Chapter 15

Mutualism

Outline

*P*ositive interactions between species are found throughout the biosphere. A hummingbird darts among the red blossoms of a plant growing at the edge of a forest glade. As it inserts its bill into a flower, hovering to sip nectar, the hummingbird head brushes up against the anthers of the flower and picks up pollen (fig. 15.1). This pollen will be deposited on the stigmas of other flowers as the hummingbird goes about gathering its meal of nectar. The hummingbird disperses the plant's pollen in trade for a meal of nectar.

Belowground we encounter another partnership. The roots of the hummingbird-pollinated plant are intimately connected with fungi in an association called *mycorrhizae.* The hyphae of the mycorrhizal fungi extend out from the roots, increasing the capacity of the plant to harvest nutrients from the environment. In exchange for the nutrients, the plant delivers sugars and other products of photosynthesis to its fungal partner.

Meanwhile, back aboveground a deer enters the forest glade and wanders over to the plant recently visited by the hummingbird. The deer systematically grazes it to the ground, lightly chews the plant material, and then swallows it. As the plant material enters the deer's stomach, it is attacked by a variety of protozoans and bacteria. These microorganisms break down and release energy from compounds such as cellulose, which the deer's own enzymatic machinery cannot handle. In return, the protozoans and bacteria receive a steady food supply from the feeding activities of the deer as well as a warm, moist place in which to live.

These are examples of **mutualism,** that is, interactions between individuals of different species that benefit both partners. Some species can live without their mutualistic partners and so the relationship is called **facultative mutualism.** Other species are so dependent upon the mutualistic relationship that they cannot live in its absence. Such a relationship is an **obligate mutualism.** It is a curious fact that though observers of nature as early as Aristotle recognized such mutualisms, mutualistic interactions have received much less attention from ecologists than have either competition or exploitation. Does this lack of attention reflect the rarity of mutualism in nature? As you will see in chapter 15, mutualism is virtually everywhere.

Mutualism may be common, but is it important? Does it contribute substantially to the ecological integrity of the biosphere? The answer to both these questions is yes. Without mutualism the biosphere would be entirely different. Let's remove some of the more prominent mutualisms from the biosphere and consider the consequences. An earth without mutualism would lack reef-building corals as we know them. So we can erase the Great Barrier Reef, the largest biological structure on earth, from our hypothetical world. We can also eliminate all the coral atolls that dot the tropical oceans as well as all the fringing reefs. The deep oceans would have no bioluminescent fishes or invertebrates. In addition, the deep-sea oases of life associated with ocean floor hydrothermal vents (see chapter 6) would be reduced to nonmutualistic microbial species.

Figure 15.1 Hummingbirds feeding on nectar transfer pollen from flower to flower.

On land, there would be no animal-pollinated plants: no orchids, no sunflowers, and no apples. The pollinators themselves would also be gone: no bumblebees, no hummingbirds, and no monarch butterflies. Gone too would be all the herbivores that depend on animal-pollinated plants. Without plant-animal mutualisms, tropical rain forests, the most diverse terrestrial biome on the planet, would be all but gone. Many wind-pollinated plants would remain. However, many of these species would also be significantly affected since approximately 90% of all plants form mycorrhizae. Those plants capable of surviving without mycorrhizal fungi would likely be restricted to the most fertile soils.

Even if wind-pollinated, nonmycorrhizal plants remained on our hypothetical world there would be no vast herds of African hoofed mammals, no horses, and no elephants, camels, or even rabbits or caterpillars. There would be few herbivores to feed on the remaining plants since herbivores and detritivores depend upon microorganisms to gain access to the energy and nutrients contained in plant tissues. The carnivores would disappear along with the herbivores. And so it would go. A biosphere without mutualism would be biologically impoverished.

The impoverishment that would follow the elimination of mutualism, however, would go deeper than we might expect. Lynn Margulis and René Fester (1991) have amassed convincing evidence that all eukaryotes, both heterotrophic and autotrophic, originated as mutualistic associations between different organisms. Eukaryotes are apparently the product of mutualistic relationships so ancient that the mutualistic partners have become cellular organelles (e.g., mitochondria and chloroplasts) whose mutualistic origins long went unrecognized. Consequently, without mutualism all the eukaryotes, from *Homo sapiens* to the protozoans, would be gone and the history of life on earth and biological richness would be set back about 1.4 billion years.

But back here in the present, let's accept that mutualism is an integral part of nature and review what is known of the ecology of mutualism. The first part of this brief review emphasizes experimental studies. Then, in the last part of chapter 15, we examine some theoretical approaches to the study of mutualism.

