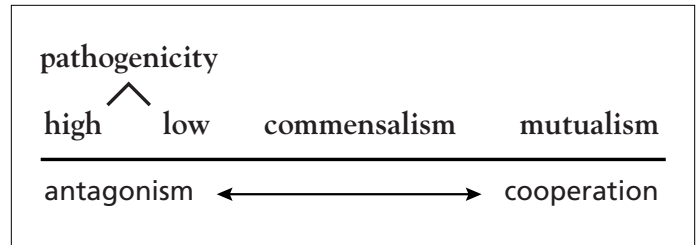# Evolving together: the biology of symbiosis, part 1

GREGORY G. DIMIJIAN, MD

Symbioses, prolonged associations between organisms often widely separated phylogenetically, are more common in biology than we once thought and have been neglected as a phenomenon worthy of study on its own merits. Extending along a dynamic continuum from antagonistic to cooperative and often involving elements of both antagonism and mutualism, symbioses involve pathogens, commensals, and mutualists interacting in myriad ways over the evolutionary history of the involved "partners." In this first of 2 parts, some remarkable examples of symbiosis will be explored, from the coral-algal symbiosis and nitrogen fixation to the great diversity of dietary specializations enabled by the gastrointestinal microbiota of animals.

Figure 1. A continuum can be visualized between antagonistic and cooperative symbiotic relationships. Antagonistic relationships occur between hosts and parasites (or pathogens), cooperative relationships between mutualists, and mixed interactions between commensals, either of which may benefit from the relationship. Exact assignment of roles is usually difficult and tentative and reflects our incomplete understanding of most symbiotic relationships.

D erived from the Greek word for living together, *symbiosis* refers to a close and prolonged association between 2 or more organisms *of different species* that may last for the lifetime of 1 or all "partners." The definition of symbiosis is not universally agreed upon; in this review, it will be considered in its broadest sense, encompassing associations varying widely in intimacy and types of interaction. Symbioses can be mutualistic (all partners benefiting), commensalistic (one benefiting and the others unharmed), or parasitic, although many symbiotic associations are complex or poorly understood and do not fit neatly into 1 category (1). A continuum can be envisioned that spans a dynamic bridge from antagonism to cooperation. Relationships may shift gradually or abruptly along the continuum (*Figure 1*).

Many pathogens and parasites clearly fulfill the definition of symbiosis. Those of relatively low virulence (in most healthy hosts), such as intestinal worms, herpesviruses, *Chlamydia* bacteria, and *Helicobacter pylori*, may colonize a host for much of the host's lifetime. Those of high virulence can become symbiotic only if virulence is limited to a subset of the host population or if there is a long period before host death, as with HIV-1. An asymptomatic carrier state, as in the case of some hepatitis viruses, also allows a high pathogen to become symbiotic with its host for much of the host's lifetime.

Commensal organisms are symbionts that are believed to do little or no harm to the host and provide no benefits. Examples include some of the hundreds of species of bacteria colonizing the mouth and gastrointestinal tract. Many bacteria, including tiny mycoplasmas, may be commensals colonizing the skin and mucous membranes of healthy persons. Tiny ectoparasites colonize the skin of animals and usually do little or no harm.

It was thought until recently that the malaria parasite was a commensal of mosquitoes, doing the insects no harm. This presumption may be naive; a study has shown that mosquitoes with malaria parasites bite more often and more aggressively than they need to for their own blood meal (2). Like many other hosts, the mosquitoes appear to be manipulated by the parasites to behave in a manner benefiting the parasite. Perhaps parasites don't "treat their vectors with kindness" after all, as we once thought.

At the opposite end of the spectrum from pathogenicity, *mutualisms* are extraordinary in their variety and ubiquity and will constitute the principal kind of symbiosis covered in this review.

Where a symbiont settles along the continuum depends on ever-changing variables. There is no "normal" or "abnormal" place on the continuum.

Symbiotic relationships exist everywhere we look; they are beginning to seem like the very essence of biology. They occur between the most distantly related organisms, such as bacteria and eukaryotic cells, as well as between closely related species, such as ants. Relationships encompass all degrees of intimacy, from the tight symbioses of mitochondria with their host eukaryotic cells to the more loosely crafted partnerships between entirely separate organisms. Examples covered in this review range from marine invertebrates with their symbiotic algae to the gastrointestinal tracts of animals with their associated microbial symbionts.

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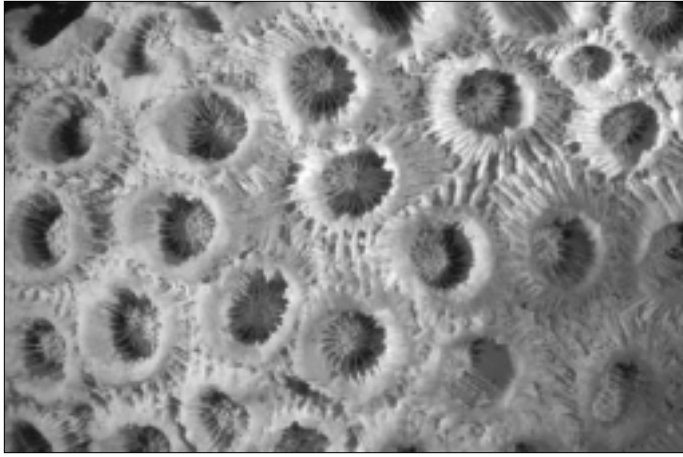
Figure 2. After the living veneer of coral dies, the underlying limestone skeleton is seen to be sculpted in the shape of the polyps which laid it down. (All photographs and illustrations by Gregory G. Dimijian, MD.)
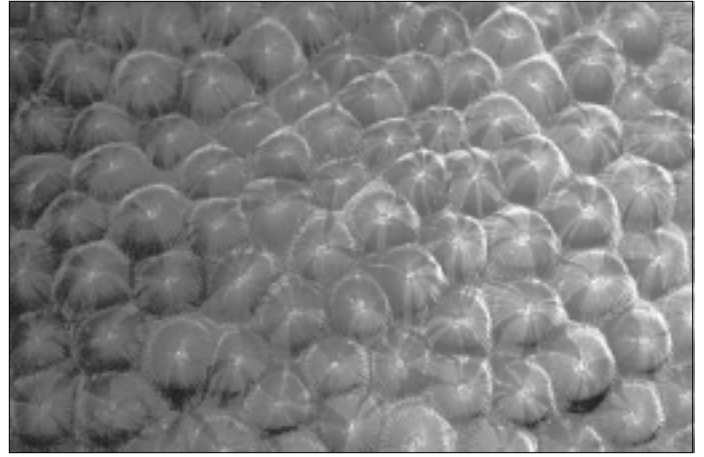
Figure 4. Closed coral polyps provide maximal exposure of symbiotic photosynthetic algae to sunlight during the day, in the shallow water of a Caribbean coral reef. The polyps will open at night and feed on zooplankton.
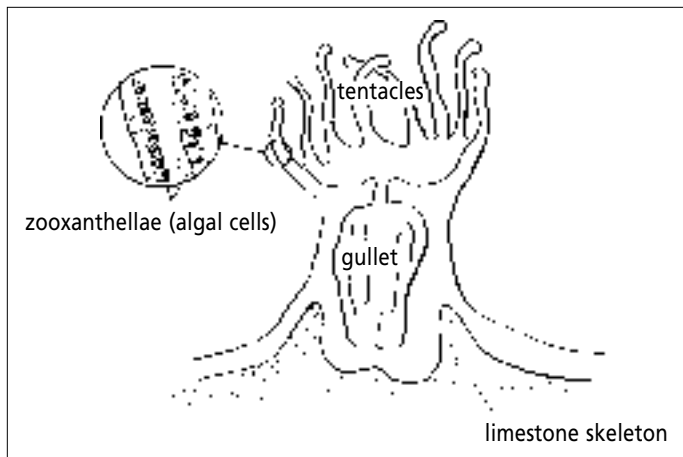
Figure 3. Drawing of coral polyp with tentacles extended. Single-celled algae reside inside the cells of the coral and contribute to the high rates of calcium carbonate deposition in the coral skeleton.

