★ NEWS FEATURE

Do hosts and their microbes evolve as a unit?

A group of evolutionary biologists sees evidence for a hologenome whereas others dismiss it entirely. One thing's certain: the debate remains heated.

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Tilapias like their baths balmy. These tropical fish are happiest in warm pools. But they can be made to adapt to tanks as cold as 12 °C, where they express a set of genes different from their warm-water-dwelling counterparts. Their gut microbes turn out to be different as well—and it may be that these unique microbes play a part in helping fish cope with frigid surroundings, according to the results of a recent study (1).

But which is actually responsible for the adaptation—a change in the animal's gene expression, or a change in its microbiome? According to one theory of evolution—which proposes that hosts and their resident microbes

function as an evolutionary unit—the answer might be both.

This unit, dubbed the holobiont, carries what some have termed a hologenome, meaning the genetic information encoded by both a host and its microbes. The hologenome theory suggests that evolutionary pressure acts on holobionts, not hosts or microbes alone, and so the two should be considered a single unit of selection.

Studies of fish, wasps, corals, and several other animals provide evidence to support the provocative idea that creatures and their microbial inhabitants are linked as holobionts through evolutionary time. Some

Various research groups have suggested in multiple articles that wasps, aphids, tilapia, and coral (clockwise, top left to bottom left) are among the creatures that exhibit the hallmarks of a hologenome. But many researchers remain skeptical. Image credit (clockwise from top left): Wikimedia Commons/M.E. Clark and Shutterstock/Frances van der Merwe/Piriya Gutsch/Stephan Kerkhofs.

Fig. 1. According to hologenome theory, holobionts encompass the host and all of its symbiotic microbes, including those affecting the holobiont's phenotype that have coevolved with the host (blue), those that affect the phenotype but have not coevolved with the host (red), and those that do not affect the holobiont's phenotype at all (gray). Microbes in the environment are not part of the holobiont (white). Reprinted from ref. 10.

researchers endorse the concept because it offers a better way to represent the importance of microbes to plant and animal evolution. But others question whether the idea is more confusing or distracting than useful, suggesting that concepts such as ecological filtering, in which the environment (or in this case, the host) selects for or against certain microbial species, can already account for the dynamics researchers have documented.

In the case of the tilapia study, although the study authors write that their results are "consistent with the hologenome concept," the accompanying decision letter from *eLife* reviewers pointed out that the results only confirm a correlation between a fish's cold tolerance and the hardiness of its microbiome. Perhaps, skeptics say, such results show something much less surprising: a capacity to adapt to the cold might be the result of a species' ability to tweak its gut microbiome in a beneficial way.

Critics note further that such a correlation could just as easily be explained by other ideas, such as traditional coevolutionary theory, and, hence, give no credence to the hologenome concept. Indeed, some suggest the concept isn't just unhelpful but is plain wrong.

The debate on what the term *hologenome* means—and whether it's even necessary—remains heated. Just last year, Jeffrey Morris of the University of Alabama at Birmingham published a review describing the hologenome concept and its potential implications (2). Getting the manuscript accepted "was an adventure," he says. "I've never seen two reviewers as angry. . . I don't think I appreciated how controversial this topic was until I wrote that review."

Evolution Under the Sea

The earliest use of the word holobiont dates back to biologist Lynn Margulis's description of the essential links between a fungus and algae in lichens, where neither partner can survive without the other (3).

But the modern hologenome theory began with corals. Millennia ago, corals teamed up with algae to take over the oceans: the coral skeleton protects the algae while the algae provide corals color and nutrients in a wide range of environments. In addition, corals carry a slew of beneficial bacteria within their skeletons and in a surface mucus layer. In the late 1990s, researchers studying a coral-bleaching disease, caused by the bacterium *Vibrio shiloi*, found that one species of coral grew resistant to the infection—simply by picking up new microbial members (4).

Tel Aviv University microbiologist couple Eugene Rosenberg and Ilana Zilber-Rosenberg, who had coauthored studies on bleaching, as well as the protective probiotic microbes, were discussing the data over dinner more than a decade ago, when "my wife pointed out that there's nothing special about these corals," Rosenberg says. "This interaction could occur with any organism that acquires the right bacteria."

