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Symbiotic Organs: Extreme Intimacy with the Microbial World

All multicellular creatures interact with bacteria, but some have taken the relationship to another level with highly specialized structures that house, feed, and exploit the tiny organisms.

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The journey into a squid isn't an easy one. But the bioluminescent marine microbe *Vibrio fischeri* is up for the challenge. Usually a free-living bacterium, *V. fischeri* has evolved a part-time symbiotic relationship with the Hawaiian bobtail squid (*Euprymna scolopes*). The latter stands to gain from the microbe's bioluminescence to disguise its silhouette against a moonlit backdrop from predators lurking below. *V. fischeri*, meanwhile, can benefit from a safe place to feed, grow, and divide—something the squid offers in the tiny nutrient-filled crypts of a specialized structure called the light organ.

For the bacteria, getting to these crypts is a multistep affair, and fraught with peril, explains Spencer Nyholm, a biologist at the University of Connecticut and an expert on symbioses. To find its future host, *V. fischeri* has to swim up a trail of "mucus goo" secreted by baby squid upon leaving their eggs, while avoiding being killed by the goo's abundant antimicrobial compounds. If it reaches the animal's surface, the microbe next faces what's known as the gauntlet. "There's this little ciliated pore that's like the door to the light organ, and there's six of these doors on each squid—three on each side," Nyholm explains. Each bacterium must navigate through one of the pores, dodging the beating cilia, and then swim along a duct pumped full of toxic compounds known as reactive oxygen species. Survivors pass through an antechamber and then have to squeeze through a microscopic bottleneck guarding the crypts themselves, Nyholm says. Only a handful of bacterial cells ever make it.

The complexity of this journey and of the light organ itself reflects the extraordinary intimacy of the relationship between these two organisms, which have been evolving together for millions of years. The obstacle course makes sure it's only the specialized, flagellated, stress-resistant *V. fischeri*—and not any of the other billions of marine bacteria floating around the squid—that make it to the food-filled crypts. Once the hardiest *V. fischeri* individuals arrive and start forming a colony, the light organ becomes a communication center between them and their host, producing and receiving vast numbers of signaling molecules and metabolites—the functions of which researchers are still uncovering.

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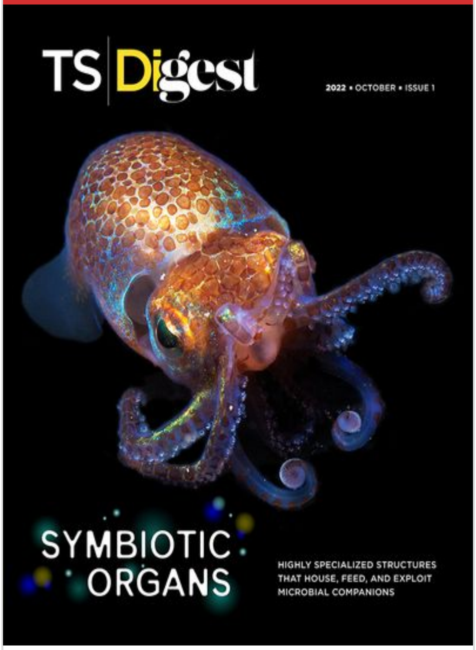

Nyholm and other biologists refer to the light organ as a "symbiotic organ" for its specialized role in housing and talking with the squid's luminescent guests. And squid aren't the only animals to have such structures. Nyholm also studies deep-sea anglerfish, which use bacteria-powered light organs dangling over their heads to attract food and mates in the sunlight-deprived depths. Various other animal and plant species have also evolved their own specialized structures to take advantage of completely different microbial functions: the production of particular antimicrobial compounds, say, or the ability to metabolize hard-to-digest food.

While many of these symbiotic organs have traditionally been studied as peculiarities of particular species, some researchers are now pushing to consider

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>
> **—Joel Sachs, University of California, Riverside**

