**Microbes take charge**

Aided by modern sequencing techniques, scientists are discovering that microorganisms can exert a powerful influence over animal behavior.

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Across the African savannah you can find strands of grass coated with a peculiar brown goop. It’s a calling card, left by hyenas that smear it from a special scent gland in a pouch under their tails. Many people don’t like its smell—rather like fermenting mulch—but the hyenas clearly find it fragrant and interesting.

So does ecologist Kevin Theis of Michigan State University in East Lansing. He suspects each scent gland contains a unique population of bacteria that produces a signature odor, which could indicate the animal’s sex and pack affiliation to other hyenas. If Theis is right, hyenas are co-opting their passengers as communications tools. For example, dominant males signify their top status by spreading more of their stinky paste than others do.

The study of how microbes influence animal behavior is a fairly new, small field. “It’s not commonly studied, largely because the techniques weren’t available in the past,” says Theis. Most microorganisms that grow in rumps, guts, or other nooks and crannies are difficult to culture in the laboratory; either scientists do not offer the right combination of nutrients and oxygen, or the bacteria of interest are swamped by others that grow more quickly in Petri dishes. So researchers simply had no way of investigating the tremendous diversity of microbes that could affect an animal’s conduct.

That has changed with the advent of next-generation DNA sequencing, which can quickly identify an organism from just a few cells in a mixed sample. Other techniques that catalog RNAs, proteins, and metabolites also help scientists to quickly profile a population of microbes, giving them another tool to test hypotheses about bacteria and behavior. Growing a pure culture is no longer the only option.

The technology has ushered in a “revolution of sorts in the study of animal behavior,” Theis says (1). Examples of bacterial influence on animals’ lives turn up in a wide range of species, including humans. Many of these interactions can be mutually beneficial, as in the hyenas’ communication system. However, if bacterial communities are altered, it can spell bad news for the host; for example, some researchers suspect that abnormalities in gut microbes might contribute to autism.

Furthermore, elsewhere in the animal kingdom, some behavior-modifying microbes can even drive their carriers to an early death.

**Attack of the Zombie Ants**

David Hughes, a behavioral ecologist at Pennsylvania State University, has crawled through the rainforests of five continents to understand the effects of parasitic fungi on host ants. In a recent study he tracked *Camponotus leonardi* ants infected by *Ophiocordyceps unilateralis* fungi in Thailand (2). As the ant forages for food, the fungal spores land on the insect’s body, then germinate and burrow inside. About nine days later, infected ants suffer convulsions that cause them to drop out of their canopy nests and meander aimlessly around the forest floor. Eventually, the fungus-driven ants grip onto a leaf. Muscle atrophy in the mandible leads to a lockjaw condition, so that the ant holds tight to the leaf even after its death, giving the fungus a solid platform to sprout from. “It’s a fungus in ant’s clothing,” says Hughes. “It’s an organism without a brain controlling an organism with a brain.”

Hughes’ work on what he calls “zombie ants” has led to some unusual gigs: consulting for the filmmakers of the recent zombie movie *World War Z*, and for developers of the video game *Last of Us*, a postapocalyptic tale of a fungal outbreak. In the real world, though, his next challenge is to understand exactly how fungi take control of their unsuspecting hosts. By culturing the fungus on isolated ant brains, he is now examining which genes are activated during the infection, and identifying the metabolites that the fungi produce. The fungus likely uses chemical neuromanipulators to take over the ant’s nervous system, and Hughes expects to soon publish more details on those chemicals and the host systems they target. This would hardly be the first example of a fungal chemical altering an animal’s brain, he adds.

*www.pnas.org/cgi/doi/10.1073/pnas.1400859111*

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[Image: Hyenas get to know each other in Kenya’s Masai Mara National Reserve. Image courtesy of Kay Holekamp (Michigan State University, East Lansing, MI).]
Rye grains infected with the ergot fungus *Claviceps purpurea* historically caused outbreaks of ergotism, in which a range of unpleasant symptoms, including psychosis, affliction, and deadly outbreaks of what's also sometimes called “Baccharis.” While the fungal chemicals responsible for ergotism were subsequently adapted to create the hallucinogenic drug LSD, not all microbe–animal interactions are parasitic: animals typically use the microbes in their guts to aid digestion, for example.

### Each to Their Own

Not all microbe–animal interactions are parasitic: animals typically use the microbes in their guts to aid digestion, for example. Research published in PNAS has revealed that those gut microbes can also influence mating habits, at least in fruit flies on restricted diets (3). The study authors followed up on a decades-old report showing that *Drosophila pseudoobscura* reared on starch preferred to pair up with other starch-eaters, rather than flies that dined on the sugar maltose. The maltose-raised flies also preferred each other (4). When independent nutritionist Ilana Zilber-Rosenberg showed the paper to her husband Eugene Rosenberg, a microbiologist at Tel Aviv University in Israel, “both of us jumped up and down and yelled, ’Bacteria!’” Eugene recalls. They knew altering an animal’s diet could trigger a change in its gut microbiome, and that microbes produce odors, which flies consider when choosing mates. The researchers hypothesized that gut microbes were responsible for the flies’ different mating selections.

To test their idea, the researchers repeated the earlier experiments, rearing some *Drosophila melanogaster* on starch and others on molasses. Flies preferred mates with similar diets after a single generation, and maintained those choices for at least 37 further generations, until the researchers added antibiotics to the flies’ food. Then the flies selected mates of either diet. “It was, in fact, bacteria determining sexual behavior,” Eugene Rosenberg concluded. Clinching the result, the researchers inoculated the antibiotic-treated flies’ food with *Lactobacillus plantarum* cultured from starch-raised flies. Mating preference returned.