Concepts

15.1 Plants benefit from mutualistic partnerships with a wide variety of bacteria, fungi, and animals.

15.2 Reef-building corals depend upon mutualistic relationships with algae and animals.

15.3 Theory predicts that mutualism will evolve where the benefits of mutualism exceed the costs.

15.1 Plant Mutualisms

Plants benefit from mutualistic partnerships with a wide variety of bacteria, fungi, and animals. Plants are the center of mutualistic relationships that provide benefits ranging from nitrogen fixation and nutrient absorption to pollination and seed dispersal. It is no exaggeration to say that the integrity of the terrestrial portion of the biosphere depends upon plant-centered mutualism. However, to understand the extent to which ecological integrity may depend upon these relationships we need careful observational studies and experiments. Here are some drawn from studies of mycorrhizae.

Plant Performance and Mycorrhizal Fungi

The fossil record shows that mycorrhizae arose early in the evolution of land plants, perhaps as long as 400 million years ago. Over evolutionary time, a mutualistic relationship between plants and fungi evolved in which mycorrhizal fungi provide plants with greater access to inorganic nutrients while feeding off the root exudates of plants. The two most common types of mycorrhizae are (1) **arbuscular mycorrhizal fungi (AMF),** in which the mycorrhizal fungus produces **arbuscules,** sites of exchange between plant and fungus, **hyphae,** fungal filaments, and **vesicles,** fungal energy storage organs within root cortex cells, and (2) **ectomycorrhizae (ECM),** in which the fungus forms a mantle around roots and a netlike structure around root cells (fig. 15.2). Mycorrhizae are especially important in increasing plant access to phosphorus and other immobile nutrients (nutrients that do not move freely through soil) such as copper and zinc, as well as to nitrogen and water.

Mycorrhizae and the Water Balance of Plants

Mycorrhizal fungi appear to improve the ability of many plants to extract soil water. Edie Allen and Michael Allen
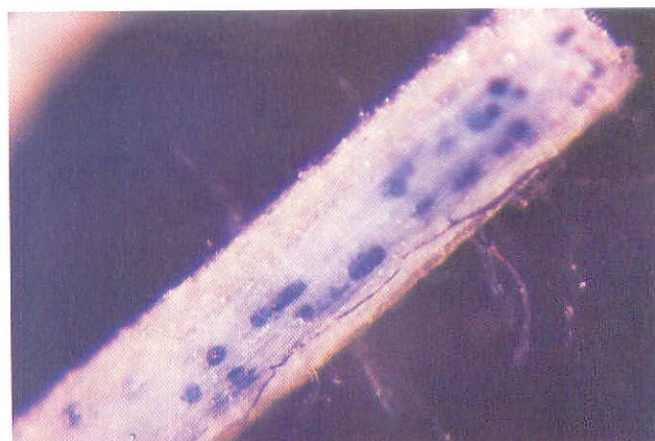
(a)

(b)

Figure 15.2 Mutualistic associations between fungi and plant roots: (*a*) arbuscular mycorrhizal fungus stained so that fungal structures appear blue; and (*b*) ectomycorrhizae, which give a white fuzzy appearance to these roots.

(1986) studied how mycorrhizae affect the water relations of the grass *Agropyron smithii* by comparing the leaf water potentials of plants with and without mycorrhizae. Figure 15.3 shows that *Agropyron* with mycorrhizae maintained higher leaf water potentials than those without mycorrhizae. This means that when growing under similar conditions of soil moisture, the presence of mycorrhizae helped the grass maintain a higher water potential. Does this comparison show that mycorrhizae are directly responsible for the higher leaf water potential observed in the mycorrhizal *grass?* No, they do not. These higher water potentials may be an indirect effect of greater root growth resulting from the greater access to phosphorus provided by mycorrhizae.

Plants with greater access to phosphorus may develop roots that are more efficient at extracting and conducting water; mycorrhizal fungi may not be directly involved in the extraction of water from soils. Kay Hardie (1985) tested this hypothesis directly with an ingenious experimental manipulation of plant growth form and mycorrhizae. First, she grew mycorrhizal and nonmycorrhizal red clover, *Trifolium*
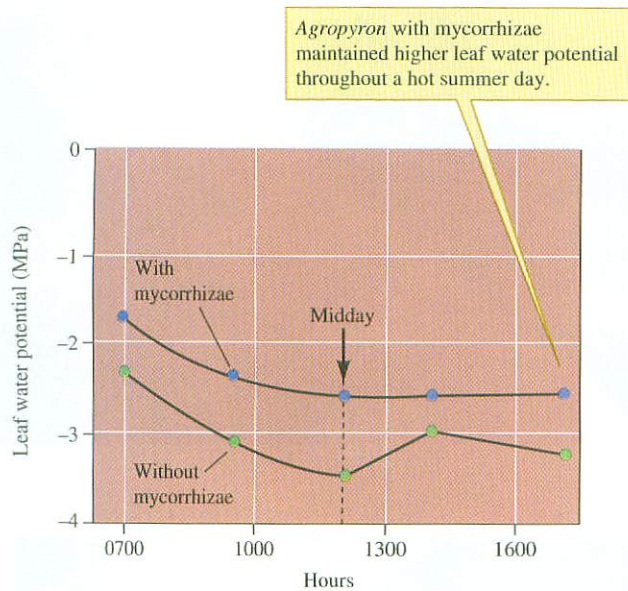
Figure 15.3 Influence of mycorrhizae on leaf water potential of the grass *Agropyron smithii* (data from Allen and Allen 1986).