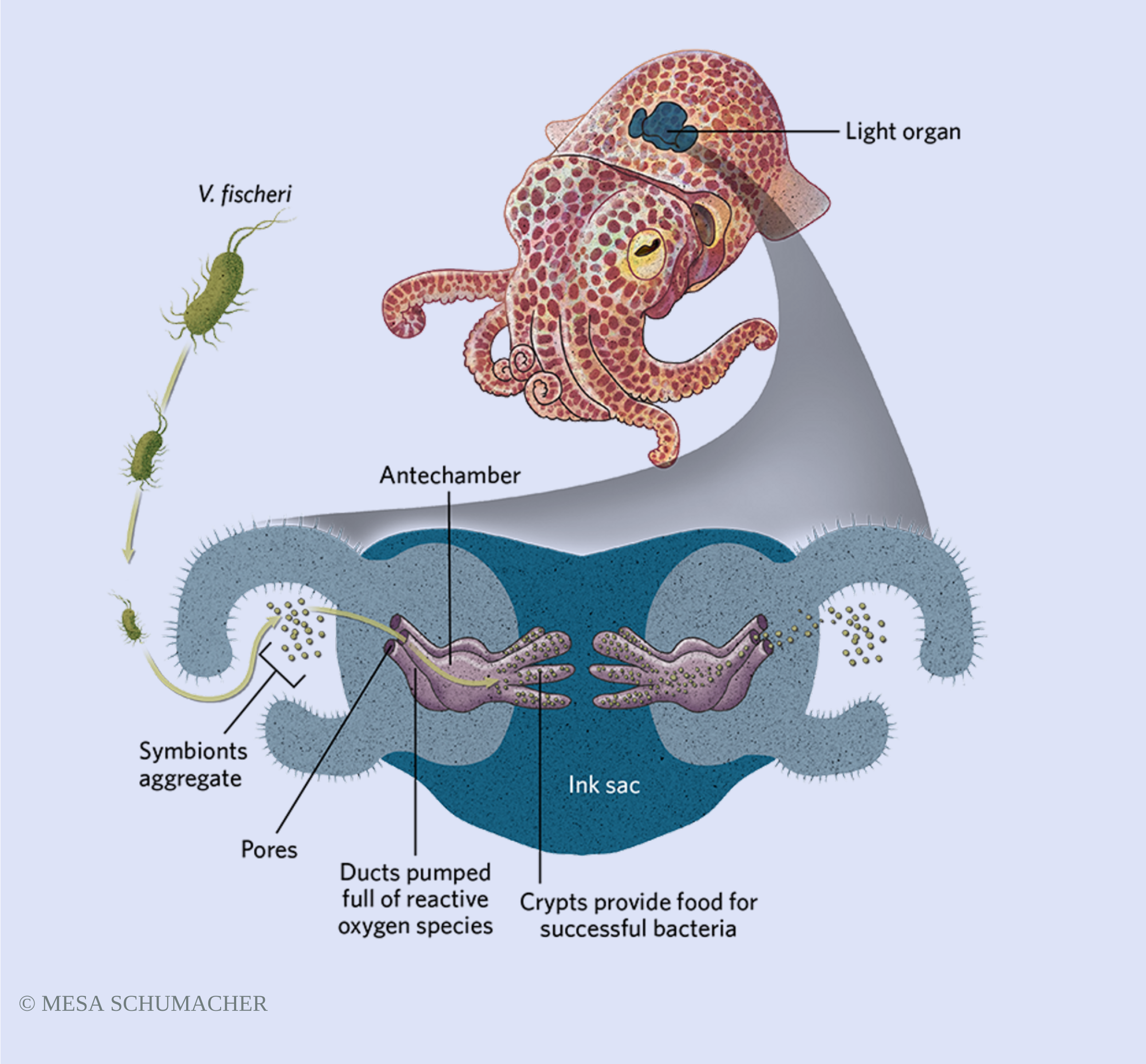
them collectively, as extreme examples of what happens when multicellular organisms develop intricate relationships with the microbes around them. In all of these cases, “you create this emergent organ that would only exist in the context of the interaction,” says Joel Sachs, an evolutionary biologist at the University of California, Riverside (UCR) who studies bacteria-housing root nodules that endow many plant species with the ability to fix nitrogen. “Once that occurs, it reshapes the evolution of both the host and the symbiont. And that’s the commonality where I think it makes sense to join these crazy, diverse systems and start to compare them side by side to see these similar dynamics.”

Let There Be Light

Host: Hawaiian bobtail squid (*Euprymna scolopes*)

Symbiont: *Vibrio fischeri*

The Hawaiian bobtail squid gets *Vibrio fischeri* into its light organ by means of chemical signals and a complicated obstacle course that blocks out other bacteria. Once established in the squid’s organ, bacterial symbionts are fed by their cephalopod host, while the microbes luminesce—a trait the squid uses to disguise its silhouette from predators beneath it in the water.



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Symbiotic organs share rules of engagement with bacteria

Symbiotic organs are remarkably diverse both in terms of function and in terms of the species that possess them. One of the best-known examples comes from a marine organism that lives at deep-sea hydrothermal vents: the giant tubeworm (*Riftia pachyptila*). At some point in its evolution, the tubeworm ditched its own gut and now depends entirely on an internal symbiotic organ called the trophosome—home to intracellular, sulfur-oxidizing bacteria that the worm acquires from its surroundings in the first few days after settling at a vent.