CORALS AND OTHER MARINE INVERTEBRATES

Coral reefs, the most massive structures on earth built by biological organisms other than humans, consist of a foundation of limestone (calcium carbonate, or $CaCO_3$) laid down by a thin living veneer of coral tissue that covers the surface *(Figure 2)* and by limestone from a few other reef organisms, such as coralline algae. Eniwetok Atoll in the Pacific was drilled to determine the thickness of the limestone, and the drill entered basalt bedrock at a depth of 1283 m, indicating a structural reef >1 km tall atop volcanic bedrock. This finding confirmed the subsidence theory, which states that a slowly sinking island supports a reef that is limited in its upward growth by the sea surface. Coral reefs exist over large areas of shallow tropical seas; the Great Barrier Reef of Australia extends continuously for >2000 km.

In complexity, the reef may have its closest terrestrial counterpart in the tropical rain forest. Both evoke an image of exuberant fertility and biomass, and both depend on sunlight filtered down through a stratified structure. Associations at successive levels consist of organisms with needs matching the prevailing illumination and shelter.

Reef construction is driven by photosynthesis. Most reef-building corals are hosts to unicellular photosynthetic algae that live inside their cells *(Figure 3)*. These algae are collectively called *zooxanthellae*, a colorful term that has survived the winnowing influence of a fastidious taxonomy. Zooxanthellae are mostly dinoflagellate algae of a single genus, *Symbiodinium*, and achieve striking concentrations of a million algal cells per cm^3 of coral tissue. The chloroplasts inside the algae, once free-living cyanobacteria which themselves have become symbionts, provide the service of photosynthesis, making glucose, glycerol, and amino acids which are useful to both the algal cell and the coral host. These photosynthesizing algae, inside the very cells of the coral, have been shown to be vital to the high rates of calcification seen in reef-building corals. Calcification rates on sunny days may be 2 to 3 times higher than rates on cloudy days. Many corals can form reefs only when they maintain a viable dinoflagellate population in their tissues.

During the day, when the symbiotic algae are bathed in sunlight, most reef-building corals keep their polyps closed *(Figure 4)*. After dark they open their polyps and actively feed on zooplankton, which are relatively scarce in tropical waters.

In contrast to the cold, nutrient-rich waters of higher latitudes, tropical waters are warm and buoyant, tending not to sink to depth and be replaced by bottom water. Sinking and replacement, called overturn, stirs up bottom sediments and brings nutrients to the surface waters, triggering plankton growth. Because overturn is hindered in the tropics, the waters around coral reefs are usually low in nutrient and plankton content and have been called "deserts."

Photosynthetic products from the symbiotic algae close the nutrient gap (3). Symbionts may release virtually all of these products to host tissues. This is often sufficient to fuel the entire respiratory carbon requirements of the host, making the coral independent of the food sources available in the ambient water.

Under stressful conditions, such as unusually warm water, coral "bleaching" may occur, in which corals expel their algae and lose their green color (4). The partnership is thus dynamic and provisional, the coral host tolerating its partner only under stress-free conditions. Bleaching may sometimes involve exchange of 1 algal type with another, a kind of choosiness on the part of the coral enabling adaptation to a changing environment.

Figure 5. Photographed in 1992, a remarkable landscape of lichen covers a lava flow of 1855 on the island of Hawaii. No large plants had colonized this dry, inhospitable terrain in the 137 years since it was created.

Figure 6. Drawing of a lichen with a framework of fungal filaments enclosing individual cells of its symbiotic alga.

Symbiodinium algae can live independently of corals in seawater. When corals reproduce vegetatively the algal symbionts spread with the growing coral, but when corals reproduce sexually by broadcasting gametes into the water, the algae are delivered in the eggs in some species and obtained later from the seawater in others, after dispersal from the parent colony.

Human activities are killing coral reefs around the world by drowning them in freshwater runoff, pollution, and sediment. Loss of coral reef habitat will have potentially disastrous consequences for the conservation of global biodiversity.

Single-celled algae enter into symbiotic relationships with many other marine animals, including sea anemones, jellyfish, soft corals, mollusks, and tunicates. Like corals, the giant clam *Tridacna*, which can attain a length of 1.5 m, may owe its huge size and rapid growth to light-enhanced calcification. The marine world abounds in symbiotic partnerships of great variety; the closer we look, the more we find.

Lichens

Lichens grow in the leftover spots of the natural world that are too harsh or limited for most other organisms (*Figure 5*). They are pioneers on bare rock, lava flows, cleared soil, dead wood, and newly emerged volcanic islands in the sea. Capable of shutting down metabolically under unfavorable conditions, they can survive extremes of heat, cold, and drought.

With ≥15,000 species, lichens are a successful partnership between a fungus, on the one hand, and either an algal or a cyanobacterial species on the other, and sometimes both. The fungal partner usually constitutes 90% to 95% of the lichen biomass and encloses the cells of the photosynthetic symbiont within a network of filaments (*Figure 6*). The nonphotosynthetic fungus provides a sturdy structure, while the algae and cyanobacteria contribute the products of photosynthesis; cyanobacteria also fix atmospheric nitrogen and contribute this element to the partnership. Over 500 species of lichens contain *both* algal and cyanobacterial symbionts.