Figure 15.4 Effect of removing mycorrhizal hyphae on rate of transpiration by red clover (data from Hardie 1985).

pratense, in conditions in which their growth was not limited by nutrient availability. These conditions produced plants with similar leaf areas and root:shoot ratios. Under these carefully controlled conditions, mycorrhizal red clover showed higher rates of transpiration than nonmycorrhizal plants.

Hardie took her study one step further by removing the hyphae of mycorrhizal fungi from half of the red clover with mycorrhizae. She controlled for possible side effects of this manipulation by using a tracer dye to check for root damage and by handling and transplanting all study plants, including those in her control group. Removing hyphae significantly reduced rates of transpiration (fig. 15.4), indicating a direct role of mycorrhizal fungi in the water relations of plants. Hardie suggests that mycorrhizal fungi improve water relations of plants by giving more extensive contact with moisture in the rooting zone and provide extra surface area for absorption of water.

So far, it seems that plants always benefit from mycorrhizae. That may not always be the case. Environmental conditions may change the flow of benefits between plants and mycorrhizal fungi.

Nutrient Availability and the Mutualistic Balance Sheet

Mycorrhizae supply inorganic nutrients to plants in exchange for carbohydrates, but not all mycorrhizal fungi deliver nutrients to their host plants at equal rates. The relationship between fungus and plant ranges from mutualism to parasitism, depending on the environmental circumstance and mycorrhizal species or even strains within species.

Nancy Johnson (1993) performed experiments designed to determine whether fertilization can select for less mutualistic mycorrhizal fungi. Before discussing her experiments, we have to ask what would constitute a "less mutualistic"
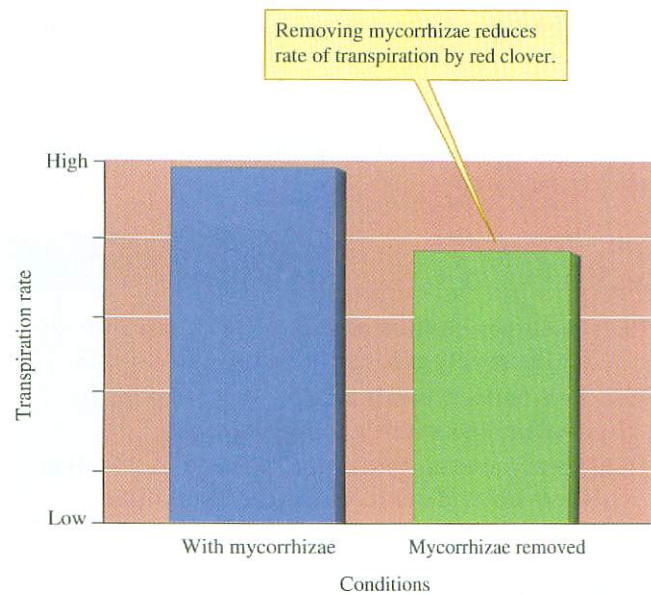
association. In general, a less mutualistic relationship would be one in which there was a greater imbalance in the benefits to the mutualistic partners. In the case of mycorrhizae, a less mutualistic mycorrhizal fungus would be one in which the fungal partner received an equal or greater quantity of photosynthetic product in trade for delivering a lower quantity of nutrients.

Johnson pointed out that there are several reasons to predict that fertilization would favor less mutualistic mycorrhizal fungi. The first is that plants vary the amount of soluble carbohydrates in root exudates as a function of nutrient availability. Plants release more soluble carbohydrates in root exudates when they grow in nutrient-poor soils and decrease the amount of carbohydrates in root exudates as soil fertility increases. Consequently, fertilization of soils should favor strains, or species, of mycorrhizal fungi capable of living in a low-carbohydrate environment. Johnson suggested that the mycorrhizal fungi capable of colonizing plants releasing low quantities of carbohydrates will probably be those that are aggressive in their acquisition of carbohydrates from their host plants, perhaps at the expense of host plant performance. She addressed this possibility using a mixture of field observations and greenhouse experiments.

