Fast Friends, Sworn Enemies

Organisms that work together, researchers are finding, sometimes have a falling out

Even as the bride and groom walk down the aisle, they—or at least their guests—know that marital bliss can be short-lived. Wrinkles can appear in the smoothest relationships and turn lovers into adversaries. Biologists are now realizing that the same holds true in symbiotic relationships. What starts out as a mutually beneficial arrangement can turn into a commensal one, in which just one partner benefits. In the worst case, one symbiont begins to parasitize the other. But sometimes the partners work through adversity to restore balance in their alliance.

A new awareness of the complexity of these interactions is shaking up the ecology and evolutionary biology communities, which are used to thinking of interspecies interactions as stable. "We've been stuck classifying these things as mutualist, commensal, or parasitic, but we've come increasingly to understand how variable [these relationships] are," explains Angela Douglas, a symbiologist at the University of York, U.K. Given the range of behaviors covered, the word "symbiosis" needs redefining, says Douglas Zook of Boston University: It should be applied to any interactions that use one or both partners' resources.

The more biologists look, the more symbiosis they see. Forests thrive only when fungi blanket their roots. Corals rely on photosynthesizing algae. Gut symbionts help humans and other animals digest food. "Symbiosis is a major phenomenon extending across all kingdoms," says Zook.

The idea that different organisms live and work together dates to 1868, when German botanists Albert Bernhard and Heinrich Anton de Bary independently developed the symbiosis concept. The term applied to any association between different organisms, including parasitism. Later mutualism (both parties profit) and commensalism (one benefits but not at the expense of the other) joined parasitism as subsets of symbiosis. For decades, all were included under a single term. In the early 1900s, biologists decided the word symbiosis should apply only to relationships in which both partners benefit, and that's what most textbooks teach today.

Only recently have researchers begun a wholesale investigation of how these relationships change over time. Plant pathologists have made a few key observations in studying grasses and microscopic fungi that live between their cells. Others have noticed that pathogens in one species or individual are partners in another. All this can lead to complex relationships that sometimes involve more than just two species. Moreover, the cause of a relationship switch is not clear-cut. But environmental factors can play a role, such as food shortages, new hosts, alterations in the chemical milieu, or changes in the local community.

Turncoat. Sometimes helpful fungi can stifle reproduction in their benefactor by spreading along the outside of the stem (bottom).

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Dynamic relationships

Fungi that live inside grasses can be fickle. These endophytes provide protection and stamina to the grass, deterring insects and livestock and making the grass drought-tolerant and disease-resistant. In return, the grass provides sustenance. But in August at the Fourth International Symbiosis Society Congress, held in Halifax, Nova Scotia, Christopher Schardl of the University of Kentucky, Lexington, reported that endophytes sometimes abort seed production.

Endophytes spread by inhabiting seed-bearing stalks. Schardl and his colleagues found that the "friendliness" of the fungus can vary stalk by stalk, depending on the fungus's mode of reproduction. Those that follow the asexual route remain within the stalk, spreading to the next generation of grasses by hiding out in the developing seeds. They are the friendly sort. But the same organism may reproduce sexually on the outside of other stalks, leading to the production of fungal spores. These fungi rob the plant of nutrients it needs for its own reproduction. In some cases, they choke off seed development entirely. "The [fungi] clearly span the range between mutualistic and antagonistic," Schardl notes.

The stable grass-fungi relationship can be disrupted by a fly. The insect relies on the fungus for its own reproduction, laying eggs on developing fungal fruiting bodies. As the fly travels from stalk to stalk, it transfers fungal spermatia, allowing for cross-fertilization, which benefits the fungus. The fly's larvae also benefit because they do best on fungi that have been fertilized. In this way both the fly and fungus maximize reproduction, but the plant may lose out. When flies are not present, sexual reproduction may become too inefficient for the fungus, and thus, over time, an amicable relationship with the grass is restored.

Nutrient supplies can likewise upset the balance between certain plants and their endophytes. When the fungi take more than their usual carbon allotment, they can overrun and kill a plant host. Conversely, fungi that are normally aggressive carbon-takers can't spread on nursery trees, likely because these trees get nutrients from the nursery and don't need the fungi. All in all for these two species, there's no alliance, just détente.

Colonizing a different host, meanwhile, may enable a microbe with a history of hostility to develop a friendly collaboration. *Colletotrichum magna* is a plant pathogen that attacks cucumbers, watermelons, and squash. Yet when Regina Redman, a geneticist at the U.S. Geological Survey in Seattle, Washington, infected tomatoes with this fungus, the plants thrived, producing bigger fruit and resisting diseases. The fungus's lifestyle "depends on the interaction with the plant's genotype," she says.

Fungal genes, too, can make a difference in how species interact. Redman created mutants by randomly knocking out each gene in *C. magna*, then testing each mutant's interactions with its native plant. Some 200 mutations rendered the pathogens harmless or even beneficial. In recent work, Redman knocked out one of the genes responsible for harmful effects in five more *Colletotrichum* species. In each case, the genetic alteration

Complex alliances. The fate of bark beetle larvae, shown in their burrows with nourishing fungi, depends on a mite hitchhiking on adult beetles.

tamed the pathogen.