The couple combed the literature but failed to find a term that they felt encapsulated this idea. They coined the term *hologenome* and introduced the concept at a lecture in Munich. It asserts that any animal or plant could acquire microbes that might confer an evolutionary advantage to their hosts (5). "The beauty of this is that the host genome evolves slowly, and microbiota changes quickly," Rosenberg notes, meaning the holobiont can adapt faster to changing external conditions.

Teasing Apart Terminology

Although terms such as *superorganism* or *metagenome* have been used to describe the combined properties of a host and its microbes, prefixes such as *super* or *meta* imply that the collective is somehow more than the sum of its parts. That's not always the case, Zilber-Rosenberg says. The word *hologenome*, however, simply implies a sum total.

Not everyone agrees on that definition. "Some people just use the term because it sounds nice, and it's a way of describing an association between an animal or a plant and its community of microorganisms," says entomologist Angela Douglas of Cornell University in Ithaca, NY. "Others use it with a lot of mechanistic baggage, implying evolutionary or ecological processes. That's one of the difficulties with the term: it's tough to know which way a colleague is using it."

Precision is paramount when introducing a new term, says evolutionary biologist Nancy Moran of The University of Texas at Austin. "Being able to say what a term means in one short sentence, not a long paper—that's kind of the test of whether a word is useful."

Experimental Evidence

Several groups have tried to define exactly what the hologenome or holobiont is, both with experimental studies and conceptually (see Fig. 1).

The term first caught Seth Bordenstein's attention when he was studying parasitoid wasps of the *Nasonia*

species. Bordenstein, an evolutionary geneticist at Vanderbilt University in Nashville, TN, found that hybrids formed by crossbreeding closely related lineages of *Nasonia* died. But when treated with antibiotics that cleared gut bacteria, these same hybrids could survive—suggesting that the gut microbiomes of *Nasonia* species prevented interspecific breeding. This reproductive barrier is often the first step to forming a new species. Because gut microbiota appeared to drive the process in *Nasonia*, the researchers attributed the case to the hologenome—that is, neither the host nor its microbes worked alone (6).

"For me, this wasn't a host problem or a microbiome problem—you can't explain it with one or the other because both entities are required for the hybrid lethality that occurs," Bordenstein says. "It's clearly a result of the host and microbiome no longer working together properly in the hybrids."

Other studies have reported similar phenomena in fruit flies. And in mice, researchers have found that microbiomes can direct what reproductive partner an animal will find attractive (7). With mounting evidence, Bordenstein, Rosenberg, Zilber-Rosenberg, and others have refined the hologenome concept to explain how it might account for various aspects of evolution.

For example, they have clarified that unlike a multicellular organism's genome—which must be handed down from one generation to the next—the microbial part of a hologenome can be acquired either from the parent or from an organism's environment. "It really doesn't matter as long as hologenome is reconstituted in every generation," Zilber-Rosenberg says.

Moreover, the microbes don't even have to be the same species. They only need to carry the genes needed to confer properties specific to the holobiont. And the terms *holobiont* and *hologenome* are "structural," Bordenstein explains, and they don't aim to encompass all the complex interactions—symbiotic, commensal, or otherwise—between a host and its microbes. As a result, the hologenome concept encompasses the potential to account for genetic drift, meaning the chance variations that arise randomly over time. These variations create a base of holobiont diversity that natural selection then acts upon. "This is really important yet under-discussed," Bordenstein says. "Microbes may come and go or may be redundant, performing the same functions for the holobiont. This neutrality potentially explains a lot of the interindividual variation that may occur between two members of a host species."

Complex Connections

Despite the explanations and data offered by proponents of the hologenome concept, not everyone is convinced the terminology is needed—or even useful.

Microbiologist Rebecca Vega Thurber of Oregon State University in Corvallis first heard the term as a postdoc studying corals. "Being a little naïve at the time, I wholeheartedly embraced this idea that organisms could be working together and actually evolving to some group end," she says. "Now, I see some real problems with the concept, at least in terms

of how we evaluate it. I'm more skeptical of its utility now."