The thallus (leaflike or threadlike structure) of a lichen forms only when the fungus encounters an algal or cyanobacterial candidate; the developmental program is present in the genome of the fungus, but without the symbiont the genes remain turned off.
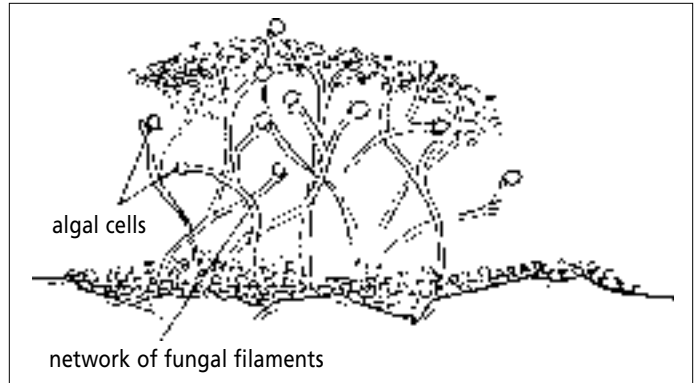
Lichens reproduce vegetatively by breaking off particles, or soredia, composed of fungal threads and algae. They also reproduce by spores produced in fruiting bodies made by the fungus; when these spores germinate, they must capture new algal or cyanobacterial cells to form new lichens. They may even "steal" them from other lichens.

Molecular studies show that lichens have evolved many times, arising from parasitic, mutualistic, and free-living fungi (5). The case for mutualism in some lichens is supported by the finding that neither partner by itself enjoys the same survival success in extreme environments. Yet a free-living fungus may overtake a colony of the cyanobacterium *Nostoc* and incorporate it into a lichen; the fungal partner appears to be parasitizing the bacterium. Some lichens thus appear to be a case of "controlled parasitism," with the hostages providing a measured resistance but no dramatic standoff. This interpretation could be changed, however, if we discover that a chemical "dialogue" between the 2 species initiates the symbiosis.

A lesson to be learned from lichens is that neither mutualism nor parasitism should be considered endpoints in the evolution of symbiotic relations. As indicated earlier, there is no "normal" or "abnormal" point on the continuum.

Words such as "cooperation," "partnership," and "service" have been criticized as teleological, hinting of purpose. The term "service," for example, might be replaced by the more scientific but cumbersome label "capability acquired by symbiosis." The choice is arbitrary; the shorter terms are useful descriptive shortcuts, as long as we understand their limitations and explain that we are not imputing a goal or purpose. Symbiotic partners are responding to each other only as a part of their environment, no differently than a free-living organism responds to its environment. Natural selection moves genes into the future without consulting a dictionary.

Lichens also present a challenge to our concept of *individuality*. Is the lichen itself an individual organism or a composite of 2 species? Do we view it as 1 or 2 organisms? The 2 partners can often be teased apart and survive without the other in less harsh environments. Yet lichens have been given scientific names, betraying our bias toward individuality.

If natural selection acts fundamentally at the level of genes, it also acts on a composite organism. One lichen species, for example, may outcompete another.

CHEMOSYNTHESIS: TUBEWORMS IN HYDROTHERMAL VENT ECOSYSTEMS

The mid ocean ridge system slices through the Pacific, Atlantic, Indian, and Southern Oceans and is home to most of the hydrothermal vent sites that have been studied. Miles down, these strange ecosystems have been known only since the 1970s.

Near ridge systems, cold, dense water on the ocean floor percolates into the earth's crust and is heated by magma just below the surface, making it less dense and more buoyant. Now as hot as 400°C (752°F), the water surges up through the sea floor into the deep ocean, where the surrounding water is nearly freezing. Minerals dissolved in the plume precipitate to form chimneys called "black smokers," which discharge thick clouds of suspended minerals into the water. A thriving community of animals surrounds the vent while it is active; destined to cool eventually, the vent and its living community will die. It behooves vent animals to produce young which disperse regularly across the ocean floor, where some will chance upon other vent ecosystems during their ephemeral existence.

An extraordinary animal in these ecosystems is the giant tubeworm, a 3-foot-long invertebrate housed in its own 6-foot-tall tube and rooted near the black smokers. As adults, these tubeworms have no mouth, gut, or anus, obtaining all their energy and food from *intracellular symbiotic bacteria* that live in the highly vascularized tissue filling the bulk of their body cavity. The worms have a unique hemoglobin in their blood that binds oxygen and sulfide and transports both from the surrounding seawater to the bacteria, which number a billion cells per gram of worm tissue. Carbon isotope data show that the tubeworms depend on carbon compounds synthesized by the bacteria as the main source of their nutrition (6).

The bioenergetics of the bacteria are as follows: hydrogen sulfide plus oxygen yields sulfate plus energy. The energy is utilized to synthesize organic molecules from the carbon dioxide in vent water and seawater. The key part of this energy equation is the oxidation of sulfide to obtain energy; this is *chemosynthesis* instead of photosynthesis. It achieves the same thing as photosynthesis, making organic molecules out of carbon dioxide, but gains energy from a chemical reaction instead of from photons and captures carbon dioxide from water instead of air.

The cozy picture we once had of hydrothermal vents as warm oases in a frigid ocean has changed a bit. The vent field is a harsh environment with shifting currents, scalding water, near-freezing water, and water alternately rich and poor in sulfide and oxygen. Changes occur over seconds to hours. This makes it easier to appreciate the real oasis found by the bacteria in the protected bodies of the host tubeworms, where they enjoy a stable shelter from thermal and chemical extremes, a place where they won't be swept away into the rest of the ocean, and a table to feast at, where a special variety of hemoglobin delivers sulfide, oxygen, and carbon dioxide right to their front door. It is not surprising that the bacteria deliver attractive benefits to the worms. As a result, we have a remarkable mutualism that provides survival benefits to both parties in a harsh and primitive environment with special dangers *and* special resources.

Hydrothermal vent ecosystems are models of very old and sheltered environments that may have been the least perturbed places on earth during mass extinctions. Asteroid impact, sur-

Figure 7. Thorn trees, *Acacia tortilis,* lend a unique beauty to the open landscape of the Serengeti Plains of Tanzania. They obtain much of their nitrogen from symbiotic nitrogen-fixing bacteria in their root nodules.

face volcanism, and other causes of mass extinctions may have left the vent biota alone. In these migrating hotspots on the ocean floor, we may have the ancestors of some of the earliest life forms.