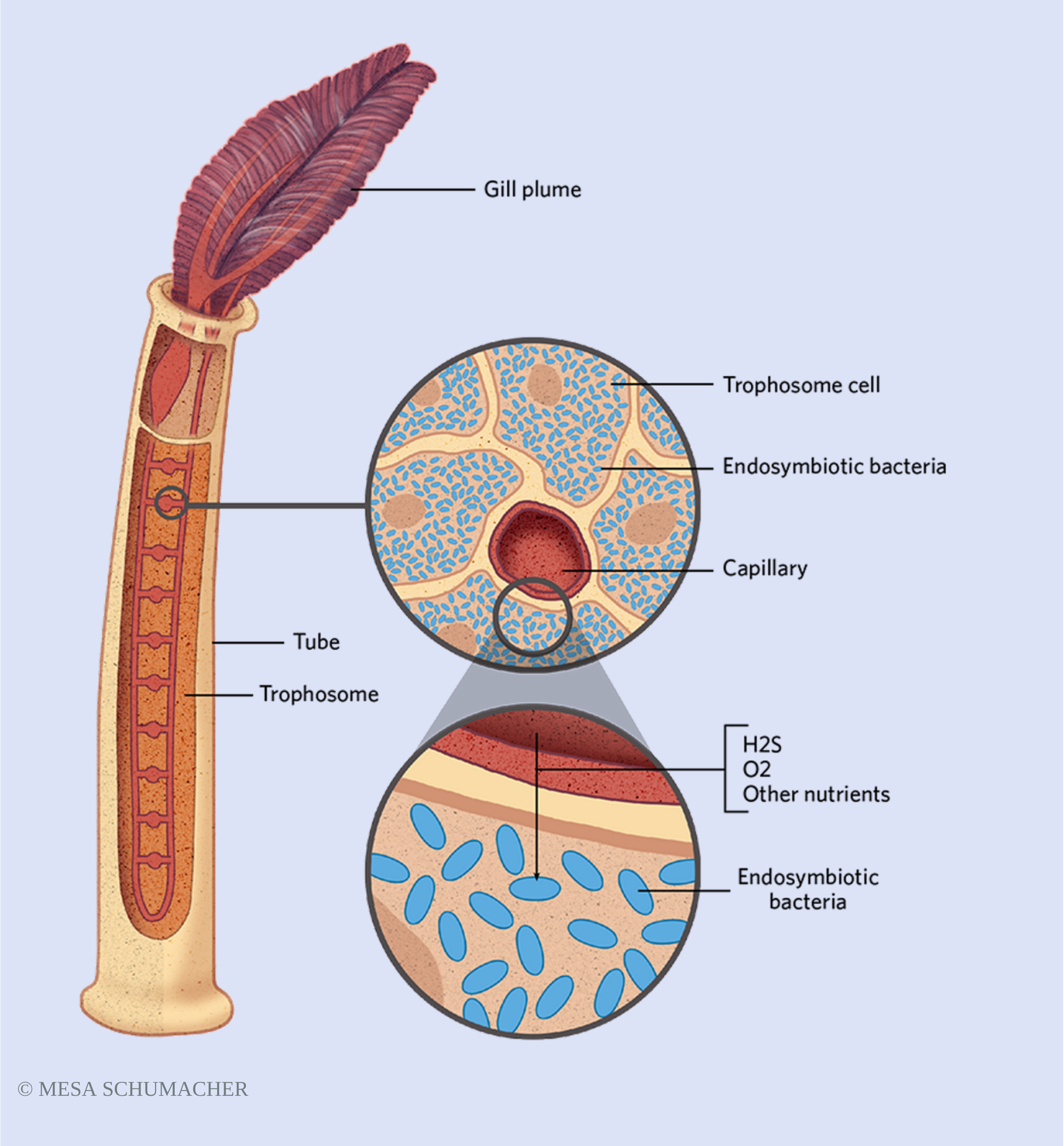
These 1- to 1.5-meter-long worms "have no mouth and no gut," explains Colleen Cavanaugh, a microbial ecologist at Harvard University who first described the symbiosis more than 40 years ago. Instead, they funnel sulfur compounds from the mineral-rich seawater to their hungry resident bacteria, while digesting a portion of those bacteria and their metabolites as a source of organic carbon. This symbiosis is still a focus of intensive study. Last year, researchers published a highquality draft genome and full mitochondrial sequence for the tubeworm, along with new data revealing how tubeworm hemoglobin binds sulfide as well as oxygen—all the better to deliver sulfur to the symbionts.