In the first phase of her project, Johnson examined the influence of inorganic fertilizers on the kinds of mycorrhizal fungi found in soils. She collected soils from 12 experimental plots in a field on the Cedar Creek Natural History Area in central Minnesota that had been abandoned from agriculture for 22 years. Six of the study plots had been fertilized with inorganic fertilizers for 8 years prior to Johnson's experiment, while the other six had received no fertilizer over the same period.

Johnson's samples of mycorrhizal fungi from fertilized and unfertilized soils showed that the composition of mycorrhizal fungi differed substantially. Of the 12 mycorrhizal

species occurring in the samples, unfertilized soil supported higher densities of three mycorrhizal fungi, *Gigaspora gigantea*, *G. margarita*, and *Scutellispora calospora*, while fertilized soil supported higher densities of one species, *Glomus intraradix*. Spores of *G. intraradix* accounted for over 46% of the spores recovered from fertilized soils but only 27% of the spores from unfertilized soils.

Johnson used greenhouse experiments to assess how these differences in the composition of mycorrhizal fungi might affect plant performance. She chose big bluestem grass, *Andropogon gerardii*, as a study plant for these experiments because it is native to the Cedar Creek Natural History Area and is well adapted to the nutrient-poor soils of the area. Seedlings of *Andropogon* were planted in pots containing 980 g of a 1:1 mixture of sterilized subsurface sand from the Cedar Creek Natural History Area and river-washed sand. Johnson added a composite sample of other soil microbes living in the soils of fertilized and unfertilized study plots. She prepared the composite by washing a composite soil sample from all fertilized and unfertilized study plots with deionized water and passing this water through a 25 μm screen.

To each pot Johnson added a mycorrhizal "inoculum" of 30 g of soil of one of three types: (1) a fertilized inoculum consisting of 15 g of soil from fertilized study plots mixed with 15 g of sterilized unfertilized soil, (2) an unfertilized inoculum consisting of 15 g of soil from unfertilized study plots mixed with 15 g of sterilized fertilized soil, or (3) a non-mycorrhizal inoculum consisting of 30 g of a sterilized composite from the soils of fertilized and unfertilized study plots. The first two inocula acted as a source of mycorrhizal fungi for colonization of *Andropogon*. The design of Johnson's experiment is summarized in figure 15.5.

Pots were next assigned to one of four nutrient treatments in which Johnson (1) added no supplemental nutrients (None), (2) added phosphorus only (+P), (3) added nitrogen only (+N), or (4) added both nitrogen and phosphorus (+N+P). The subsurface sand from the Cedar Creek Natural History Area contained a fairly low concentration of nitrogen but considerably higher concentrations of phosphorus. Nutrient additions were adjusted so that the supplemented treatments offered nitrogen and phosphorus concentrations comparable to those of the topsoil in the fertilized study plots.

Johnson harvested five replicates of each of the treatments at two points in time: at 4 weeks, when *Andropogon* was actively growing, and at 12.5 weeks, when the grass was fully grown. At each harvest she measured several aspects of plant performance: plant height, shoot mass, and root mass; and at 12.5 weeks she also recorded the number of inflorescences per plant.

At 12.5 weeks shoot mass was significantly influenced by nutrient supplements and by whether or not plants were mycorrhizal but not by the source of the mycorrhizal inoculum (fig. 15.6). Shoot mass was greatest in the double nutrient supplement treatment (+N+P), somewhat lower in the nitrogen supplement (+N), and very low in the other two treatments (None and +P). Figure 15.6a also indicates a definite influence of mycorrhizae on performance. Shoot mass

Question: Does fertilizing soil select for less mutualistic mycorrhizal fungi?

Experimental Design

Two sources of mycorrhizal fungi
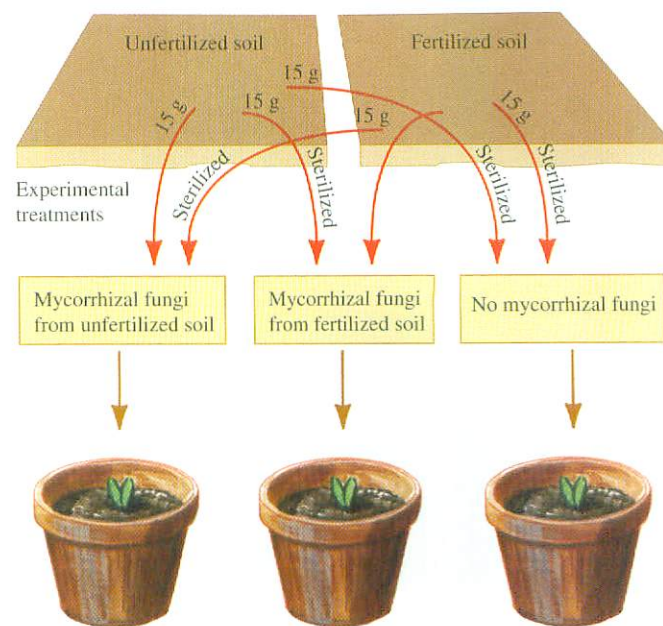
Compare: Growth, root:shoot ratios, and number of inflorescences produced by three treatments.

