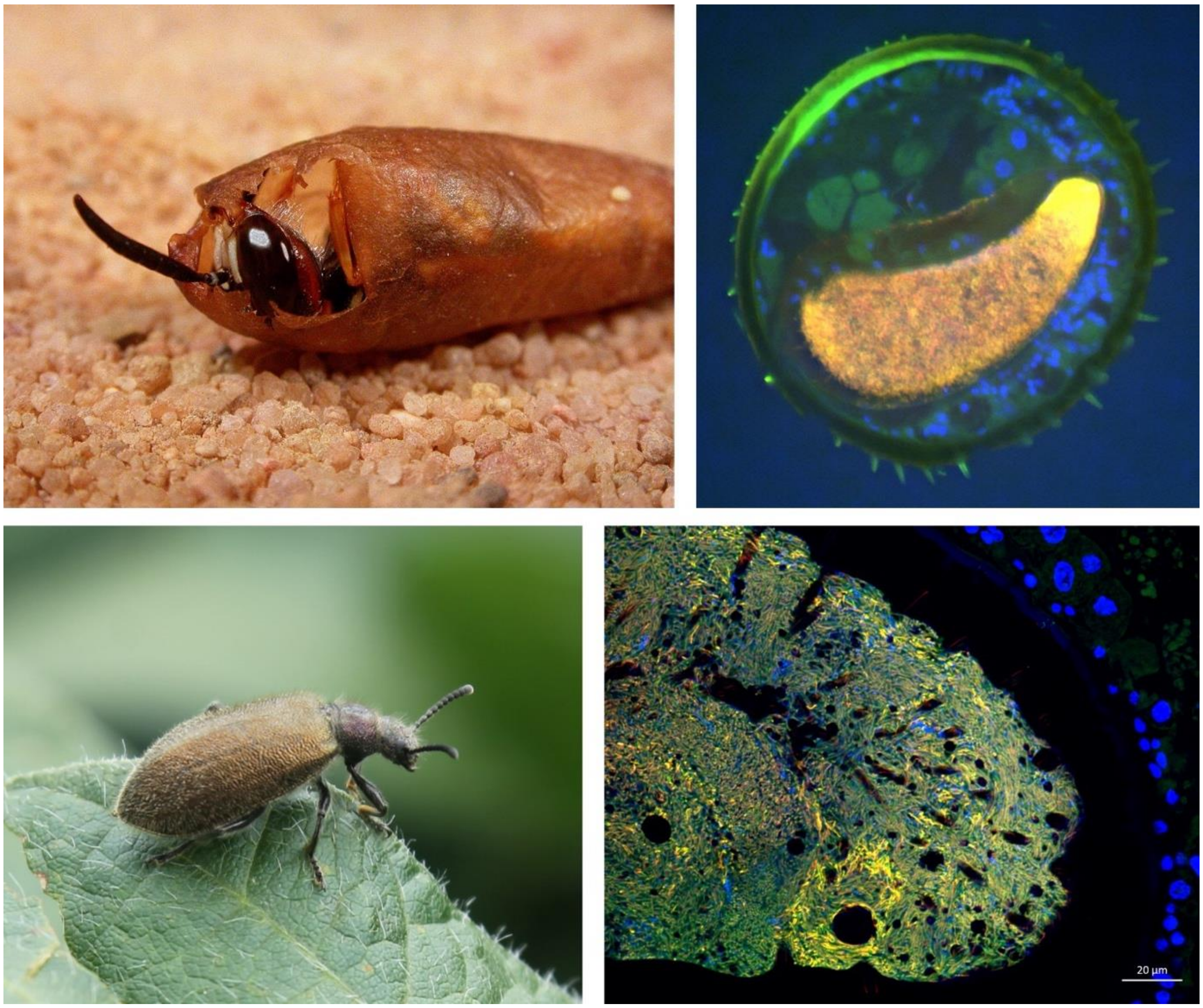
Insects with Microbial Helpers for Defense

Mom: When we are sick, we get medicine from the pharmacy. Where do animals get their medicines?