Deep-Sea Symbiosis

Host: Giant tubeworm (*Riftia pachyptila*)

Symbiont: *Candidatus Endoriftia persephone*

Giant tubeworms house sulfur-oxidizing bacteria in a specialized organ called the trophosome. The worms funnel sulfides from the mineral-rich water at deep-sea hydrothermal vents to their symbionts, which metabolize those compounds. In exchange, the worms feed on a portion of the bacteria.



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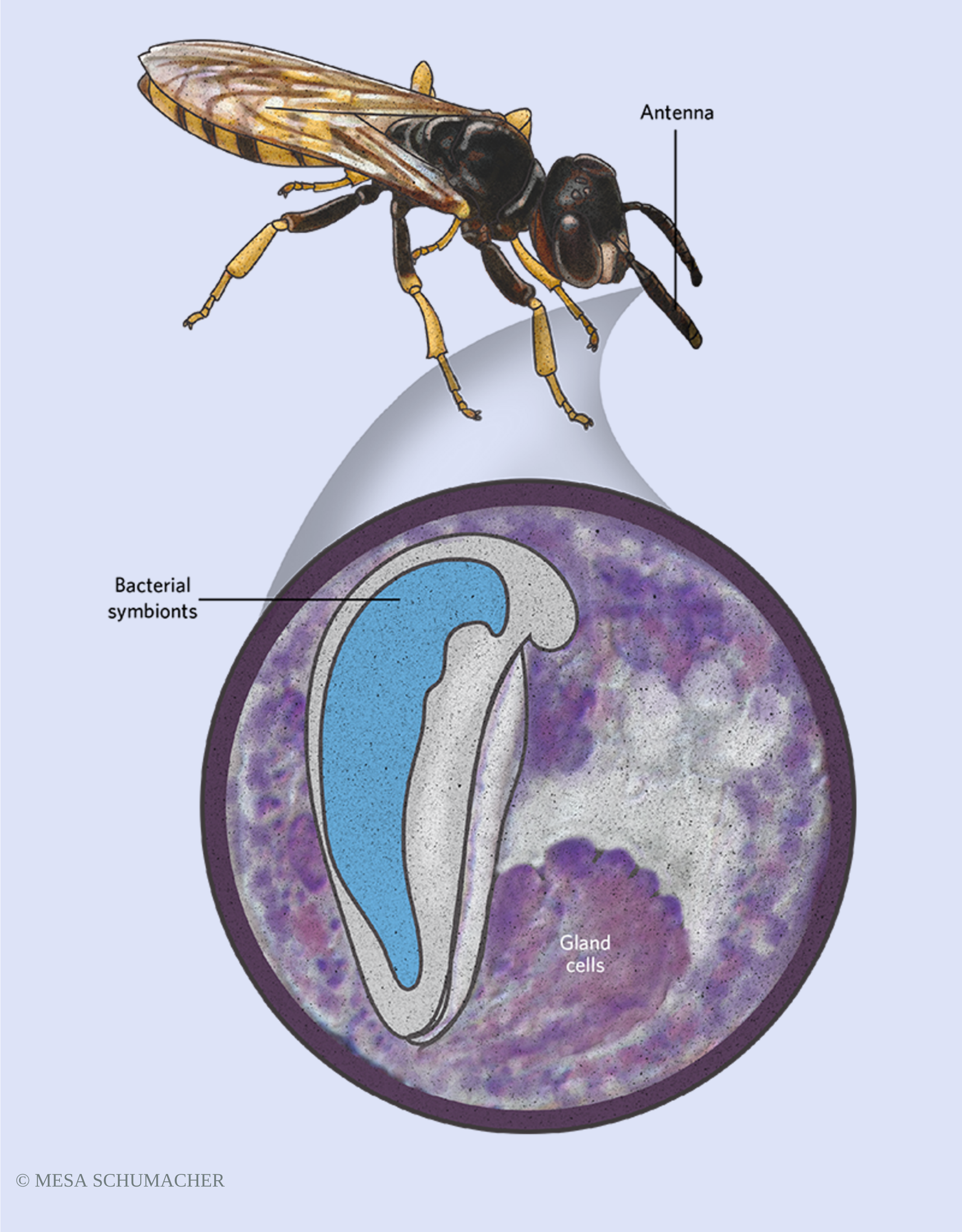
Up on land, and at a tinier scale, are insects that rely on microbes to provide protection from environmental pathogens. The beewolf, a predatory wasp that kidnaps bees to feed to its own offspring, has specialized antennae that house *Streptomyces* bacteria. After digging her underground nest, a mother beewolf paints the ceiling of each brood cell with *Streptomyces*-filled goo, then provisions the cell with one or more unfortunate, paralyzed bees, onto which she lays an egg, explains Martin Kaltenpoth, an evolutionary ecologist at the Max Planck Institute for Chemical Ecology in Jena, Germany, who first described this symbiosis in 2005.