Figure 15.5 Testing the effects of long-term fertilizing on interactions between mycorrhizal fungi and plants on agricultural lands.

was significantly greater for mycorrhizal plants across all nutrient treatments.

Nutrient supplements and mycorrhizae also significantly influenced root:shoot ratios (fig. 15.6b). As we saw in chapter 6, plants invest differentially in roots and shoots depending on nutrient and light availability. It also appears that variation in investment is aimed at increasing supplies of resources in short supply. For instance, in nutrient-poor environments many plants invest disproportionately in roots and consequently have high root:shoot ratios, which decline with increasing nutrient availability. The results of Johnson's experiments are consistent with this generalization. Root:shoot ratios were highest in the treatments without nitrogen supplements (None and +P) and lowest in the treatments with nitrogen supplements (+N and +N+P). In other words, higher plant investment in roots in the low-nitrogen treatments suggests greater nutrient limitation than in the high-nitrogen treatments.

Now let's look a bit deeper into Johnson's results, where we find evidence for increased nutrient availability to mycorrhizal plants. In both the +N and "None" treatments, root:shoot ratios were significantly lower in plants with mycorrhizae (fig. 15.6b). Mycorrhizal plants in these treatments invest less in roots, suggesting that they have greater access to nutrients. Here we also see a hint that the source of
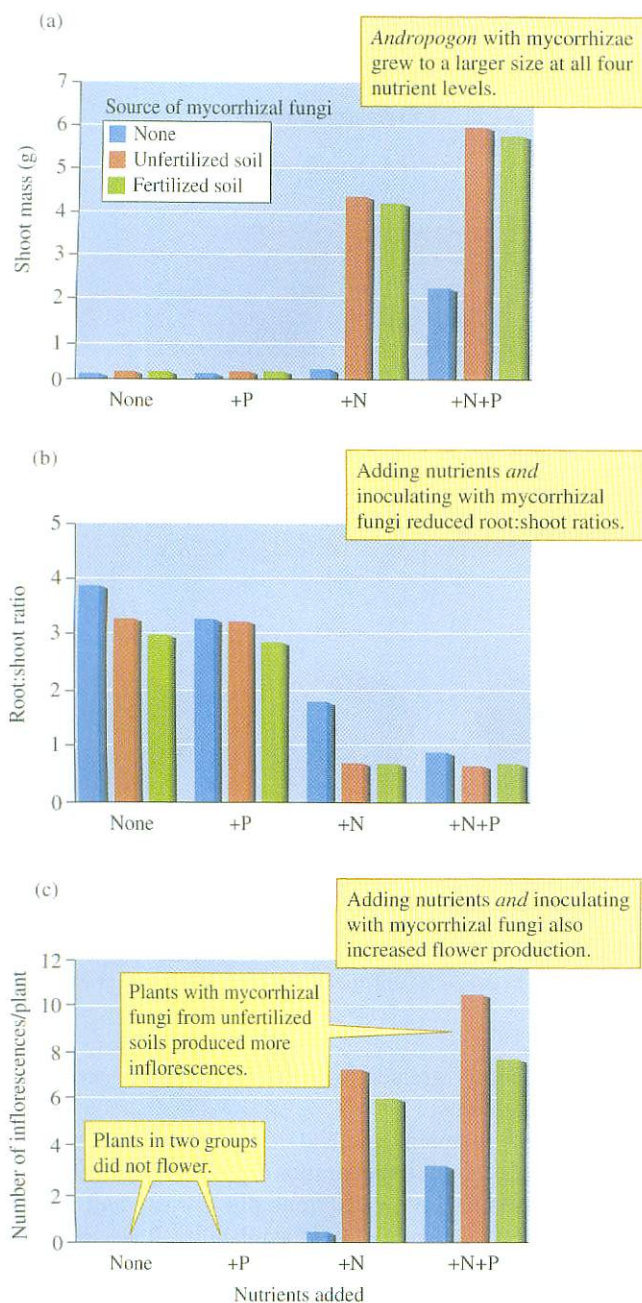
(a)

> *Andropogon* with mycorrhizae grew to a larger size at all four nutrient levels.

Source of mycorrhizal fungi
- None
- Unfertilized soil
- Fertilized soil

(b)

> Adding nutrients *and* inoculating with mycorrhizal fungi reduced root:shoot ratios.

(c)

> Adding nutrients *and* inoculating with mycorrhizal fungi also increased flower production.