Beewolf wasps (upper left) carry bacterial helpers in their antennae (upper right, bacteria in yellow/orange) and transfer them to the cocoon, where the bacteria protect the developing wasp from harmful microbes ©Martin Kaltenpoth. Similarly, some beetles (lower left) smear bacteria (lower right) onto the surface of their egg to protect them from overgrowth mold fungi ©Laura V. Flórez.

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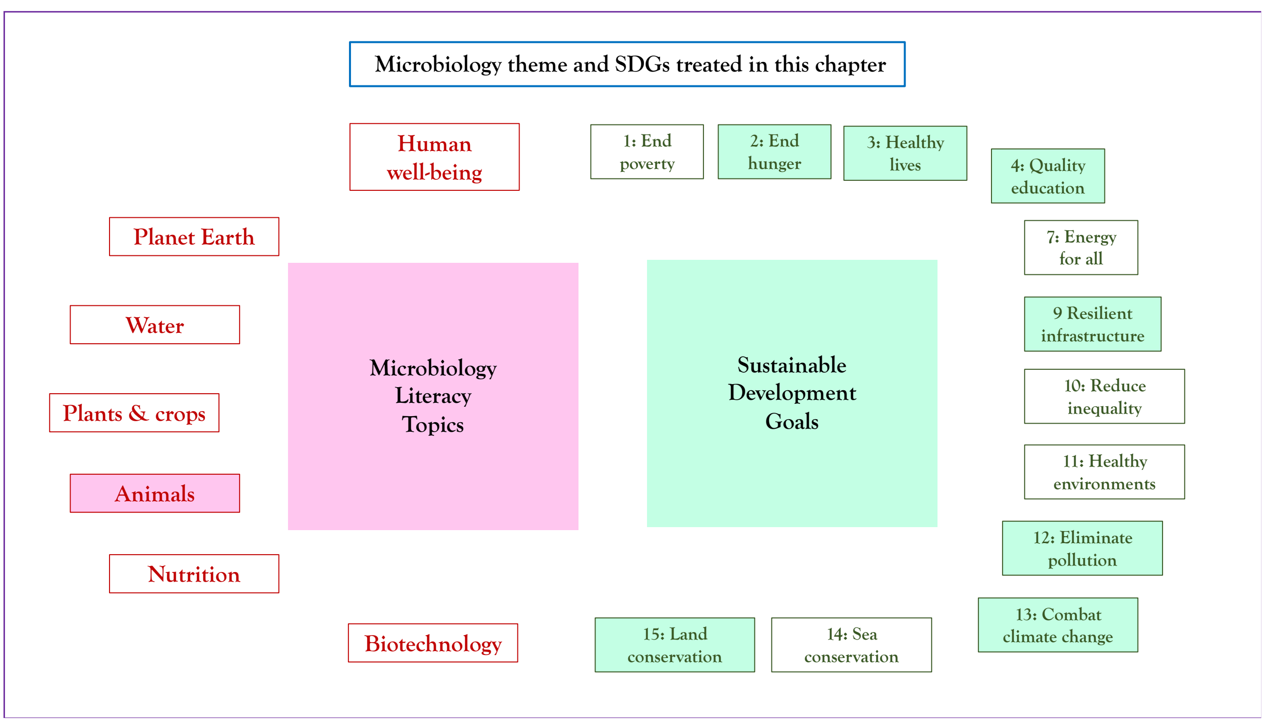
Insects with Microbial Helpers for Defense

Storyline

For millennia, humans have used natural products, like mold and plant extracts, to treat infections. However, it was not until around a century ago that scientists identified specific chemicals produced by microbes that were responsible for this activity, and mass production of so-called antibiotics began. Since then, these drugs revolutionized disease treatment and have saved countless lives. However, humans are not the only animals that use microbial natural products as a treatment against infections. In fact, some other animals have had a similar strategy in place for much longer. Several different insects, like wasps, beetles and ants, team up with microbes that play the role of bodyguards, providing chemical protection for them, their offspring, or their stored food resources. Such long-lasting relationships, called symbioses, can be key for some insects to thrive in specific environments. The associated microbes, or symbionts, are not only an important component of the natural history of their insect hosts but also a source for us of new antimicrobials that might be useful for human medicine or food production. Insect symbionts are additionally a valuable reference to study how antibiotics can remain effective against infections in the long-term. Also, as microbes can be important for insect survival, it is relevant to be aware of and understand these relationships for conservation purposes, particularly in light of the recent declines in insect populations due to anthropogenic disturbances. This may contribute to minimizing the impact of human activity and properly manage conservation measures that sustain the fascinating diversity of insects.