His team has shown that wasp larvae later transfer the bacteria to their cocoons. There, the microbes produce a cocktail of antibiotics that protects the developing insects from pathogens until they emerge months later as adults. Females also acquire the bacteria to use in their own future reproduction. The precise composition of the bacterial antibiotic mixture may even be adapted to the specific pathogens present in a particular beewolf species’ environment. “It seems like it’s been a very successful strategy to have these symbionts,” Kaltenpoth notes, adding that all of the 40 or so beewolf species his lab has studied harbor *Streptomyces* bacteria.

Bacterial Sentinels

Host: Beewolf (genus *Philanthus*)
Symbiont: Genus *Streptomyces*

Beewolves keep their microbial symbionts in specialized antennal gland reservoirs. These multi-segment structures supply food to the bacteria, which in return produce antimicrobial compounds that protect a beewolf’s offspring from environmental pathogens.



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Despite the obvious differences across scales and phyla, there are important similarities in how these organs establish their symbioses, Sachs and UCR postdoc David Fronk argue in a recent paper. For a start, symbiotic organs are well equipped to control where a symbiont can and can't settle. Nutrient-filled crypts, for example, appear in symbiotic organs across the animal kingdom, suggesting that there are benefits to confining bacteria in this way. Restricting interactions to these specific areas stops a symbiont from taking over other host tissues while letting the host focus its energy expenditure on feeding and housing the microbes in that space, Sachs says.

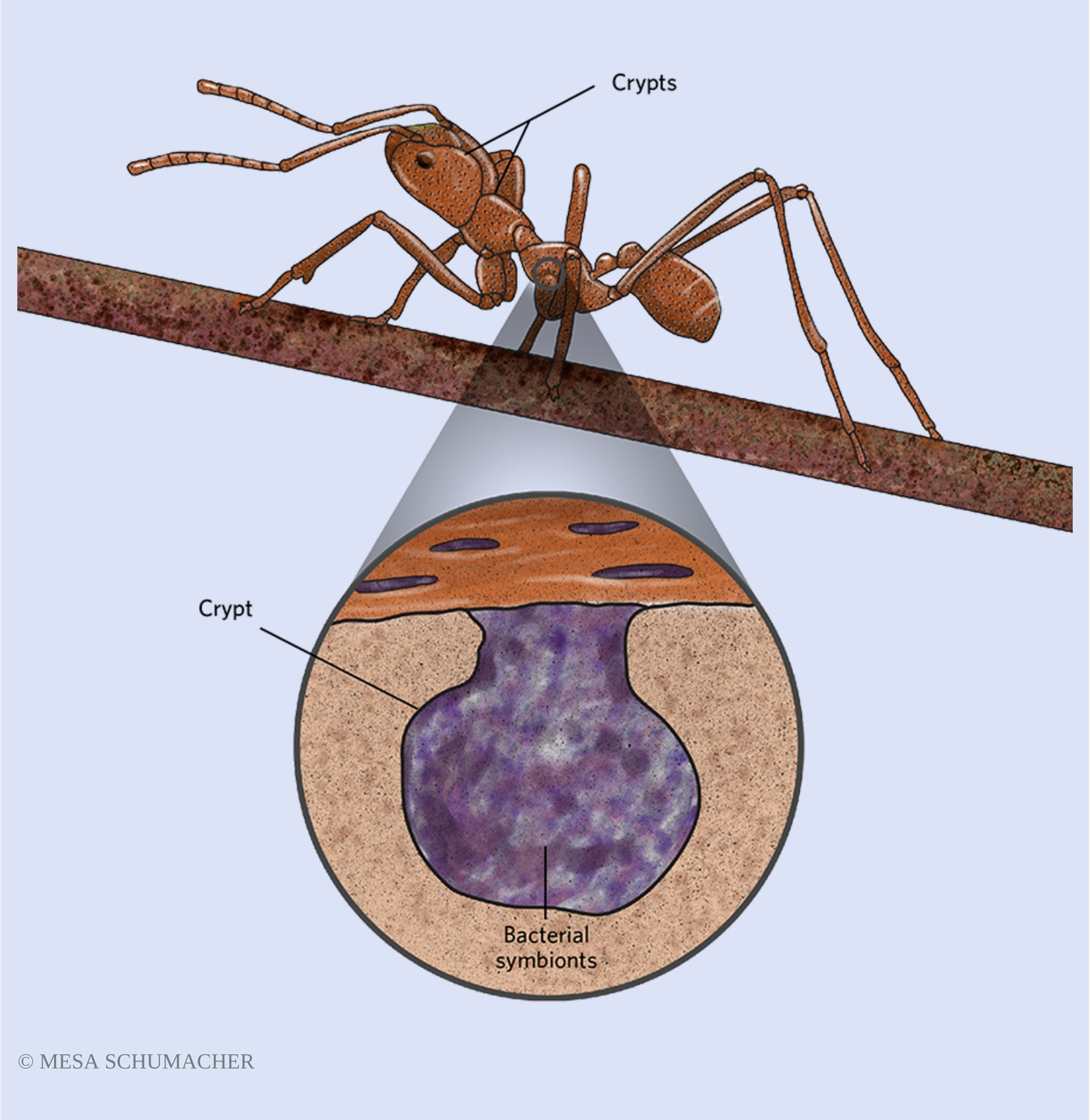
Some of these microbe-housing structures seem to show remarkably consistent features, notes Cameron Currie, a microbiologist and evolutionary biologist at McMaster University. Currie studies symbioses in attine ants, which culture fungus gardens for food, similar to how humans grow crops. These ants depend on antibiotic-producing bacteria to protect their gardens from a fungus-attacking pathogen, and they host these bacteria in highly specialized cuticular structures such as crypts that are scattered across their exoskeletons. Using micro-examinations of nearly 70 attine ant species—both living and extinct—Currie and his colleagues found evidence that almost identical structures have evolved on at least three independent occasions in the last 50 million years or so. “That was a huge surprise for me,” he says. “I was heavily assuming that there was a single origin.”