> Plants with mycorrhizal fungi from unfertilized soils produced more inflorescences.

> Plants in two groups did not flower.

Nutrients added

Figure 15.6 Effect of nutrient additions and mycorrhizae on the grass *Andropogon gerardii* (data from Johnson 1993).

the inoculum significantly influenced plant performance. Plants in the +N+P treatment that were inoculated with soils from the unfertilized plots had slightly lower root:shoot ratios than those inoculated with soil from fertilized plots. These lower root:shoot ratios suggest that the mycorrhizal fungi from unfertilized soils were supplying their plant partners with more nutrients, freeing the plants to invest more of their energy budget in aboveground photosynthetic tissue.

Inflorescence production provides the strongest evidence for an effect of inoculum source on plant performance (fig. 15.6c). *Andropogon* produced inflorescences only in treatments with nitrogen supplements (+N and +N+P).

Within these treatments, the mycorrhizal plants produced the greatest number of inflorescences. In addition, *Andropogon* inoculated with mycorrhizal fungi from the unfertilized plots and grown in the +N+P treatment produced the greatest number of inflorescences of all.

In summary, Johnson's study produced two pieces of evidence that bear on the question posed at the outset of her study: Can fertilization of soil select for less mutualistic mycorrhizal fungi? First, in the early stages of her experiment, *Andropogon* inoculated with fertilized soil had lower shoot mass than those inoculated with unfertilized soil. Second, *Andropogon* inoculated with unfertilized soils produced more inflorescences than did *Andropogon* inoculated with fertilized soils. In other words, *Andropogon* inoculated with mycorrhizal fungi from unfertilized soils showed faster shoot growth as young plants and reproduced at a higher rate when mature. These results suggest that plants receive more benefit from association with the mycorrhizal fungi from unfertilized soils. Johnson's simultaneous studies of the mycorrhizal fungi indicate the mechanisms producing these patterns. It appears that altering the nutrient environment does alter the mutualistic balance sheet, an influence of potential importance to agricultural practice.

Plants engage in a wide variety of mutualisms with many other organisms. One of those involves associations that provide protection from exploiters and competitors. Writing about the natural history of mutualism, Daniel Janzen (1985) included "plant-ant protection mutualisms" as one of his general categories of mutualism. Janzen (1966, 1967a, 1967b) himself is responsible for studying one of the best known of these mutualisms, the obligate mutualism between ants and swollen thorn acacias in Central America.

Ants and Swollen Thorn Acacias

The ants mutualistic with bullhorn, or swollen thorn, acacias are members of the genus *Pseudomyrmex* in the subfamily Pseudomyrmecinae. This subfamily of ants is dominated by genera and species that have evolved close relationships with living plants. *Pseudomyrmex* spp. are generally associated with trees and show several characteristics that Janzen suggested are associated with arboreal living. They are generally fast and agile runners, have good vision, and forage independently. To this list, the *Pseudomyrmex* spp. associated with swollen thorn acacias, or "acacia-ants," add aggressive behavior toward vegetation and animals contacting their home tree, larger colony size, and 24-hour activity outside of the nest. This combination of characteristics means that any herbivore attempting to forage on an acacia occupied by acacia-ants is met by a large number of fast, agile, and highly aggressive defenders and is given this reception no matter what time of the day or night it attempts to feed. Janzen listed six species of *Pseudomyrmex* with an obligate mutualistic relationship with swollen thorn acacias and refers to three additional undescribed species. His experimental work focused principally on one species, *Pseudomyrmex ferruginea*.

Worldwide, the genus *Acacia* includes over 700 species. Distributed throughout the tropical and subtropical regions around the world, acacias are particularly common in drier tropical and subtropical environments. The swollen thorn acacias, which form obligate mutualisms with *Pseudomyrmex* spp., are restricted to the New World, where they are distributed from southern Mexico, through Central America, and into Venezuela and Columbia in northern South America. Across this region, swollen thorn acacias occur mainly in the lowlands up to 1,500 m elevation in areas with a dry season of 1 to 6 months. Swollen thorn acacias show several characteristics related to their obligate association with ants, including enlarged thorns with a soft, easily excavated pith; year-round leaf production; enlarged foliar nectaries; and leaflet tips modified into concentrated food sources called Beltian bodies. The thorns provide living space, while the foliar nectaries provide a source of sugar and liquid. Beltian bodies are a source of oils and protein. Resident ants vigorously guard these resources against encroachment by nearly all encroachers, including other plants.