Thurber's skepticism began when she and her team launched the Global Coral Microbiome Project in 2014, an effort to understand corals and their microbial diversity around the world. Their samples represent nearly 400 million years of evolution. In teasing apart host, environment, and microbes, Thurber and her colleagues found that the skeletal microbiome was largely governed by a host's genes, but the microbes in a coral's mucus layer were determined by its environment. They found that some microbial and host genes appear to reciprocate changes in one another, suggesting a coevolutionary process, whereas others show no such changes despite remaining associated over long periods of time. One microbial species that showed strong evidence of evolving with its host was a predator that eats other bacteria. "We started asking, 'what's the biological meaning of this evidence?'" Thurber recalls. "Biologically, there could be many mechanisms that drive these patterns."

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—Seth Bordenstein

In the case of the cold-adapted tilapias, for example, it's possible that the cold-adapted microbes in the fish are selected by host changes rather than with them—an effect also known as ecological filtering. Similarly, hologenome critics point out that other ecological or evolutionary concepts such as symbiosis can be used to describe—perhaps in more precise ways—different kinds of host-microbe relationships (see, for example, ref. 8).

And while the hologenome theory suggests that a holobiont is a true unit of selection, there's little conclusive proof of that so far, according to microbial ecologist Forest Rohwer of San Diego State University in California, who was also Thurber's postdoctoral mentor. The host and its microbes are essentially "separate units that maintain their individual identity as they evolve," he says. "It's not as if the whole system is evolving. There's never been any proof that the holobiont evolved any differently than we would think of them classically evolving together, which is just as individual species with some horizontal gene transfer thrown in."

Evolutionary biologist Ford Doolittle of Dalhousie University in Halifax, Nova Scotia, goes further, saying there's no value in thinking about a system of animal and microbe—and in fact, there may be a downside. Looking at hosts and their microbes as a single unit can cause researchers to lose sight of symbiosis, mutualism, or other patterns of interaction. And those

interactions—not the identities of the interacting players—are more critical to evolution (9). Doolittle, while a skeptic, does see a sliver of merit to the theory. If the collective activities do persist across lineages, then perhaps, he speculates, the unit of selection could be the “processes and patterns” of metabolic interactions and the like. This might constitute a holobiont that’s consistent with Darwinian principles.

Indeed, few suggest completely abandoning the hologenome idea just yet. Morris and others agree that the holobiont may be a unit of selection in certain circumstances, although it’s unlikely to be the only one—and it’s certainly not the primary unit of selection, Moran says.

Nonetheless, Moran notes that discussions of the term have “energized the field.” And despite problems with practical experiments and her own skepticism, Thurber says discussions of the hologenome concept have helped the research community “push the envelope to better understand how individuals and groups of organisms evolve alongside each other—and perhaps together.”

Proof of Concept

To better investigate the concept, researchers need better ways to gauge microbial complexity. Thurber and others have found that the sequence of the 16S ribosomal RNA gene, which is commonly used as a marker of bacterial evolution, is inadequate to understand how complex microbiomes adapt in hosts. “It’s not a good marker to study how genes diversify,” she says. “There’s just not enough sequence variation to look at these very long patterns of evolution.”

Also crucial: better delineating the boundaries of the hologenome, Morris says. In studies of marine

ecosystems, for example, it can prove tricky to identify if a fish’s microbiome might include some species that live near the host but not on it. Is a microbe that spends its entire life 1 millimeter away from the surface of a fish part of the fish holobiont?

Being able to quantify the nature and strength of the interaction between a host and its microbes would also help, Morris suggests. Because microbes can span a gamut from obligate symbionts to transient inhabitants, “there needs to be a way to relate those interactions to how we think about the holobiont,” he says. “The obligate symbionts should perhaps be weighted a little bit more heavily in terms of our thinking about the holobiont than ones that are just passing through.”

And perhaps most importantly: those trying to figure out whether tilapias, corals, or other organisms can reasonably be categorized holobionts must consider other explanations of their data. “I think the hologenome should be the alternative hypothesis being tested, not the null hypothesis,” says Moran. “Sometimes, of course, it really does hold up.”

With increasing evidence of the importance of microbes, there’s no doubt that the microbiome should be factored into evolutionary studies. But whether the hologenome framework is the way to do so remains to be seen. “The hologenome concept is not the answer to any particular question, but it’s an idea that has inspired a lot of conversation, thought and debate,” says biologist Jessica Bolker of the University of New Hampshire in Durham. “Ultimately, such concepts should be judged on the basis of the research they end up driving,” Bolker adds. “Moving the science forward is what really matters.”

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