The Microbiology and Societal Context



A child-centric microbiology education framework

The microbiology: Microbial production of secondary metabolites for signaling and defense; antimicrobial compounds as cures of infectious diseases; symbioses of insects with microorganisms; protection of developing life stages against pathogens in beewolf wasps and darkling beetles; insect symbionts as potential sources of new antimicrobial compounds. *Sustainability issues*: role of antibiotics in food security and health improvement; foster innovation in antibiotic research; lessons from insects on sustainable production; impact of climate change on insects and their microbiota; protect terrestrial ecosystems and halt biodiversity loss.

Insects with Microbial Helpers for Defense: The Microbiology

1. Bacteria and fungi produce antibiotics to communicate or compete with each other. Like all other living organisms, microbes must keep functions running that enable them to survive, grow, and reproduce. However, most can also produce non-essential substances that help them to interact with other living beings. In some cases, these substances are used to send signals, or communicate, while in others they act as weapons that stall the growth of microbial competitors or even kill them. These are therefore collectively termed antimicrobial compounds (including antibiotics and antifungals). In many cases, a single microorganism can produce multiple antimicrobials that act against the same or multiple different antagonists. Because there has been a long natural process of trial and error over evolutionary time in different environments, microbes can produce an astounding diversity of antimicrobials that allow them to interact and compete with other organisms.

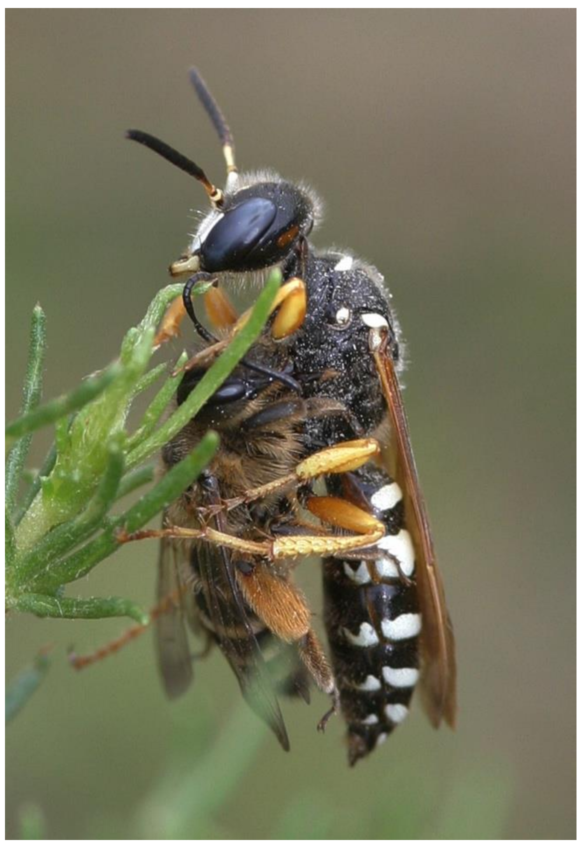
2. Humans have learned how to produce and use some of these antimicrobial substances to fight harmful infections. Since the discovery of penicillin by Sir Alexander Fleming in 1928, humans have discovered, described, and exploited a number of these microbe-produced antimicrobials to develop treatments against many infectious diseases. Around 150 antibiotics have been approved for treating infections in humans, but the discovery of new antimicrobials has slowed down in recent years. In the meantime, the number of infectious bacteria that can cope with the already available antibiotics has increased tremendously; this effect is called antimicrobial resistance (AMR).

But, are we the only animals that make use of the arsenal of bioactive chemicals that are produced by microbes? And if there are others, can we learn from their experience?

3. Some insects and other animals house microbes which produce antibiotics that protect them from enemies. Insects are the most species-rich animal class on earth, with an estimate of up to ten million species. Many insects engage in long-term associations with microorganisms, i.e. symbioses. Often, the symbiotic microbes are important for their insect hosts by providing limiting nutrients, digestive enzymes, or detoxifying capabilities. However, several insect groups have also evolved symbiotic associations with antibiotic-producing microorganisms that protect them against pathogens.