Janzen's detailed natural history of the interaction between bullhorn acacia, *Acacia cornigera,* and ants paints a rich picture of mutual benefits to both partners (fig. 15.7). Newly mated *Pseudomyrmex* queens fly and run through the vegetation searching for unoccupied seedlings or shoots of bullhorn acacia. When a queen finds an unoccupied acacia, she excavates an entrance in one of the green thorns or uses one carved previously by another ant. The queen then lays her first eggs in the thorn and begins to forage on her newly acquired home plant. She gets nectar for herself and her developing larvae from the foliar nectaries and gets additional solid food from the Beltian bodies. As time passes, the number of workers in the new colony increases, and while they take up all the chores of the colony, the queen shifts to a mainly reproductive function; her abdomen enlarges and she becomes increasingly sedentary.

In exchange for food and shelter, ants protect acacias from attack by herbivores and competition from other plants. Workers have several duties, including foraging for

Figure 15.7   Split thorn of a bullhorn acacia, revealing a nest of its ant mutualists.

themselves, the larvae, and the queen. One of their most important activities is protecting the home plant. Workers will attack, bite, and sting nearly all insects they encounter on their home plant or any large herbivores such as deer and cattle that attempt to feed on the plant. They will also attack and kill any vegetation encroaching on the home tree. Workers sting and bite the branches of other plants that come in contact with their home tree or that grow near its base. These activities keep other plants from growing near the base of the home tree and prevent other trees, shrubs, and vines from shading it. Consequently the home plant's access to light and soil nutrients is increased.

Once a colony has at least 50 to 150 workers, which takes about 9 months, they patrol the home plant day and night. About one-fourth of the total colony is active at all times. Eventually colonies grow so large that they occupy all the thorns on the home tree and may even spread to neighboring acacias. The queen, however, generally remains on the shoot that she colonized originally. When the colony reaches a size of about 1,200 workers, it begins producing a more or less steady stream of winged reproductive males and females, which fly off to mate. The queens among them may eventually establish new colonies on other bullhorn acacias or one of the other Central American swollen thorn acacias. Colonies may eventually reach a total population of 30,000 workers.

Experimental Evidence for Mutualism

While much of the natural history of this mutualism was known at the time Janzen conducted his studies, no one had experimentally tested the strength of its widely supposed benefits. Janzen took his work beyond natural history to experimentally test for the importance of ants to bullhorn acacias. It was clear that the ant needs swollen thorn acacias, but do the acacias need the ants? Janzen's experiments concentrated on the influence of ants on acacia performance. He also tested the effectiveness of the ants at keeping acacias free of herbivorous insects. Janzen removed ants from acacias by clipping occupied thorns or by cutting out entire shoots with their ants. He then measured the growth rate, leaf production, mortality, and insect population density on acacias with and without ants.

Janzen's experiments demonstrated that ants significantly improve plant performance. Suckers growing from stumps of acacias occupied by ants lengthened at seven times the rate of suckers without ants (fig. 15.8). Suckers with ants were also more than 13 times heavier than suckers without ants and had more than twice the number of leaves and almost three times the number of thorns. Suckers with ants also survived at twice the rate of suckers without ants (fig. 15.9).

What produces the improved performance of acacias with ants? Differences in plant performance were likely the result of increased competition with other plants and increased attack by herbivorous insects faced by acacias without their tending ants. Janzen found that acacias without ants had more herbivorous insects on them than did acacias

Acacia shoots inhabited by ants grew much faster.
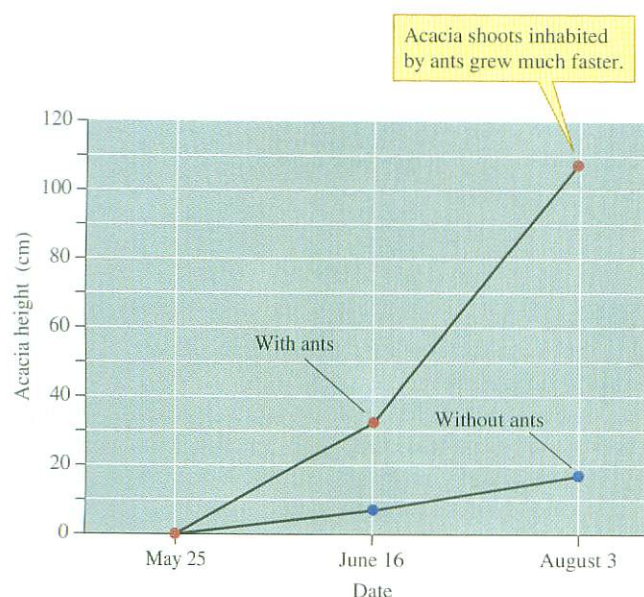
Figure 15.8 Growth by bullhorn acacia with and without resident ants (data from Janzen 1966).

Acacia shoots without ants have much larger numbers of herbivorous insects.