Symbiotic organs also employ common mechanisms for ensuring that they only welcome desired guests. In beewolves and attine ants, for example, symbionts are transmitted directly among individuals in a population, eliminating some of the risk of environmental contamination. (While the beewolves have their brood cell secretions, the ants propagate microbes largely through physical contact between adult ants.) This sort of inheritance can have important consequences for bacterial evolution, notes Kaltenpoth. His group showed recently that beewolf symbionts are undergoing a reduction in genome size and complexity, consistent with their protected existence and reliance on hosts for transmission.

Farming Aids

Host: Attine ants (genera *Atta* and *Acromyrmex*)
Symbiont: Genus *Pseudonocardia*

Fungus-growing ants use microbial symbionts to produce antibiotics to protect their fungus gardens from environmental pathogens. Microbes are stored and fed in crypts all over the ants’ bodies, and are easily transferred through contact between worker ants.



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Some hosts with symbiotic organs instead filter their prospective partners from the environment using physical barriers, chemical attractants, or selective antibiotics. Plants with root nodules, for example, secrete compounds such as flavonoids when nitrates are scarce in the soil, helping to activate signaling pathways in nitrogen-fixing bacteria that then communicate back to the plant and kickstart a symbiosis. The baby bobtail squid, meanwhile, uses its combo of mucus and an obstacle course to specifically acquire *V. fischeri*.

These indirect modes of acquiring symbionts also affect the ecology and evolution of bacteria, which must be able to handle the journey to, and life in, the host organ, in addition to their regular environment in the ocean or soil. Clotilde Bongrand, a microbiologist at the University of Florida, has studied how different strains of *V. fischeri* compete with one another to access and colonize the squid light organ. Her research with Edward Ruby of the University of Hawai‘i at Mānoa has found that there are “dominant strains” of *V. fischeri* that “have a tendency to reach [the crypt] earlier,” Bongrand tells *The Scientist*. In lab experiments with squid, these strains seem to block out any competitors, she notes. In the field, however, she’s observed squid colonized by multiple strains—something that could happen if nondominant strains have a significant head start on the obstacle course and reach the light organ crypts first.

Getting bacteria into the symbiotic organ is only the initial stage of the partnership, of course. The real relationship begins once the microbes, settled into their new home, start doing the job they were hired for—or not, as the case may be. This much longer part of the symbiosis provides rich possibilities for host-symbiont conflict, Sachs notes, and thus can have major effects on the evolution of both parties.

Hosts and microbial symbionts keep the communication going

However hard a host tries to attract the symbiont it wants, there’s always a risk that the microbes won’t hold up their side of the bargain. Bacteria reproduce much faster than the host they live in, and any strain that manages to hold onto its house without doing the costly work the host wants is likely to gain an advantage over its hardworking peers. Consequently, many multicellular organisms with symbiotic organs have evolved mechanisms to monitor and punish microbial cheaters.