4. A type of solitary wasp carries bacteria in their antennae that protect their young from fungi. One of these protective symbioses occurs in the so-called beewolves, which are solitary digger wasps that hunt bees or wasps as prey and provide them as food to their offspring. Female beewolves carry bacteria of the genus *Streptomyces* in specialized pockets in their antennae and transmit them to underground chambers in which the larvae develop. When the larvae have eaten the food provided by the mother, they take up the bacterial helpers and incorporate them into their cocoon during the spinning process. On the cocoon surface, the bacteria produce multiple antimicrobials that protect the larva in the cocoon from invasion and attack by mold fungi during the long time of diapause during the winter and spring. The protection provided by the bacterial symbionts strongly increases the probability of the beewolf offspring surviving until adulthood and successfully emerging from the cocoon.

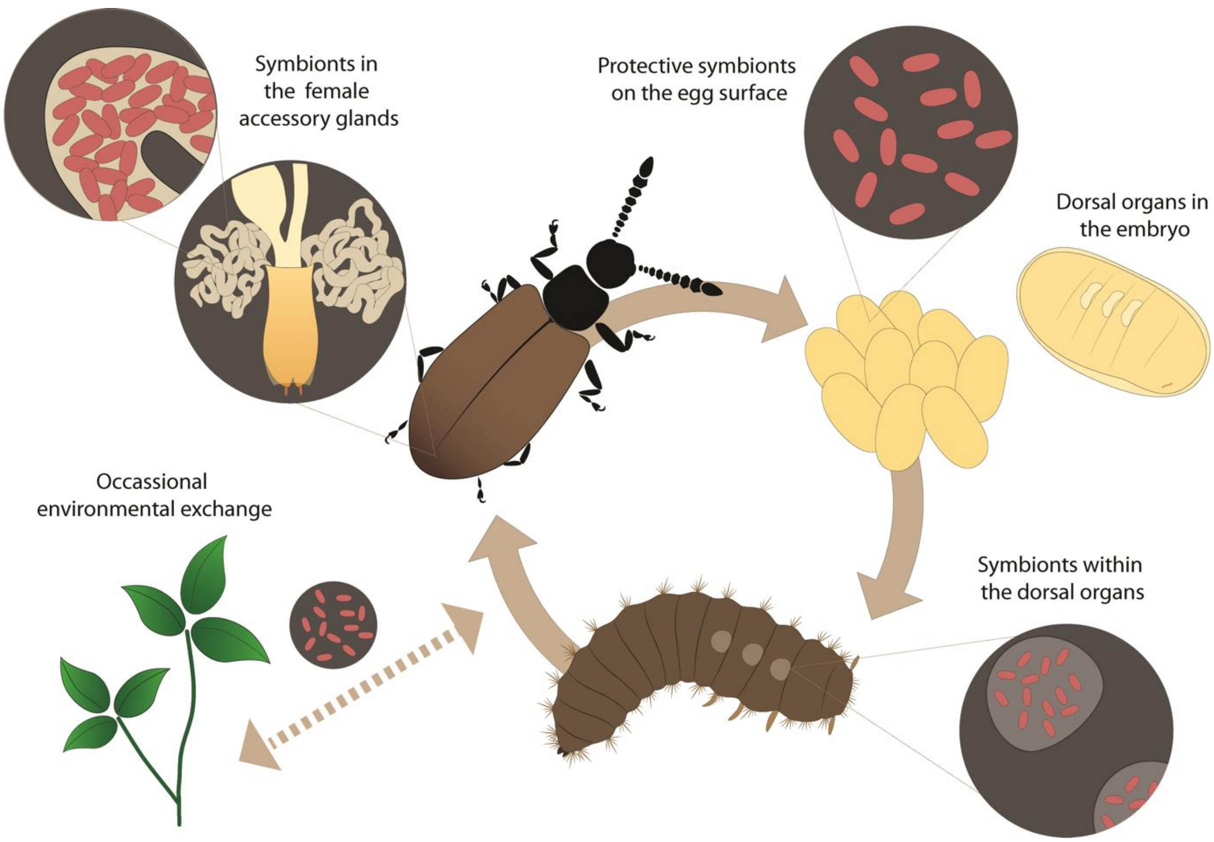
In a sense, the beewolves carry their pharmacies around with them to provide medicines that prevent childhood diseases. Interestingly, the bacterial genus *Streptomyces* that the beewolves team up with also contains the most prolific species of antibiotic-producing bacteria that we humans exploit for medical purposes, being responsible for the production of roughly half of the antibiotics we use to fight diseases. However, while we humans have been using microbe-produced antibiotics systematically for not even a hundred years, the protective beewolf symbiosis evolved about 68 million years ago, around the time of the dinosaurs' mass extinction.