Living closer to the sea surface, other marine invertebrates such as bivalve mollusks have also established symbioses with chemosynthetic bacteria, where sulfide and oxygen are present in the water perfusing the sediments. Sulfur-oxidizing epibacteria have been found on nematodes living in marine sands, at the oxic-anoxic interface; the nematodes appear to derive their nutrition by feeding directly on their symbionts, and they track the chemocline (chemical gradient in the water column) to provide their symbionts with both sulfide and oxygen. The closer we look at marine organisms, the more symbioses we find, each one more strange than the last.

NITROGEN FIXATION

The nitrogen in proteins of every living thing comes ultimately from thin air. Yet that freely circulating dinitrogen molecule is glued together by a triple bond that is nearly impossible to break, and it must be broken for atmospheric nitrogen to become available to living organisms.

Lightning provides a tiny amount of atomic nitrogen. More is freed up by microorganisms that break down dead organisms and their excreta. But a select few bacteria have evolved the secret of how to pull it right out of the air all the time, by means of *nitrogenase* enzymes. These enzymes break the triple bond of
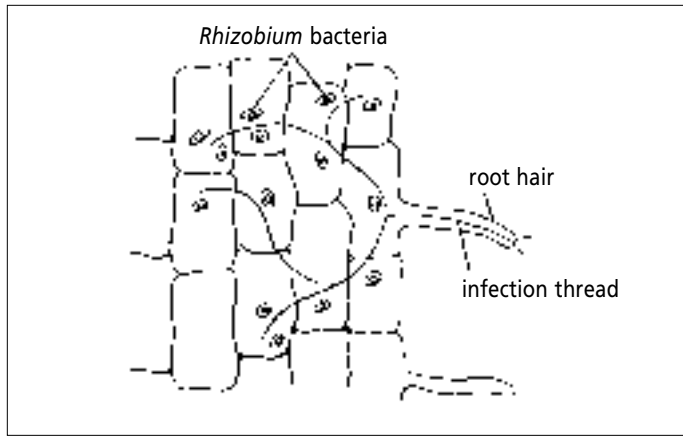
Figure 8. Drawing of the cellular matrix of a plant root nodule, with root hairs and an infection thread laid down by the plant. *Rhizobium* bacteria follow the infection thread into the root hair and take up residence in the root nodule cells, where they will multiply and fix nitrogen in the specialized, low-oxygen environment of the nodule.

Figure 9. Floating *Azolla* ferns are symbiotic with filaments of nitrogen-fixing cyanobacteria tucked away in pockets of their tiny scalelike leaves, providing them with nitrogen captured from the atmosphere. Each plant in the photo is about 1 cm wide.

N_2 and allow reduction of nitrogen to ammonia, which is used by plants to synthesize amino acids. Some of these nitrogen-fixing bacteria form partnerships with green plants, which provide them with a specialized shelter called the root nodule. The nodule provides the low-oxygen environment needed for reducing nitrogen to ammonia.

The collective term "rhizobia" (from one of their genera, *Rhizobium*) has been given to those soil bacteria that form symbioses with most plants in the legume family. Legumes include soybean, alfalfa, clover, pea, peanut, mesquite, mimosa, and acacia (*Figure 7*); with the help of symbiotic rhizobia in their root nodules, most legumes can grow in poor soils without addition of nitrogen-containing fertilizers.

A sprouting legume enters into a *reciprocal molecular conversation* with soil rhizobia (7, 8). The first step is the release of a molecular signal by the legume, which induces a bacterial gene to synthesize a morphogen. The morphogen triggers the formation of root nodules in a narrow infection zone located just behind the growing root tip. The bacteria invade these developing nodules through *infection threads* laid down by the plant (*Figure 8*). The 2 partners have coevolved in the context of a mutualistic symbiosis, manipulating each other with chemical signals. To the best of our knowledge both partners derive benefits, natural selection having favored reciprocal changes in both.

The plant host nevertheless *regulates the infection* by means of feedback inhibition of nodulation. A mutant legume that fails to regulate infection has been found, and its roots are virtually taken over by the bacteria, to the detriment of the plant.

Plants make their own oxygen, so it was surprising when a hemoglobin was found in plants. The hemoglobin found in root nodules of legumes is called *leghemoglobin* (*leg* for legume); it is now believed that this protein supplies oxygen to the bacteria while keeping it away from the nitrogen-fixing machinery to which it is toxic (9). Like hemoglobin, leghemoglobin is red, and nodules are often pink when opened.

Rhizobia reproduce slowly for long periods in the soil, but if they encounter a compatible legume they begin to multiply rapidly; successful infection by a single bacterium can initiate formation of a nodule containing >10 million bacterial progeny.

The root nodule is like a lichen: it is a structure unique to the symbiosis, formed only when the potential partners encounter each other (10).

Rhizobia are the primary nitrogen fixers on land. In the oceans the job is done mainly by an extraordinary class of microorganisms that alone on earth perform both photosynthesis and nitrogen fixation: cyanobacteria. It is remarkable that a single organism can perform both functions, because photosynthesis generates oxygen, and nitrogen fixation must be carried out in a low-oxygen environment. Cyanobacteria have specialized, walled-off cells called heterocysts in which nitrogen fixation is carried out.

Cyanobacteria also fix nitrogen inside leaf cavities of the aquatic fern *Azolla*, which floats on the water in our cypress swamps (*Figure 9*) and has for centuries been added as "green manure" to rice paddies in Asia; fish are often added to these rice-*Azolla* systems and feed on the *Azolla*. *Azolla* is also added to animal feed as a protein supplement.

Occasional nitrogen-fixing symbioses occur between bacteria and *animals*. Wood-feeding termites provide a home for nitrogen-fixing bacteria in their anaerobic hindgut and benefit from the ammonia synthesis because the wood consumed by the termites is low in nitrogen.

For high agricultural yields, we enrich soil with still more nitrogen by adding nitrate in the form of fertilizer or feces. In the 1800s whole cliffs of guano were transported by sailing ships to New York from islands where seabirds nested. Early methods for synthesizing nitrate were motivated by its use in gunpowder. In 1909 the Haber-Bosch process, which harvests nitrogen right out of the air and converts it to ammonia, revolutionized the agricultural world; this process was nominated in a recent *Nature* millennium essay as the most important invention of the

20th century. (An unmixed blessing? It has been estimated that without the Haber-Bosch process almost two fifths of the world's population would not be here.)

Vaclav Smil writes:

> For several decades, virtually all the fixed nitrogen added to the fields of China, Egypt and Indonesia has come from synthetic fertilizers. When you travel in Hunan or Jiangsu, through the Nile Delta or the manicured landscapes of Java, remember that the children running around or leading docile water buffalo got their body proteins, via the urea their parents spread on the fields, from the Haber-Bosch synthesis of ammonia (11).