This kind of selective mating is an early step toward splitting one species into two, and the data lend support to Eugene Rosenberg’s suggestion that bacterial inhabitants are major contributors to evolution as well as behavior (5).

### Gut Feeling

Insects are not the only animals in which microbes guide behavior. For example, gut microbiota affect the brains of young mice, altering their activity and anxiety levels. In a 2011 PNAS study, neuroscientist Rochellys Diaz Heijtz and colleagues at the Karolinska Institute in Stockholm, Sweden, compared mice raised in sterile environments, whose guts were free of any microbial occupants, with mice that contained normal gut microbiota. The microbe-free mice spent more time exploring well-lit, open environments than the other animals, indicating reduced anxiety (6).

The researchers found that mice without a gut microbiome processed neurotransmitters, such as noradrenaline and dopamine, faster than normal, and produced more of the proteins involved in making synapses. Diaz Heijtz expects to publish follow-up results soon, singling out specific molecules made by gut microbes that are active in the brain.

In people, a few researchers suspect gut microbes might contribute to autism. Children with autism appear to have different gut microbiomes than those who develop normally. The emerging theory about autism and gut flora has generated some excitement, but not been clearly demonstrated or accepted, says Eugene Rosenberg. Although scientists don’t agree on exactly what changes—different cohorts and study methods yield different results—they often see that certain bacterial families are increased or reduced in stools from kids with autism. Furthermore, autistic children often have gastrointestinal problems, such as constipation or diarrhea, because of a syndrome called “leaky gut” that allows substances to escape the intestines into the bloodstream.

In a study published in December, microbiologist Sarkis Mazmanian and neuroscientist Paul Patterson at the California Institute of Technology in Pasadena examined the gut microbes of a mouse model for autism, which exhibits leaky gut as well as core autism-like behaviors, such as repetitive actions and limited communication and socialization (7). When the researchers treated the animals with the probiotic *Bacteroides fragilis*, a beneficial bacterium, it corrected not only the leaky gut but also some of the abnormal behaviors.

Compared with control mice, the autism model mice had 46-times the normal amount of a molecule called 4-ethylphenyl sulfate (4-EPS), a predicted output of gut bacteria. It is also a close chemical relative of *p*-cresol, a metabolite found at relatively high concentrations in the urine of autistic children. Treatment with probiotics reduced the 4-EPS levels of the autism model mice to normal, probably by fixing their leaky guts and keeping the 4-EPS in the intestine.

Moreover, treating healthy mice with 4-EPS resulted in anxious behaviors similar to those seen in the autism model: they were more easily startled and spent less of their time in the middle of an open space than untreated mice. Perhaps, Mazmanian speculates, people with autism have excess 4-EPS, or similar molecules, leaking into their bloodstream and reaching the brain, affecting their behavior. The researchers
are now planning to look for elevated 4-EPS in the blood of children with autism.

Like Rosenberg, Mazmanian cautions that although a few studies have linked the gut microbiome to autism in people, intestinal microbes are one of many genetic and environmental factors that could contribute to the condition. “There’s still more work to be done before we’ve nailed it down and are confident that the microbiome is different in autistic subjects, but it looks to be that way,” he says.

**Savannah Conversation**

Back in Africa, Theis and colleagues camped out in Kenya’s Masai Mara National Reserve, tracking hyenas and occasionally darting them to collect samples. Researchers use the handle of a scalpel to scoop a bit of paste from the scent pouches of anesthetized hyenas, and drop the sample in liquid nitrogen for later analysis back home. Theis can determine which bacteria inhabit each hyena’s pouch by sequencing the genes for structural RNA in the ribosome, the cell’s protein factory, which differs between taxonomic groups and acts as a sort of barcode to identify the bacterial types.

If bacteria underlie scent communication, then each hyena should contain a different population. Indeed, Theis found a range of bacteria, including types that are likely to produce odors, in adult female spotted hyenas. These populations lined up, albeit imperfectly, with the clans they came from, so that the scents of the so-called Southern Comfort clan were distinct from those in the Mara River or Fig Tree hyenas (8).

Theis expanded on these findings in a recent PNAS study (9). He used mass spectrometry to examine paste odor profiles of males and females belonging to two species: striped and spotted hyenas. The bacterial communities, as well as the odor molecules they produced, distinguished the striped from the spotted animals. Different bacterial and odor patterns also signaled the sex of the animal, and whether females were pregnant or lactating.

Although the data back up the hypothesis that hyenas use bacteria to mediate scent-based communication, Theis is still looking for conclusive proof. That would require experiments, such as applying antibiotics to disrupt communication: not easy with large, wild carnivores. Instead, Theis plans to follow up his work by studying bacteria in the preening oil of captive dark-eyed juncos, songbirds that rely on chemical communication.

Theis’ plans illustrate how the field is already circling back to basic microbiology. Sequencing of genes coding for ribosomal RNA and other techniques have made it possible for scientists to catalog the diverse microbes that live in or on animals, and correlate these populations with behaviors. However, to prove causal links between individual microorganisms and specific actions, researchers still need to culture those bacteria wherever possible or apply antibiotics to test their hypotheses.

So, researchers need to team up. Understanding the behavioral symbiosis between microbes and animals requires another symbiosis of sorts, between microbiologists and behavioral ecologists. “It probably wouldn’t work if you didn’t have a mix,” Theis says.

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