A female beewolf (*Philanthus coronatus*) with a halictid bee as prey. © Martin Kaltenpoth.

5. A group of beetles cover their eggs with a fluid containing antibiotic-producing bacteria. In a symbiosis similar to that of the beewolves, darkling beetles of the genus *Lagria* are protected by bacteria against mold fungi during their most vulnerable life stage. Female beetles carry the bacterial symbionts in pouches that are connected to the reproductive organs and – when laying eggs – release them onto the egg surface. There, symbiont-produced antimicrobials stop mold fungi from growing, thereby increasing the chances of the offspring to survive and successfully hatch from the eggs as larvae.

Interestingly, even though the bacterial symbionts are transmitted from mother to offspring, new symbionts can occasionally be taken up from the environment. These additional bacteria can produce different antimicrobials, which may help the beetles to cope with changes in the community of mold fungi that surrounds them.



The defensive symbiosis of *Lagria* beetles with bacteria. Reproduced from Kaltenpoth & Flórez 2020, with permission from the Annual Review of Entomology, Volume 65 © 2020 by Annual Reviews, http://www.annualreviews.org.

6. Protective microbial partners have been found in many different types of insects. Beewolves and *Lagria* beetles are not the only insects that team up with microbial partners for defense. Many others, including aphids, psyllids, fruit flies, mosquitoes, ants, bees, locusts, bugs, weevils, bark beetles, short-winged beetles, and burying beetles also cultivate microbial symbionts that protect them from enemies, including predators, pathogens, parasites, and parasitoids. As such, protective symbioses between insects and microorganisms are not only interesting for scientists studying their biology, but they may also represent promising and as yet underexplored sources of novel bioactive chemicals with potential relevance for human medicine. Importantly, antimicrobial compounds that have been used for millions of years in a symbiosis with insects are less likely to have harmful effects on animal cells (including human cells) than compounds from bacteria living freely in the environment.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 2: End hunger, achieve food security and improved nutrition and promote sustainable agriculture.** While many may think of antibiotics exclusively as medicines to control infections in our body, these substances also play an important role in the food industry. Due to our growing population and economy, global food production has strongly increased in the past decades and, along with it, also the use of antibiotics in agriculture. Antimicrobials are used to ward-off or treat diseases in livestock, farmed fish and crops, and their use is expected to continue expanding. Importantly, increased use in food production contributes to the rise of antimicrobial resistance (AMR). While managing AMR relies on a combination of strategies, searching for new antibiotics produced in nature is one useful approach to tackle the problem. Therefore, research that explores novel antimicrobials in natural systems —like defensive symbionts of insects— and explains how this is a long-term sustainable strategy for an insect or other animals, can contribute to improve food security.
- **Goal 3: Ensure healthy lives and promote well-being for all at all ages.** Since the discovery of penicillin in the early twentieth century, antibiotics have revolutionized the treatment of infectious diseases: antimicrobials are very important for ensuring health. However, every year drug-resistant (AMR) infections are responsible for around 700,000 deaths worldwide. As is the case for goal 2, investigating how and when antibiotics are produced and act in nature, as well as screening sources of potentially novel antimicrobials, is pivotal to health program development and counteracting the effects of AMR. Symbioses are a largely untapped resource for antibiotic discovery. As study systems for prospective drugs, defensive symbioses have the advantage that many of the antimicrobials that play a role in protecting an animal have already been selected by nature to not harm the animal host. Thus, they are less likely to have harmful side effects on animals and humans than antimicrobials derived from free-living microbes in the environment. Also, the antimicrobial properties can be studied in the context of the animal where it has been discovered and its diseases, to later translate this knowledge to medical research for humans.
- **Goal 4: Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all.** High quality education must involve the promotion and accessibility to fundamental knowledge, where curiosity is a key driver. Therefore, it is crucial to provide students and individuals in general with the tools to inquire and learn about biological systems and set the stage for becoming fascinated by them. Insects are astonishingly varied in form, color and size, and their apparently "invisible" microbial partners have participated in shaping this wonderful diversity. This is one of countless examples of the intrinsic value in addressing our curiosity for nature and understanding it as part of lifelong learning.
- **Goal 9: Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation.** Innovation relies on exploration. Although the resources directed to science and development are growing, achieving Goal 9 requires more attention and efforts towards both fundamental and applied research. As mentioned for goals 2 and 3, insect-associated microbes are promising as a source of antibiotics as well as other naturally