NEMATODE-BACTERIA SYMBIOSIS

Some tiny parasitic nematodes have evolved a remarkable skill: they farm bacteria for food, using the body (larva) of their parasitized insect host as the "soil" in which to cultivate the bacteria. The bacteria feed on the larva, killing it, and the nematodes feed on the bacteria. The bacteria are both symbionts and food and have never been found apart from their nematode hosts.

The larval body is preserved from invasion by other bacteria by antibacterial compounds produced by the nematodes, to which its own symbiotic bacteria are resistant.

When the nematodes reproduce, thousands of juveniles leave to locate new larval hosts, carrying some of the bacteria in their own gut.

The ability of these nematodes to kill insect larvae has not been overlooked by the global agricultural community (12, 13). Species from 2 genera of insect-parasitic nematodes, *Steinernema* and *Heterorhabditis*, are sprayed on crops around the world to control the larvae of plant-eating insects and are valued as natural biological pesticides.

LUMINESCENCE ORGANS

If you scuba dive in Hawaii at dusk, you might see some tiny Hawaiian bobtail squid, only 1½ inches long, which emerge to forage as the light fades. If you were beneath them looking up, you might lose sight of them because their undersides glow dimly with the deep blue light of millions of luminescent bacteria, housed in special light organs. In the downwelling light from above they are almost invisible to predators from below. The bacteria providing this *ventral bioluminescent countershading*, as it is called, glow with the colors of moonlight and starlight.

A single Hawaiian bobtail squid maintains about a billion cells of *Vibrio fischeri*, a gram-negative luminous bacterium related to *V. cholerae*, in the extracellular epithelial crypts of 2 light organs called photophores, which branch out over the underside of the squid. Shutters constructed of borrowed flaps of the ink sac modulate the amount of light emitted by the light organs.

Unlike our intestines, which shelter >400 species of bacteria, the light organs of the squid use only 1 species of bacterial symbiont, which also exists as part of the bacterioplankton. A baby squid hatches without the symbionts but acquires them within hours from the surrounding seawater. It accepts only 1 species, rejecting all others offered to it in controlled experiments. *V. fischeri* occurs as only about 0.001% of the bacteria in the water column, yet only this particular species colonizes the light organs. The juvenile squid has appendages covered with cilia that waft the bacteria into the light organs, where they multiply rap-

idly. Within hours, both the bacteria and the squid undergo specific developmental changes in response to the symbiosis: the bacteria begin to produce a high level of bioluminescence (greater, in fact, than the level that can be achieved by these cells in laboratory culture) and the light organs begin to differentiate, forming a lens and reflecting surface. After 4 days the collecting structures undergo apoptosis and disappear. This is a rare example of a specific body structure used only for acquisition of symbionts, which regresses after symbiosis is achieved (14).

Clearly the 2 organisms are *communicating* in some way and are inducing changes in each other's gene expression. This is reminiscent of the chemical cross-talk between nitrogen-fixing bacteria and their legume host, which provides the bacteria with an infection thread. Also like legumes, the squid controls overgrowth of its bacterial symbiont by elaborating an antimicrobial protein and by dumping some of the bacteria every morning.

The squid provides a nutrient-rich environment for this heterotrophic bacterium, and the host acquires *luminescence* rather than a nutritional benefit.

When foraging over sand in shallow water, where twilight or moonlight provides light for predators swimming above, bobtail squid carry a sand cover over the top of their bodies, mimicking the substrate.

The luminous *Vibrio* bacteria elaborate a substance which is believed to signal the squid to provide nutrients. The chemical is related to the toxin elaborated by their close relative *V. cholerae*. Does cholera toxin, so lethal in mammalian hosts, represent a bacterial signal gone wrong? Or was the gene co-opted to code for a toxin that spreads cholera bacteria by means of copious diarrhea, with host death a small price for the bacteria to pay?

Luminescent bacteria provide many other hosts with the gift of light (15). Examples include the following:
- Anglerfishes, deep-sea predators throughout the world ocean, attract prey in dimly lit waters with a lure; some lures are brightly lit by bacterial symbionts.
- Flashlight fishes, plankton feeders in shallow tropical waters, have large suborbital light organs called photophores. They use the light for several purposes: to see, to communicate, to lure prey, and to confuse predators. One species, *Photoblepharon*, forms a galaxy of glowing lights on shallow reefs in the Red Sea. The light organ of *Photoblepharon* has a fold of skin that can be raised to block light emission. A disturbed fish closes the light organ and darts off, sometimes using a blink-and-run tactic: light up on the zig, go dark on the zag.

GASTROINTESTINAL "FLORA"

The final example of symbiosis is in many ways the most remarkable—that between animals and the microorganisms that live in their gut. The terms "flora" and "microflora" are misnomers, as gut microorganisms are not plants; yet the terms are firmly established in biology and medicine.

The gastrointestinal tract is an *ecosystem* maintained by both host and colonizing symbiotic microorganisms. The latter, which enjoy a homeostatic environment supplied with regular food, provide useful services to their host in return: breakdown of cellulose cell walls of plant food (especially important for strict herbivores, but also important for omnivores like humans), synthesis of vitamins (especially vitamin K), development and main-
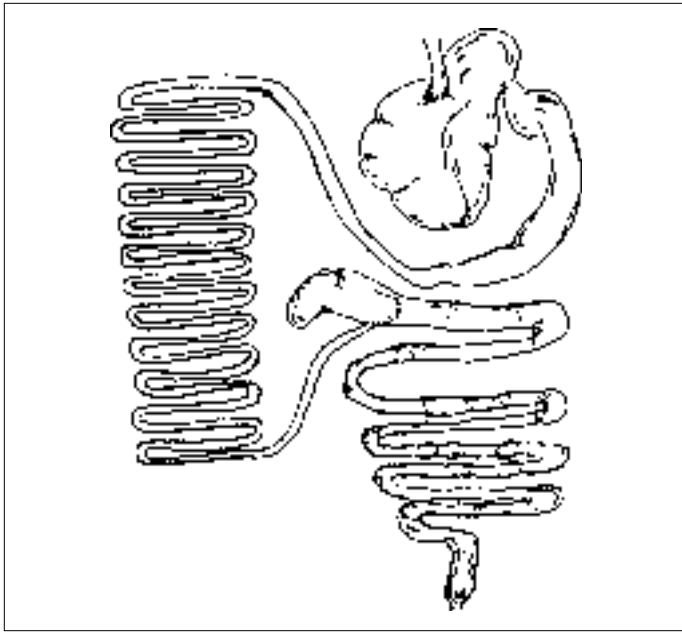
Figure 10. The gastrointestinal tract of a ruminant mammal, the sheep, is specialized for "foregut fermentation," with a complex stomach designed to extract the maximum yield from a diet of plant leaves. The multiple operations of the stomach slow down the passage of food through the gut, allowing extraction of nutrients from a relatively small amount of high-quality food but introducing a handicap if the food supply is low in nutrients.