Geographic location affects alliances as well. It shapes the relationships among the members of a threesome: a southern pine beetle, a mite that lives on the bark beetle's body, and a fungus carried by the mite. The mite can be helpful or harmful to the beetle depending on the type of fungus it carries, says Matthew Ayres of Dartmouth College in Hanover, New Hampshire, who described the complex relationship at the August meeting.

The bark beetle dumps fungi into the host tree, which help kill the tree, and lays eggs in the excavated burrows. In the case of two types of fungi, beetle larvae feed off the fungus. In return, the beetle provides a safe haven for spore growth in the form of sacks behind the beetle's head that cull all fungi except the beneficial ones.

But mites that hop onto the beetle sometimes bring with them a less generous fungal partner. Called the blue stain fungus, the interloper disrupts the beetle's reproduction by shoving out the fungi-nourishing beetle larvae. As a result, "virtually all the larvae die," says Ayres. By aiding the blue stain fungus, the mites shift their relationship with the bark beetle from a positive or neutral one—in which it carries beneficial fungi or none at all—to an antagonistic one.

Ayres and Kier Klepzig, a research entomologist at the U.S. Forest Service in Pineville, Louisiana, are now studying this three-way interaction in a different latitude. In Mexico, the beetle and the mites get along well. The researchers find no blue stain fungus, and instead the mites ferry beneficial fungi.

Thwarting cheaters

The mites that interact with pine beetles have no control over the circumstances that make or break a relationship. But in other cases, the partners themselves take steps to maintain harmony, keeping undercurrents of tension in check. Take legumes and rhizobia, the nitrogen-fixing bacteria that help feed them. Soybeans supply the bacteria with nutrients and a safe place to live. In return, the rhizobia expend an exorbitant amount of energy fixing nitrogen for the plant's consumption.

Theorists have calculated that rhizobia would do much better as freeloaders, curtailing nitrate production and devoting more energy to their own growth. Researchers wonder why the microbes don't evolve ways to sell the soybean short. "That question has been around for a long time," says Frans de Bruijn, a microbiologist at INRA-CNRS in Toulouse, France.

The reason is that the plant keeps close tabs on microbial productivity, and "cooperation is maintained through coercive measures," E. Toby Kiers of the University of California, Davis, reported at the symbiosis meeting. Working with her adviser, crop ecologist R. Ford Denison, Kiers and her colleagues showed that soybeans cut off nutrient supplies to nitrogen-fixing bacteria whenever they begin to slack off. The plants can even selectively sanction the worst offenders.

The researchers turned honest bacteria into cheaters. In the lab, Kiers deprived rhizobia of nitrogen, which they normally get from the air, and watched what happened as nitrate production declined. It took just a few days to see a 50% reduction in the rhizobia's reproduction; the loss of key nutrients provided by the plants, including oxygen, caused these declines. Moreover, the plant seems able to turn off the oxygen spigot nodule by nodule, mounting surgical strikes against what it perceives as cheaters.

Occasionally cheaters do get the upper hand. Joel Sachs, a graduate student at the University of Texas, Austin, has found this to be the case with the upside-down jellyfish *Cassiopeia xamachana.* It hosts an alga, *Symbiodinium microadriaticum*, whose photosynthetic activity supplies the jellyfish with carbohydrates.

Working with Tom Wilcox of Long Key Tropical Research Center in Florida, Sachs raised algae-free jellyfish, then exposed them to algae from wild jellyfish. He tracked the well-being of both the host and its guests as they spread from parent to offspring (known as vertical transmission) or from jellyfish to jellyfish (horizontal transmission).

Transmission mode shaped the partnerships over time. Declines in jellyfish growth and reproduction "revealed the evolution of exploitation in the horizontal treatment," Sachs explained. Sometimes the cheater algae that spread from jellyfish to jellyfish got carried away. The algae robbed so much that they killed their host, compromising their own reproduction.

In contrast, vertical transmission "selected for symbiont cooperation," says Sachs. In each successive generation, jellyfish and alga survived better and were more prolific. As long as each took just enough from its partner, the relationship remained balanced and productive.

Work in coral has suggested that these creatures eject algae that don't suit their purposes. Some researchers think that when the weather gets too warm, corals bleach because they kick out their algal symbionts and, possibly, take on others that can tolerate the heat better. If so, Sachs questions the long-term effectiveness of that strategy. Replacing the original algae with others from the surrounding water, a case of horizontal transfer, "could lead the coral to pick up cheaters."

Telling experiment. Algae colonizing immature algae-free jellyfish (*above*) added color to their hosts (*left*) but sometimes caused harm as well.

Sachs is now exploring what jellyfish and possibly other hosts can do to curtail freeloading. But at least, he notes, work by his team and others is helping reshape current thinking about symbiosis. "A lot of biologists think the relationship [between two species] is static," he points out. "But it's much more dynamic." But then, what relationship isn't?

–ELIZABETH PENNISI

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