produced substances valuable for industry. For many developing countries that harbor high biodiversity — including that of insects and their microbiota, exploring this treasure trove of potential natural products in symbiotic microorganisms can be useful to enhance discovery and foster innovation.

- **Goal 12: Ensure sustainable consumption and production patterns.** Insects like bees, wasps, ants, and beetles are successful in preserving their food reserves under risky conditions in which pathogen attack is likely. We know that in many cases, they achieve this by applying microbes or antimicrobials, which can go along with specific behaviors or storage strategies. For example, honeybees are associated with lactic acid bacteria that produce antimicrobials important for preserving an essential food source, the beebread. The bees also line their hive with propolis containing the lactic acid bacteria, thereby protecting their brood against pathogen intruders. Not only learning about which microbes and substances are used by insects, but also the conditions in which they are effective can be valuable and might be adapted for designing sustainable strategies in product storage and processing.

Microbial symbionts of insects can also be relevant for sustainable agricultural practices. Many insect pests that affect crops worldwide rely on bacterial symbionts for their nutrition or protection. When designing environmentally friendly strategies to control these pests, it is crucial to be aware of how the microbial symbionts might interfere with or enhance these measures. Defensive symbionts could, for example, counteract application of fungi that are used in biological control. There are also reported cases in which bacterial symbionts of bugs can degrade a commonly applied insecticide, making the insect resistant to this control measure. On the other hand, microbial symbionts can provide novel targets for insecticides, because eliminating the symbionts may result in death or reduced population growth of the host insect. In conclusion, we can gain useful information from insect-microbe associations to improve and apply sustainable production and consumption strategies.

- **Goal 13: Take urgent action to combat climate change and its impacts.** Global warming and habitat disturbance can have severe effects on insect populations. We already know of strong declines in diversity of certain groups of insects, and across different regions around the globe, due to human impact on climate and environment. Given the essential roles of microbes for insects, it is important to know whether and how climate change affects specific symbiotic interactions of insects with microorganisms. Also, climate change might affect the presence of natural enemies of insects in specific habitats, altering the stability of defensive symbiosis with microbes. In other cases, associations with microorganisms might help certain insects to better cope with climate change and its effects. Understanding these challenges and their consequences is not only important for conserving insect biodiversity, but also indirectly for human communities, as insects provide key ecosystem services like pollination.

- **Goal 15: Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt biodiversity loss.** To conserve biodiversity, it is very important to have good knowledge on the natural history of the organisms we want to protect. Learning about the impact that microbes can have on animal and insect well-being is relevant to understand and shape a positive interaction with the animals or the ecosystems in which they are embedded. Insects – supported by their microbiota – are important members of food webs in virtually all terrestrial ecosystems and, as mentioned in Goal 14, play an essential role in natural and agricultural environments, due to the pollination services they provide. In addition, they are important for controlling specific pests, aid in waste decomposition, and can be a protein-rich addition to livestock feed and human nutrition.