Figure 11. The gastrointestinal tract of a nonruminant herbivore, the zebra, is characterized by "hindgut fermentation," with a large colon and cecum, both specialized to retain and process plant food. Nonruminants extract smaller yields than ruminants but have a higher "throughput," allowing them to survive on lower quality food by consuming more. Equids (horses and zebras) are bulk-feeding grazers that spend up to 18 hours a day feeding.
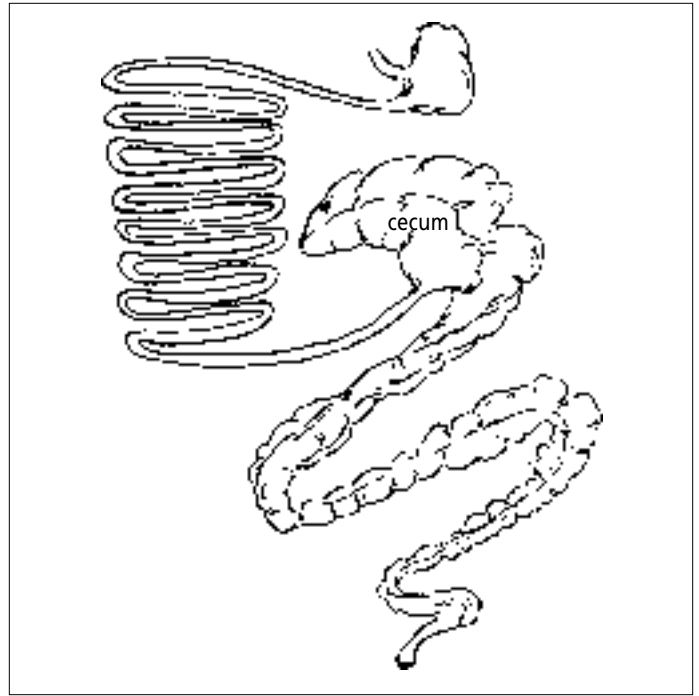
tenance of the gastrointestinal mucosa and mucosal immune system, and protection against enteric pathogens.

Approximately half of the contents of the human colon is microbial biomass. This ecosystem is so complex that it has been called an organ in itself, unique in that it comprises billions of diverse cells engaged in anaerobic fermentation. The cells are not our own but belong to hundreds of species of microorganisms, yet they have coevolved intimately with us and other animals in this unique habitat.

The gut flora of vertebrates and invertebrates extends across phyletic lines and represents *one of the most widespread and ancient of symbioses*. In the evolution of animals, these microbial symbionts enabled spectacular evolutionary radiations based on dietary specializations. Herbivores may specialize on grasses, leaves, or fruit; meat-eaters on flesh, small invertebrates, or eggs; omnivores on multiple food sources; and other specialists on plankton, tree sap, wood, fungi, microorganisms, even blood. This extraordinary diversity of food choices would not be possible without gut microorganisms.

The most abundantly distributed carbohydrate in the world is polysaccharide cellulose. Along with a few other compounds cellulose forms the primary component of plant cell walls. It is extremely insoluble and refractory to chemical attack. Some invertebrates possess endogenous cellulases, but even those that do usually require microbial activity as well. No vertebrates have yet been found with endogenous cellulases; this is a surprising fact, in light of the reliance of so many vertebrates upon plant material in their diet. The symbiotic gut flora have taken center stage in releasing the protein inside plant cells and breaking down cellulose to energy-rich carbohydrates and short-chain fatty acids.

The term *fermentation* refers to the biochemical pathway by which carbohydrates are broken down *anaerobically*, using en-

zymes. The principal products of carbohydrate fermentation are short-chain fatty acids (acetate, propionate, and butyrate), which are important in nutrition because they are a source of energy for skeletal and cardiac muscle and the brain. The gut microflora also synthesizes amino acids (from ammonia) and protein, which are utilized by both the microorganisms themselves and the host (16).

The 2 regions of the vertebrate gastrointestinal tract that have undergone the most specialization for microbial fermentation are the stomach and the large intestine (including the cecum). Stomach fermenters are called "foregut fermenters"; colon fermenters are called "hindgut fermenters."

Ruminants, which are "foregut fermenters," have a superior ability to convert cellulose into digestible carbohydrates and short-chain fatty acids and to free up the protein confined within cellulose cell walls (*Figure 10*). Other animals can also break down cellulose by virtue of their gut microflora, but ruminants are masters at extracting the maximum yield from a given amount of plant food. Much more fiber is left undigested by a nonruminant, as can be seen by comparing the coarse dung of a zebra, rhino, or elephant with the fine-grained dung of any ruminant (17).

The rumination process is mechanically and biochemically complex. The animal feeds until the rumen is full, then settles down to ruminate, either lying or standing, and chews the cud, which consists of the coarsest plant particles. Rhythmic contractions of the stomach stir the contents. Rumen microbes break down the cellulose, later becoming a major source of protein for the host as they pass through the digestive tract. Ruminants can also recycle urea, thereby retaining nitrogen which the bacteria use to synthesize the essential amino acids that nonruminants

Figure 12. The great herds of grazing herbivores, like these Burchell's zebras on Tanzania's Serengeti Plains, owe their ability to digest plant material to specializations of the gastrointestinal tract, which provide safe confinement of a veritable ecosystem of microbes. Zebras, which are nonruminants, are often the first grazing animals to move into tall, stringy grasses like these, which provide less protein but can be eaten in large quantities by herbivores with a high throughput. Ruminants such as wildebeests and Thomson's gazelles are more dependent on fresh green grass with a higher protein content.
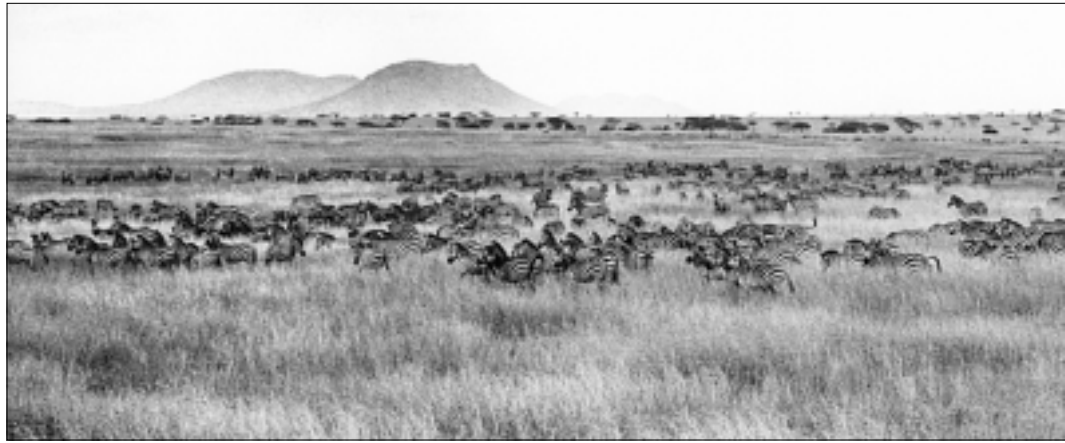
must acquire from their food. As an added bonus, recycling urea cuts down on urine excretion, conserving water (17).

