts like cadaverine and putrescine contribute to the characteristic smell of the corpse. Eventually, most of the cadaver's fluids and mass transfer into the ground and scavenging animals. The speed of decomposition slows: maggots migrate away, and scavenging activity is reduced. The CDI shows death of immediate plant life from the overload of cadaver components.

4

5

DRY REMAINS

Only dried bones, cartilage and skin remain. Plant life around the CDI now begins to surge from the cadaver nutrients, which may influence the ecosystem for years following death.

Details Make All the Difference

The timeframe of decomposition is dependent on temperature and moisture. A corpse will decay much faster in a warm, humid climate than it will during a dry, cold winter. Similarly, if a body is buried, access by worms, insects and scavengers may be limited, slowing the process.

7. Blowflies are often the first scavengers to arrive at a cadaver. They lay their eggs in orifices and open wounds, and the stage of their development can help determine time since death.
   a. How might the presence of blowflies affect the course of decomposition?
      The flies and their offspring consume part of the cadaver. They also bring new microbes to the cadaver's ecosystem and may attract other scavengers to it.
   b. Other scavengers include beetles, wasps, dogs, crows, and crustaceans. Explain how these other scavengers could also affect decomposition.
      The scavengers may consume the cadaver or spread their own microbes to the cadaver, influencing the microbial community present. Some scavengers, such as insects, may also attract larger scavengers that prey on both the smaller scavengers and the cadaver.
   c. What abiotic factors might affect the kind of scavengers that contribute to a cadaver's decomposition?
      Factors could include temperature or season, as well as the location of the cadaver (above ground or underground, in water or on land, etc.).

8. The graphs in Figure 2 show some of the data that the scientists in the video collected from decomposing mice cadavers.

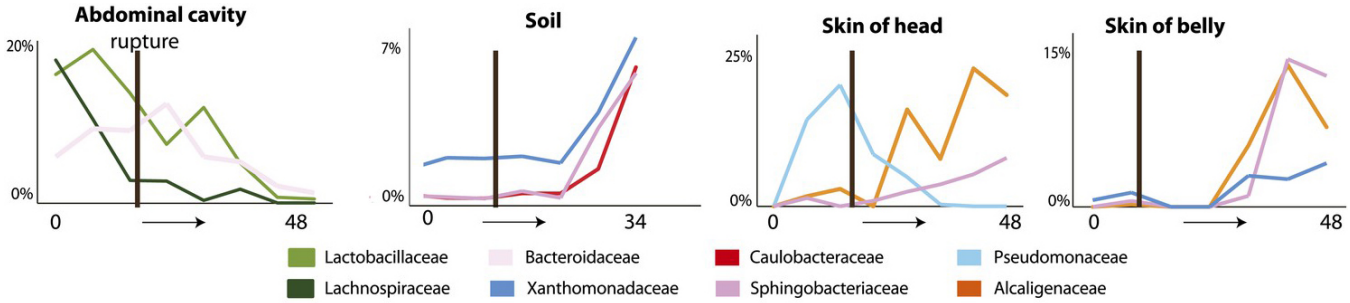

Figure 2. Abundances of different bacterial groups in and around the cadavers over time. In each graph, the *x*-axis represents the time since death in days, the *y*-axis represents the relative abundances of the bacterial groups, and the thick vertical line represents when the body ruptured. Figure from Metcalf et al. (2013).

For each of the claims below, use evidence from the graphs to either support or refute it. If you are supporting the claim, summarize a second experiment that would help to confirm it. If you are refuting the claim, summarize a second experiment that would help to rule it out.

Answers will vary, as students could make arguments for either side. For each claim, two sample answers (one that supports the claim and one that refutes it) are shown below, along with a potential experiment that could be used to either support or refute the claim depending on the results.

Claim A: The rupture of the body during decomposition is associated with changes in bacterial communities in the abdominal cavity, on the skin of the head, on the skin of the belly, and in the surrounding soil.

Sample Answer 1 (Support): The graphs show that different groups of bacteria change in abundance in these locations after rupture occurs. For example, the relative abundances of several bacterial groups decrease inside the abdominal cavity, and the relative abundances of several bacterial groups on the skin and in the soil increase.

Sample Answer 2 (Refute): The graphs show that the soil and belly bacteria do not increase for many hours after rupture. Some of the abdominal cavity bacteria were also decreasing long before rupture occurred. Since the timing of these changes do not match the timing of rupture, these changes could be associated with environmental factors or other decomposition events going on in the body.

Potential Experiment: You could monitor changes in the bacterial groups in and on decomposing bodies in a sterile vs. outdoor environment. This would allow you to see whether the changes are still associated with rupture under

different environmental conditions. Students may also propose ways to stop rupture from happening altogether, to see if the changes in bacterial abundance still occur or not.

Claim B: The changes in bacteria in the abdominal cavity caused the observed changes in bacterial abundance on the head.

Sample Answer 1 (Support): The graphs show that changes in the abundance of certain bacteria in the abdominal cavity precede changes in bacterial abundance on the head. This is consistent with what might happen if the claim were true. (Since the bacteria in the abdominal cavity changed earlier, they could have caused the changes in the bacteria on the head, once given enough time to replicate and spread.)

Sample Answer 2 (Refute): Although the changes in bacterial populations in the two locations are correlated, we cannot say if the changes in the abdominal cavity directly caused the changes on the head. Both of the changes may have been caused by rupture, or there may be other causes that are not measured here.

Potential Experiment: You could remove the head from the body of a decomposing corpse, then measure changes in bacterial abundance on the head when it is separate from the abdominal rupture.

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