NEWS FEATURE

# News Feature: Intimate partnerships

## Recent research illuminates how symbiosis has been—and still is—a major player in evolution

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*Science Writer*

Minutes after emerging from its egg, the tiny Hawaiian bobtail squid begins a remarkable symbiosis. The squid sucks a tiny amount of seawater into a cavity in its mantle, and with it, a cell or two of the bacterium *Vibrio fischeri*. The *V. fischeri* then "tell" the squid to create biochemical conditions that wipe out competing bacteria and to build an organ where the *V. fischeri* can live. "The bacteria sort of go in and close the door behind them," says Margaret McFall-Ngai, director of the Pacific Biosciences Research Center at the University of Hawaii, who has uncovered the details of this intimate partnership over decades of research.

As the squid forages at night, the eerie bioluminescent glow on its underside produced by the captive bacteria mimics the moonlight shining above, making the animal virtually invisible to hungry fish below. The bacteria, in turn, get a home with lots of nutrients. The relationship is so intimate that *V. fischeri* even influences the squid's immune system and circadian rhythm.

But the implications of such partnerships go far beyond a small squid and its bacterial sidekicks. Over the last few years, research on such mutually beneficial relationships, along with the discovery of a vast, previously unknown microbial world, has opened "the biggest frontier that biology has presented us in a long time," says McFall-Ngai. Life, it turns out, is awash in—and has been shaped by—crucial relationships between species, from nutrient-providing symbionts living inside insects and fungi that help feed plants, to the enormous populations of microorganisms within the human body.

This major biological role has implications for the theory that shapes all life: evolution.

Bacteria living within the Hawaiian bobtail squid produce a bioluminescent glow on the squid's underside that mimics the moonlight shining above, masking the animal from hungry fish below. The bacteria, in turn, get a nutrient-rich home. Image courtesy of Sara McBride.

Forging cooperative partnerships to take advantage of the genes and biochemical pathways honed by others appears to be a much more common path to successful adaptations than scientists previously realized. Recent discoveries are, among other things, breathing new life into the age-old debate about the evolution of mitochondria and chloroplasts. Symbiosis was the underlying mechanism behind these two great biological leaps, which made complex life and plants possible; examples of more recently evolved symbiotic pairings may offer new clues into how these organelles developed. "I think we are just scratching the surface of appreciating the role of symbiosis in evolution," says John Thompson, Distinguished Professor of Ecology and Evolutionary Biology at the University of California, Santa Cruz.

### From Cooperating to Co-Opting

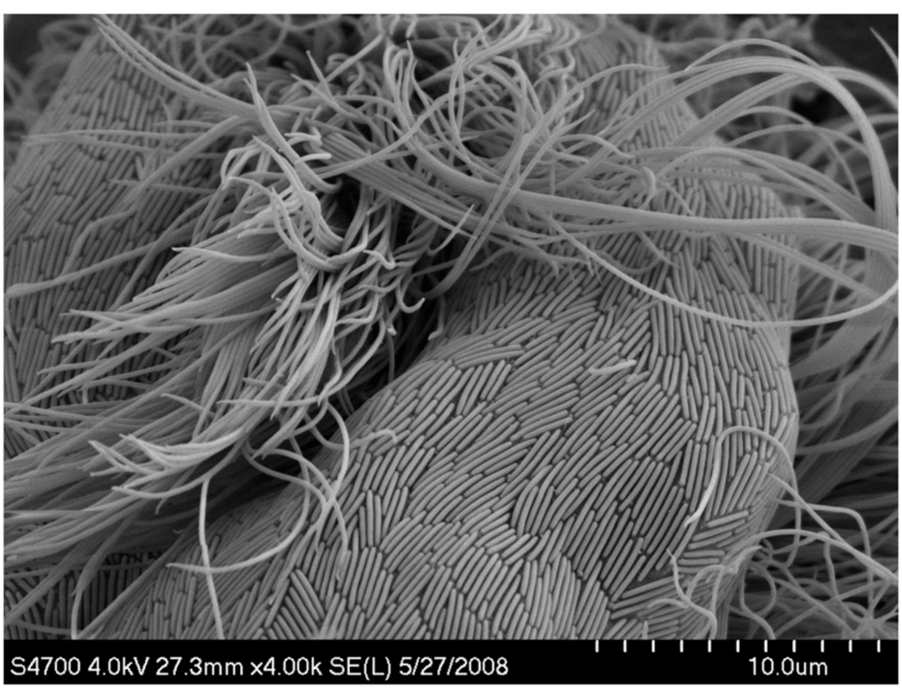
Consider the simple textbook view of evolution: Natural selection acts on mutations in genes or on chromosome rearrangements in populations, perpetuating genetic changes that make individual organisms, in isolation from other species, better adapted to their environments and produce more offspring. But there's another possible path, explains Christian Kost, a bio-organic chemist at the Max Planck Institute for Chemical Ecology in Jena, Germany. Instead of becoming better adapted through felicitous mutations, "an organism could interact with someone who has solved the problem already," he says. Plants that suddenly find themselves in an environment short of nitrogen, for example, could forge a symbiotic partnership with fungi that can suck the nutrients out of the air, gaining a selective advantage for both partners.