The ruminant esophagus is designed for controlled regurgitation, as it is in other mammals and birds which regurgitate food to feed their young. Methane and carbon dioxide are produced continuously, and the esophagogastric junction allows regular eructation, without which the grazing ruminant would literally explode. A cow can produce as much as a liter of gas per minute; about half is methane, half carbon dioxide. (Because methane and carbon dioxide are greenhouse gases, eructation contributes to global warming.)

There is one drawback to rumination: if the protein content falls to below 6%, the slow passage of food through the ruminant gut precludes adequate nutrition. The greater "throughput" of nonruminants allows survival on low-protein food, simply by virtue of eating more. A nonruminant like a zebra, for example, may extract only two thirds as much protein from a given quantity of food as a wildebeest (a ruminant), but by processing twice as much in a given time, it will assimilate four thirds that of a wildebeest *(Figure 11)*.

The difference between ruminants and nonruminants can be seen on the Serengeti Plains under hardship conditions. Three mammal species make the annual migration between the Ngorongoro Crater highlands in Tanzania and the Masai Mara of Kenya: Burchell's zebras, which (like horses) are nonruminants, and wildebeests and Thomson's gazelles, which are ruminants. When food is abundant but of low quality, such as an expanse of tall, stringy grasses, zebras will typically be found grazing those grasses *(Figure 12)*, as they can process a large quantity in a short time. When food is scarce but of higher quality, such as short green sprouts after a fire or the first rains, wildebeests and gazelles are most efficient at obtaining a maximum of nutrients from those sources, as the rumination process allows recycling and thorough extraction. At times a "grazing succession" is seen, in which the zebras graze down the tall, dry grass and the ruminants follow afterward and feed on the exposed lower-story grasses.

Most herbivorous mammals are *nonruminants*, and their principal site of microbial fermentation is usually the hindgut instead of the stomach. Hippos are an exception, with a complex, compartmentalized stomach with diverticula. Hoatzins are leaf-eating birds with elaborate foregut fermentation (18) *(Figure 13a)*.

Many animals have a large cecum, which is a blind pouch off of the large intestine *(Figure 13b)*. Birds often have paired ceca, with a complex morphology adapted to different feeding ecologies (19). The cecum provides a diversion away from the rapid mainstream passage of digesta, where microorganisms can digest cellulose more completely and synthesize vitamins and proteins and where nutrients can be absorbed. The cecum may enable some animals to switch between insect and plant foods, exploiting unpredictable environments.

Most meat-eating mammals have an amazingly "simple" gut when compared with herbivores. The stomach is simple and the colon unspecialized. A long small intestine is adapted to the readily digested animal food and is the major site of absorption of animal proteins, carbohydrates, and fats. Insect-eaters also have a relatively simple and unspecialized gut. Even in carnivores such as foxes, bears, and raccoons, which include fruits and other plant material in their diet, the gut anatomy continues to reflect the easily digested animal food.
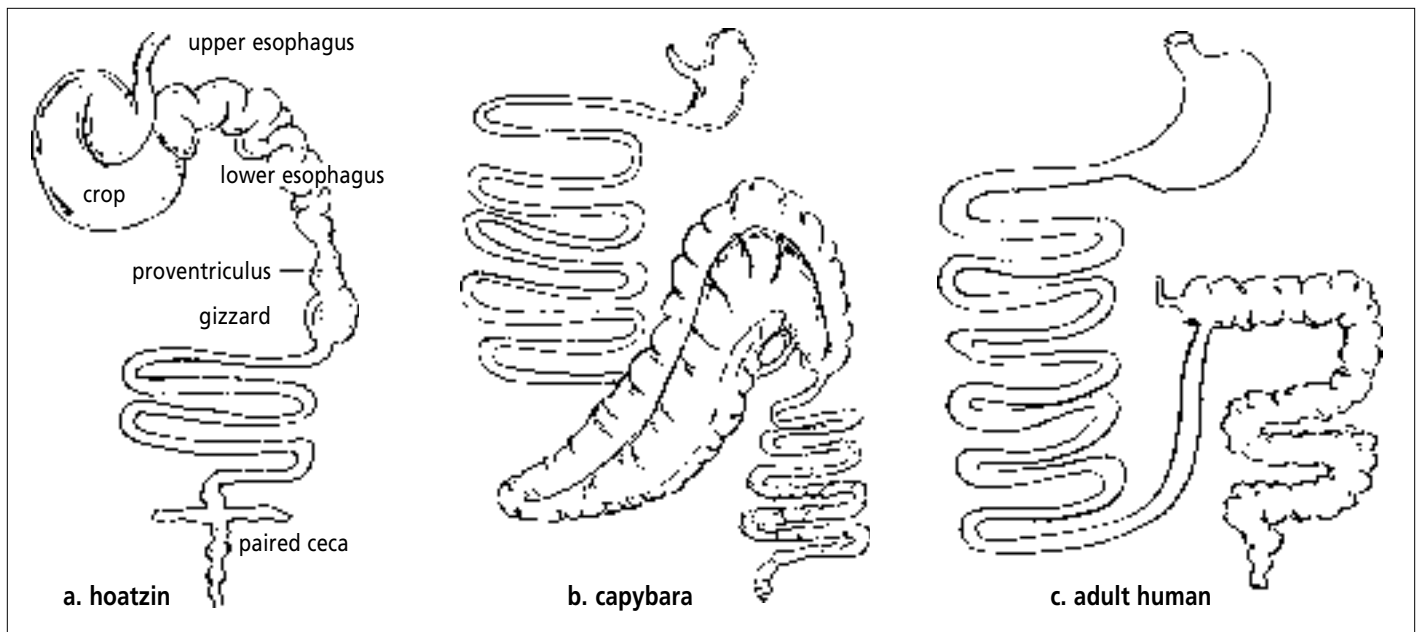
The human gastrointestinal tract *(Figure 13c)* is intermediate between that of herbivores and carnivores, with a simple stomach, unremarkable length of small intestine, small cecum, and a moderately large colon where fermentation of plant material takes place. Humans and apes are believed to have evolved from a common plant-eating ancestor; humans added animal protein to their diet, reaping nutritional advantages enjoyed by carnivores while retaining some features of digestive anatomy characteristic of herbivores (20, 21).