Pupil participation

1. ***Points for class discussion and key aspects to guide the dialogue***
   a. Why don't all insects (or animals) have defensive symbionts?
      i. The immune system of animals is useful for combating and controlling disease, in some cases this can be enough.
      ii. There are other alternative or complementary strategies for defense like mechanical protection (spines, hard shells, strong mandibles), toxins produced by the animal itself or behaviors (grooming, evading enemies).
      iii. Animals pay a price for carrying symbionts in their body. They must feed their microbial "bodyguards" and sometimes house them in special locations in their bodies.
   b. What do you think can happen if a new enemy arrives in the habitat of beewolves (or any insect hosting defensive symbionts)?
      i. The insect might switch its symbionts.
      ii. The symbionts might improve the production of different antibiotics, or new ones could be produced. However, this process requires time and sometimes many generations.
      iii. The beewolves might be decimated or die out in this habitat if they or their microbial partners do not develop a way to fight or evade the new enemy.
   c. Some darkling beetle species now occur in very high abundance in human controlled crops. How do you think this could affect the symbiosis?
      i. Microbial communities are different, usually less diverse, in agricultural fields. The microbes that the beetles could pick up might change.
      ii. Microbes causing diseases or other enemies (like predators or other insects) can be different from those encountered in a natural forest.
   d. Do you know if humans carry microbes that can protect us? Where would you expect to find them?
      i. Humans have a complex and diverse microbial community
      ii. Microbes in our gut and skin can compete with harmful microbes
      iii. Our microbiota is important in preventing diseases, and a disruption of the microbiota can result in serious health issues.

2. ***Exercises***
   a. Many people think about microbes as a threat for humans and other animals. From what you have learned in this lesson, list at least three facts that would give them a better overview on the role of microbes.
   b. Think about how bacteria can be acquired and maintained in insects, and brainstorm on body parts where you expect to find them. Then, research the

internet to find examples (hints for searching: bacteriome, insect gut microbiota, insect midgut crypts, cuticular crypts, mycangia).

The Evidence Base, Further Reading and Teaching Aids

Antibiotic Resistance. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance

Antimicrobial resistance and our food systems: Challenges and Solutions. Food and Agriculture Organization of the United Nations. http://www.fao.org/3/a-i6106e.pdf

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Glossary

Antennae: Organs on the heads of insects and other arthropods that are mainly used for smelling.
Antibiotic: Chemical substance that prevents the growth of bacteria or kills them.
Antifungal: Chemical substance that prevents the growth or germination of fungi or kills them.
Antimicrobial compounds / antimicrobials: Chemical substances that prevent the growth of microorganisms or kills them.
Antimicrobial Resistance (AMR): Ability of microbes to withstand the harmful effects of antimicrobials. Often, this ability evolves after long-term exposure of microbes to low concentrations of the antimicrobials.
Beebread: Stored provisions of honeybees in their comb that mainly consists of pollen.

Bio-control / biological control: Control of populations of pest species through the use of antagonists (pathogens, predators, parasites, parsitoids), rather than by chemical means.
Diapause: Period of delayed development during unfavorable environmental conditions, e.g. during winter in temperate climates.
Infectious disease: Disease that is caused by a pathogenic microorganism (bacterium, virus, fungus, eukaryotic parasite).
Insecticide: Chemical substance that prevents growth or reproduction of insects or kills them.
Lactic acid bacteria: Group of bacteria that are economically important due to their application in the fermentation of milk and plant products. Lactic acid bacteria also occur in the guts of several insect species.
Microbiota: Community of microorganisms belonging to different groups (e.g. bacteria, fungi, etc.) that inhabit a particular environment (e.g. an insect host).
Microorganism: Microscopically small organism that is not visible to the unaided human eye.
Parasite: Organism that lives inside or on another organism (the host) and causes it harm.
Parasitoid: Parasite that kills its host at some point during its development.
Pathogen: An organism that can cause a disease.
Propolis: A mixture of saliva and wax produced by honeybees to seal cracks in their hive.
Solitary: Living alone (instead of in groups).
***Streptomyces*:** Genus of bacteria that grow in filaments (looking more like fungi) and famous for producing many different antimicrobials. About half of the antibiotics used in human medicine are produced by different *Streptomyces* species.
Symbiosis / Symbionts: A symbiosis is the close living-together of individuals of different species over most or all of their lifetime. The smaller partner is called the symbiont, the larger is termed the host.