Indeed, much of evolution involves co-opting entire genomes of other species, notes University of California, Santa Cruz's Thompson. Plants have enlisted birds to spread their seeds, bees to carry pollen, and fungi to bring nutrients to their roots. Nile crocodiles depend on plovers to clean their teeth. Termites can't survive on a diet of wood without gut microbes called protists, and the bacteria that swarm on each protist "like a rum ball," says biologist Patrick Keeling of the University of British Columbia. The protists digest cellulose and the bacteria fix nitrogen. And the development of this termite–microbe symbiosis itself required another form of cooperation, says Keeling, because the termite's ancestors, cockroaches, had to become social insects in order for the symbionts to be transmitted down through the generations. "So we can put together this picture of cooperation," says Keeling. "It isn't contrary to natural selection; rather it's a way to improve your chances that isn't 100% competition." That makes nature seem less "red in tooth and claw," suggests E. Toby Kiers, University Research Chair and Professor of Mutualistic Interactions at Vrije Universiteit, in Amsterdam.

Certainly, cooperation can bring major fitness advantages, as Kost has shown by creating new symbioses in his laboratory. Kost knocks out the gene for an essential amino acid in one species of bacteria. Then he manipulates the genes of another species to make more of the amino acid and puts the two species together (1). The results have been very exciting, says Kost, because they don't fit into existing theory. Conventional wisdom held that the original individual species would be more fit, outgrowing the Rube Goldberg-like partnership. Not so. The symbiotic pair grew faster than the autonomous cells by a dramatic 20%. Some of the paired bacteria even constructed bridges between them to exchange nutrients directly (2).

The fitness advantage from the symbiosis is so drastic, suggests Kost, that bacteria have a powerful evolutionary incentive to lose key metabolic pathways as soon as they can get those required nutrients from others. And that finding, in turn, helps to explain why the immense microbial world—part of the "new frontier" that McFall-Ngai describes—went undiscovered for so long. "The majority of bacteria are unculturable because, we believe, they live in complex communities that depend on the cross-feeding of metabolites," says Kost. Only the dramatic plunge in the cost of gene sequencing has allowed scientists to identify previously unknown species by reading the genes of whole microbial populations collected from places like soil, oceans, or the human gut.

These symbiotic relationships microbes have with plants and animals are essential, suggesting that natural selection doesn't just operate on individuals—including each of the partners in a symbiotic relationship—it also shapes entire networks of species. That's bringing a resurgence of century-old ideas about evolution, says Thompson. Before Gregor Mendel's pioneering work on individual inheritance became widely known in the early 1900s, many of the best tests of evolution involved species interactions, he explains. "Now we have a greater appreciation of the balance between single gene changes versus co-opting genomes," Thompson says. "But we still don't know how important symbiosis is in developing major adaptations that lead to the diversification of life."




This scanning electron microscope image shows bacteria on the surface of a protist, inside a termite. Termites engage in a symbiotic interaction with protists, which digest cellulose, and bacteria, which fix nitrogen. Image courtesy of Kevin Carpenter and Patrick Keeling.