Sachs’s lab has explored this phenomenon as it relates to plant nodules, which typically store their bacterial symbionts in specialized compartments within root cells. He and his colleagues have found that nodules that house cheaters—bacteria that don’t fix nitrogen into ammonia or related compounds for the plant—launch an offensive to kill off these cells. In the process, intracellular structures collapse and bacteria are ejected from the safety of their compartments into the cell cytosol. Importantly, this nodule shutdown happens even when only a fraction of the local symbiont population is cheating, allowing the plant to solve the problem before it gets out of control, Sachs notes.

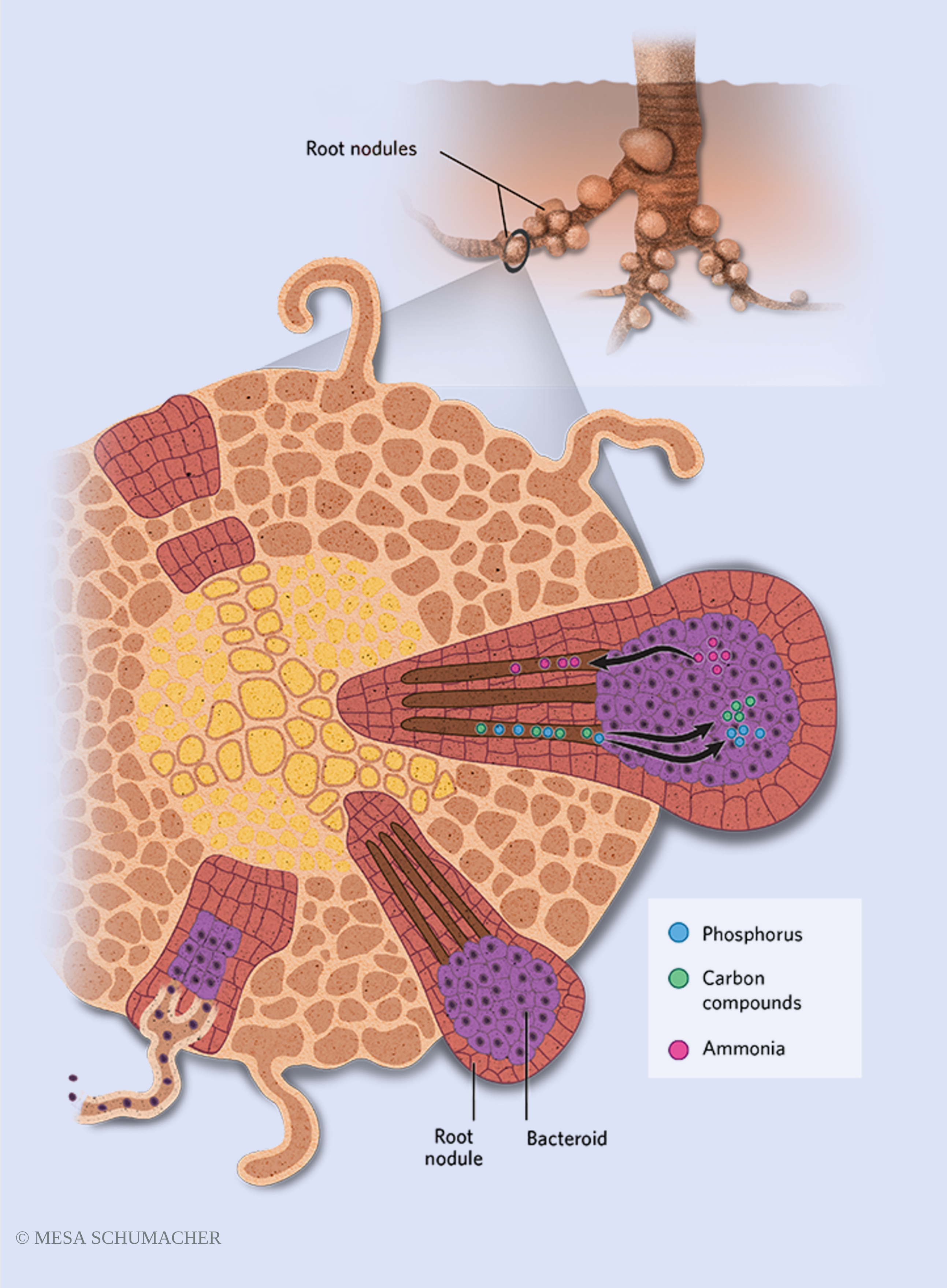
It’s not yet known how plants sense these cheaters. A simple hypothesis is that low nitrate concentrations could signal to the plant that nodule-living microbes aren’t doing enough fixation, Sachs says. But it’s probably more complicated than that. Experiments by his team have shown that even plants in nitrate-rich soil seem to know when their symbionts are shirking their responsibilities. “So [now] we are testing a hypothesis that there’s a private signal,” he says, “a very specific form of nitrogen that the host is getting from the symbiont” and nowhere else.