The small bowel normally contains few microorganisms. Jejunal cultures fail to identify any bacterial growth in about one third of healthy human volunteers. A dramatic change in the enteric flora occurs across the ileocecal valve, where the number of microorganisms explodes upward to 10^9 to 10^{12} organisms per gram of colonic contents, constituting up to 50% of fecal matter by weight. In intestinal stasis, the bacterial count rises even higher. Forty genera of bacteria, represented by at least 400 species, can be cultured from the feces of a healthy human.

An ecosystem with >400 species of bacteria, plus protists and fungi, constitutes a jungle of competitive species and individuals. Some species are mutualists, others commensals, still others potential pathogens. The species composition of this jungle changes remarkably little over the life of a healthy host.

The mouth is part of the gastrointestinal tract, and its microbial diversity is no less extraordinary. Many species are thought to be commensals, and a small number are opportunistic pathogens. Dental caries, gingivitis, and periodontitis are

Figure 13. (a) The hoatzin gastrointestinal tract uses the enlarged muscular crop and lower esophagus as fermentation organs. The greatly sacculated lower esophagus delays passage of food to the stomach (proventriculus and gizzard). Some additional fermentation occurs in the paired ceca. Among birds the hoatzin is one of the world's few obligate folivores and one of the smallest endotherms with this digestive strategy (19). **(b)** The gastrointestinal tract of a large rodent, the capybara, with a very large cecum, the major site of microbial fermentation. **(c)** The adult human gastrointestinal tract is intermediate between that of carnivores and herbivores. Microbial fermentation occurs in the colon but is less extensive than that of a strict herbivore. A small cecum and vestigial appendix extend from the proximal end of the colon.

thought to be caused in part by opportunistic bacteria. Nearly 500 bacterial strains have been recovered from just beneath the gums, a well-studied microbial niche (22).

At birth the human alimentary canal is sterile, like that of animals raised in a germ-free environment. Enteric bacteria colonize the newborn infant in an oral-to-anal direction. About 3 or 4 weeks after birth the flora characteristic for the individual host is fairly well established and, except under unusual circumstances, does not change significantly thereafter.

Studies with germ-free newborn animals show that the indigenous bacterial flora is essential for the completion of intestinal epithelial cell differentiation and the maturation of the mucosal immune system.

The normal gastrointestinal flora can be disturbed by illness, ingestion of pathogens, and antibiotic administration. Diarrheal diseases and colitis may be accompanied by an overgrowth of species such as *Clostridium difficile* or *Escherichia coli*, present in

smaller numbers in the normal flora. Overgrowth of some species may also result in malabsorption syndromes. Paradoxically, overgrowth of some components of the colonic flora may result in competitive uptake of vitamin B_{12} by bacteria, interfering with absorption of the vitamin by the intestinal mucosa (23).

An exceptional gut symbiont, present in at least half the human population, may be friend or foe: *Helicobacter pylori*. This bacterium lives for decades in the extreme environment of the stomach and is responsible for one of the most widespread chronic bacterial infections known in humans (24). *H. pylori* infection is a serious, chronic, transmissible infectious disease in which clinical illness follows a long asymptomatic interval, usually of many years. Infection typically begins in childhood and illness appears in adulthood, often late in life. *H. pylori* is believed to play a causative role in most duodenal and gastric ulcers and is associated with chronic gastritis and an increased risk of gastric adenocarcinoma. Yet it elaborates an antibiotic which may

help control colonization of the gut by foreign microbes. The great genetic variability of *H. pylori*, with its tendency to exchange genetic information among strains, makes it difficult for us to assign it a place on the continuum; there is probably considerable *host* variability as well.

Is the normal intestinal flora ever highly pathogenic? In the case of colon perforation, as from a penetrating abdominal wound, the answer is clearly yes. A balanced mix of microbial species then responds to a new environment, some multiplying as high pathogens at the opposite end of the spectrum.

CONCLUSION

Symbioses are everywhere we look in nature, usually between organisms far removed from each other phylogenetically. Unlike adaptation to the nonliving environment, adaptation to another species can produce reciprocal evolutionary responses that either thwart or reinforce the adaptation. Interactions move dynamically along the continuum in either direction, toward or away from mutualism.

Some symbioses are more intimate than others. Some are intracellular, some are extracellular, and still others occur between separate partners. Some symbioses are facultative, others obligate, and this characteristic may change through the life cycle of the host.

Our tentative assignment of a relationship to a place on the continuum is subject to regular change as we learn more. Often the interactions are too poorly known or too complex to allow a precise assignment. Mutualisms, for example, may not be perfectly "egalitarian"; a fluctuating asymmetry in the provision of benefits is probably typical. As new symbioses are discovered, an understanding of one partner requires an understanding of the other and of their joint evolutionary history.

An "Economics 101" model for mutualisms has been proposed: if a species is especially good at acquiring a resource, it may pay to specialize in that resource and trade for a second. Two unrelated species may thus collaborate in a partnership that provides the best of both worlds.

The biologist Daniel Janzen has asked: Are mutualisms delicately balanced antagonisms? Do they represent "reciprocal parasitisms," that is, mutually exploitative interactions with underlying conflicts of interest? If genes are "selfish," are partners of a mutualism also fundamentally selfish? We can use any terms we choose, as long as we understand that natural selection is moving genes into the future differentially, just as with nonsymbiotic organisms. Most terms we use will probably have an anthropomorphic slant.

Functions ("services") provided by one partner, discussed in this review, include the following:
- Photosynthesis
- Chemosynthesis
- Nitrogen fixation
- Luminescence
- Nutrition (gut flora—cellulose breakdown, vitamins, maintenance of intestinal ecosystem)

A future review will expand the coverage of this article to include mitochondria, chloroplasts, microbial pathogens symbi-

otic with humans, and partnerships between entirely separate organisms, such as ants and plants, ants and fungi, one ant species and another, and plants and their pollinators.

John Donne wrote, "No man is an island." It appears that most other organisms aren't either but instead weave intimate and complex tapestries with other life forms.

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