## Life's Giant Steps

And researchers still don't know the precise details of how symbiosis led to the evolution of mitochondria, the crucial event in the development of the eukaryotic cell, and thus, complex life. But scientists have started to make progress in addressing "one of the most enduring mysteries in all of biology," says John Archibald, professor of biochemistry and molecular biology at Dalhousie University and author of *One Plus One Equals One: Symbiosis and the Evolution of Complex Life* (3). Thanks to the new genetic tools and years of effort, scientists have confirmed (4) an idea once thought so crazy that the paper proposing it in 1967, by Lynn Margulis, was rejected 15 times before it was finally published (5). Her hypothesis: Sometime a mere billion years or so after the dawn of life, bacteria found a home inside an ancestral cell, creating a whole new type of more complex entity—the eukaryotic cell—in which the symbiotic bacteria became energy-converting organelles, or mitochondria.

Studies have also confirmed that more than a billion years ago, symbiosis enabled life to take another giant step when eukaryotes engulfed cyanobacteria that were capable of harnessing the power of sunlight (6). Those photosynthetic endosymbionts evolved into chloroplasts (also called plastids), resulting in the first single-celled algae, which greened the planet and paved the way for the evolution of plants.

By sequencing the genomes of mitochondria, chloroplasts, and many bacteria, researchers have mapped the evolutionary journey of these symbionts from free-living species to essential parts of cells. A major controversy still churns over the type of cell that hosted the original mitochondria progenitor (had it previously evolved some eukaryotic features, or was the energy produced by the symbiont essential to the evolution of those features?), but other aspects of organelle evolution are becoming clearer.

One key change is that the original symbionts lost most of their genes as they evolved into organelles, which in animal mitochondria have very small genomes that consist of only about 13 protein coding genes, according to University of Montana biologist John McCutcheon. That's a tiny fraction of the thousands of genes they need to function. So a key step in the journey was the migration of genes from the symbiont to the nucleus of the host. Then, to ensure that mitochondria and chloroplasts get what they need, the host cell has evolved a targeting mechanism to take the proteins encoded by the nuclear genome to the organelles. And sometimes the vital tasks are divided up between different organelles in the same cell.

All this genetic sleuthing has turned up some oddities. "Mitochondria and plastids are playgrounds for bizarre things," says Keeling. The genetic code matching DNA triplets to amino acids can vary, and messenger RNA can be edited in unusual ways. The mitochondria in dinoflagellates, a type of single-celled plankton, have even done away with stop codons, the three nucleotides that signal the end of a DNA message, says Keeling. The strange genetics offer a window into "a lot of interesting biochemistry" that demonstrates what's possible, he says, and suggests a constant genetic tinkering that offers alternative evolutionary paths.

### Tinkering or Transitioning?

Keeling was pondering this genetic oddity in organelles and the question of how the original symbionts became captives when he was asked to review a paper by McCutcheon and von Dohlen (7) in 2011. Keeling suddenly realized that clues to organelle evolution might be found by looking at a completely different kind of symbiosis: in the cicadas that McCutcheon studies.

Cicadas feed on plant sap, which is notoriously poor in nutrients. To survive, the cicadas depend on two species of bacteria that live inside the cicada's abdomen. McCutcheon's laboratory has shown that one species, *Sulcia*, makes 8 of 10 essential amino acids. The other, *Hodgkinia*, makes the other two. That mimics the division of labor among organelles. What's more, McCutcheon discovered that the cicada's endosymbionts display genome oddities reminiscent of those found in mitochondria and plastids. Some of the symbiont genomes see dramatic reductions in genes; others balloon up with junk.

Because of these similarities, Keeling thought that researchers in two areas of biology—the study of the ancient organelles and the work on modern symbiosis—might have more to say to each other than was currently appreciated. So Keeling, McCutcheon, and biochemist W. Ford Doolittle, professor emeritus at Dalhousie University and a pioneer in the field of cellular evolution, organized a meeting of the two fields in October 2014 at a Sackler Colloquium: *Symbioses becoming permanent: The origins and evolutionary trajectories of organelles* (www.nasonline.org/programs/sackler-colloquia/completed_colloquia/Symbioses_becoming_permanent.html).