Symbiosis in the Soil

Host: Root-nodule bearing plants (family Fabaceae, among others)

Symbiont: Multiple genera including *Rhizobium* and *Bradyrhizobium*

In nitrate-poor soils, some plants develop specialized structures to house nitrogen-fixing bacteria. After establishing communication with potential symbionts by broadcasting homegrown signaling molecules, plants and bacteria form nodules in the plant roots; bacteria live in protected compartments within cells, fixing nitrogen for the plant in return for accommodation and access to other nutrients.



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The need to keep out cheaters may also help explain one of the bobtail squid’s weirder behaviors. Like many animals with symbiotic organs, this species picks up its symbionts early in life—and then doesn’t acquire any more. But as an adult, the squid also blasts a large proportion of its symbiont population out through its siphon every dawn, before burying itself in the sand to sleep while the remaining bacterial population grows to full size again. “Ninety-five percent of the contents are expelled” in this mini-explosion each day, says Bongrand, “and then there is this five percent that is regrowing.”

Some researchers are now pushing to consider symbiotic organs collectively, as extreme examples of what happens when multicellular organisms develop intricate relationships with the microbes around them.

The ostensible reason for this “venting” is to refresh the bacterial culture, which can otherwise cause a buildup of metabolic byproducts that harm the squid, Bongrand says. It affects the composition of seawater outside the squid, too, seeding the ocean with *V. fischeri* that may go onto colonize other squid. But some researchers suggest that the behavior might also offer the squid a way of jettisoning so-called dark mutants—bacteria that skimp on producing luminescent proteins. Research has shown, for example, that squid genes expressed in the light organ are regulated in response to the light produced by bacterial symbionts, not just by the presence of the bacteria themselves. This trick could provide the squid with a reliable mechanism to detect when cheaters might be sweeping through the population, Nyholm points out.

Despite such advances in understanding the biology of symbiotic organs, much about the intricacies of host-symbiont communication have yet to be worked out. Some symbionts, such as the bacteria living in tubeworms, are still impossible to culture in the lab, notes Cavanaugh, who also studies symbioses in bivalve mollusks and anemones. Other microbes are being sequenced and scanned for clues as to how they find their hosts, signal to those hosts that they’re performing their work, or interact with the host immune system to maintain their unusual relationship. Such studies could shine a light on microbial interactions across multicellular organisms, not just those that have developed separate organs for the purpose, Nyholm says.

For example, “by understanding how the innate immune system is used to tell the difference between symbiotic and pathogenic or not-symbiotic bacteria, we can really discover some evolutionarily conserved mechanisms by which all animals detect bacteria,” he explains. “This is an open question still in symbiosis, whether you're talking about the human microbiome, or a mouse, or a squid, or a zebrafish, or a plant: How do the partners find each other, and what's the language they use to talk to each other?"

Symbioses work well, until they don't

Even with the best communication in the world, not all relationships work out. There's ample evidence that symbiosis can be lost despite the presence of a symbiotic organ—although it's rare to find examples in the wild. In the last 25 years, Currie says he's only once come across a fungus-growing ant colony that lacked the bacterial symbionts usually associated with the species. That colony wasn't doing so well, he adds. "The garden was dead and all the ants were [motionless] on the side," he says. "It looked like either they'd lost the bacteria and the garden had overgrown, or the garden overgrew and then they stopped supporting the bacteria and died."

Looking at an evolutionary scale, though, researchers have identified various examples of symbioses that have permanently broken down. In ants, this kind of loss has occurred in species "where the ants appear to have evolved other mechanisms for dealing with infections, or the infection pressure is lower," Currie says. In certain scenarios, some creatures may even coopt symbiotic organs for alternative or additional purposes. One bacteria-hosting species that Currie's group studies also grows a biomineral armor on its exoskeleton that protects it in battle against other ant colonies. Preliminary data suggest that the growth of this armor is somehow aided by the ants' crypts, Currie says. "We speculate that the structure might be maintained for the biomineral."

It can be just as useful to study collapse in symbiosis as it is to study how it arises, notes Sachs, adding that while many plant species produce nodules, others seem to have lost the trait. Studying symbiont loss can help researchers understand not only the costs and benefits of symbiotic relationships, but also the long-term effects of the relationship on a species' physiology and genetics. It's a reminder, too, that even when you evolve an entire organ to host your microbes of choice, "symbiosis is this knife-edge," Sachs says. "It's beneficial for the host under a certain set of scenarios. But you alter the ecology, and suddenly it becomes neutral or even harmful."