The major question for the two fields: Are the symbionts in sap-eating insects, bioluminescent squid, and many other creatures actually on evolutionary paths that could transform them into full-blown organelles?

> **"The origin of eukaryotes is a Big Bang event."**
> **—Nick Lane**

Or is organelle evolution a piece of ancient history, the result of a complex process that occurs extremely rarely?

The question has generated heated debate. Evolutionary biologist William Martin of Heinrich-Heine Universität in Düsseldorf, and Nick Lane, an evolutionary biochemist at University College London, emphasize that eukaryotic cells evolved from the symbiosis of two primitive cells only once in four billion years. "The origin of eukaryotes is a Big Bang event," says Lane. Plus, they argue, the phenomenon was fundamentally different from classic symbiosis because the development of mitochondria boosted the energy available to the nascent eukaryotic cells by orders of magnitude (by making ATP). In contrast, classic symbionts merely supply a few amino acids, as in cicadas. "Most other endosymbionts provide very trivial benefits compared to mitochondria and chloroplasts," says Lane.

So whereas modern symbiosis might help show how genes get transferred from symbiont to the host nucleus and how symbiosis between eukaryotes evolved, Lane argues, "we really don't learn about what made the origin of eukaryotes unique."

Most of the time the genomic experimentation seen in symbionts has no discernable effect on fitness, scientists acknowledge. "A lot of things in biology happened just because they didn't kill the organism," says Keeling. But the very act of sacrificing autonomy to enter a relationship can be a gamble as well as an opportunity for host and symbiont. The marriage can be a disaster for both partners. As University of Texas at Austin, biologist Nancy Moran writes in a 2015 PNAS paper (8), together they could "spiral down the symbiosis rabbit hole."

Or the pair can become greater than the sum of its parts. The relationship could get better and better, until the bacterial symbiont becomes indistinguishable from an organelle. "Perhaps it happens by trial and error," says Keeling. If so, he speculates that the key steps in the process are the transfer of enough genes from symbiont to the nucleus of the host, and then the targeting of protein products back from host to symbiont. Once important genes are incorporated in the host nucleus, they are protected from the genetic weirdness than can occur in the symbiont genome. "I think these events are probably more common that we realize," says Archibald. "We just have to have an open mind."

The heated debate over whether organelle evolution is incredibly rare or more common won't be resolved soon. "There's not enough evidence to nail it one way or another," explains Lane.

Another major open question is how the countless examples of modern symbiosis evolved in the first place. "What was the first symbiosis and what brings organisms together?" asks Nicole Dubilier, leader of the department of symbiosis at the Max Planck Institute for Marine Microbiology in Bremen. "It is one of our favorite beer conversations."

Answering the big questions about how these intimate partnerships begin and are maintained promises important new insights into evolution and the larger role of microbes in biology. And for the first time, scientists believe, they have the tools to finally address these questions. "We are on the edge of something here," says Vrije Universiteit's Kiers. "And it's getting more and more exciting."


**1** D'Souza G, Waschina S, Kaleta C, Kost C (2015) Plasticity and epistasis strongly affect bacterial fitness after losing multiple metabolic genes. *Evolution* 69(5):1244–1254.

**2** Pande S, et al. (2015) Metabolic cross-feeding via intercellular nanotubes among bacteria. *Nat Commun* 6:6238.

**3** Archibald J (2014) *One Plus One Equals One: Symbiosis and Evolution of Complex Life* (Oxford Univ Press, Oxford).

**4** Gray MW (1992) The endosymbiont hypothesis revisited. *Int Rev Cytol* 141:233–357.

**5** Sagan L (1967) On the origin of mitosing cells. *J Theor Biol* 14(3):255–274.

**6** Gray MW, Doolittle WF (1982) Has the endosymbiont hypothesis been proven? *Microbiol Rev* 46(1):1–42.

**7** McCutcheon JP, von Dohlen CD (2011) An interdependent metabolic patchwork in the nested symbiosis of mealybugs. *Curr Biol* 21(16):1366–1372.

**8** Bennett GM, Moran NA (2015) Heritable symbiosis: The advantages and perils of an evolutionary rabbit hole. *Proc Natl Acad Sci USA* 112:10169–10176.