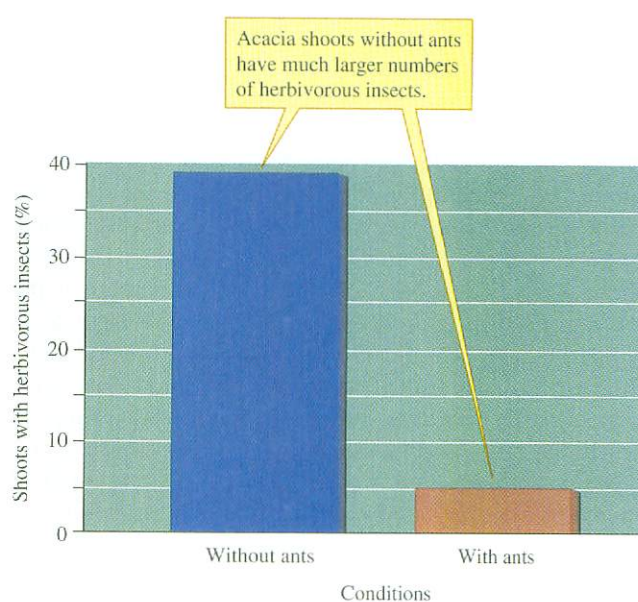**Figure 15.10** Ants and the abundance of herbivorous insects on bullhorn acacia (data from Janzen 1966).

Survival of acacia suckers was much higher where they were occupied by ants.
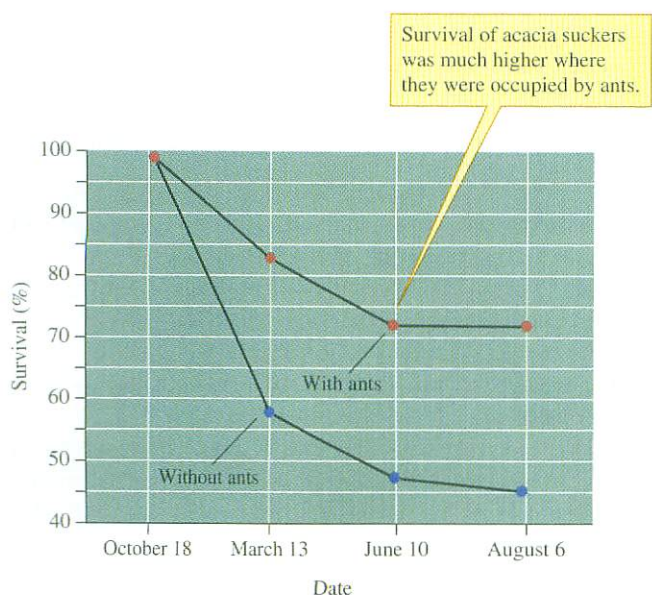

Figure 15.9 Survival of bullhorn acacia shoots with and without resident ants (data from Janzen 1966).

with ants (fig. 15.10). Janzen's experiments provide strong evidence that bullshorn acacias need ants as much as the ants need the acacia. It appears that this is a truly mutualistic situation and that it is obligate for both partners.

Potential Conflict Between Mutualists

Most research on swollen thorn acacias has focused on their ant-protection mutualisms. However, these trees depend on many other mutualistic relationships. Belowground, their roots shelter nitrogen-fixing bacteria in nodules and also harbor mycorrhizal fungi. Aboveground, besides sheltering swarms of *Pseudomyrmex* ants that drive away herbivorous

insects, the acacia's flowers depend on other insects, mainly bees, for pollination. The acacia's ant guards could come into conflict with pollinators in two ways. First, the ants could remove nectar from flowers and reduce their attractiveness to potential pollinators. Second, the ants could guard flowers, driving pollinators away.

This potential conflict between mutualists of swollen thorn acacias attracted the attention of Nigel Raine, Pat Willmer, and Graham Stone (Raine, Willmer, and Stone 2002), researchers from three different British universities. They conducted their research at the Chamela Biological Station of the Universidad Nacional Autónoma de México, where they studied *Acacia hindsii* and its ant protector, *Pseudomyrmex veneficus*. Raine, Willmer, and Stone first examined the distribution of ants and pollinators to see if they overlapped in space or time. They found that ant and pollinator activity overlaps in time. However while ants and pollinators are active on *A. hindsii* at the same time, they rarely overlap spatially. The ants rarely visit acacia inflorescences. Why is that? Raine, Willmer, and Stone observed that the foliar nectaries and Beltian bodies used by the ants occur on new growth, while flowers are restricted to older shoots. In addition, in contrast to acacia species that do not support protective ants, the inflorescences of *A. hindsii* produce no nectar. Lack of nectar would make the flowers less attractive to patrolling ants. Still because new and older shoots can grow in close proximity, the researchers wondered whether some other factor might keep the ants from patrolling the inflorescences on older shoots.

Since Willmer and Stone (1997) had discovered previously that the flowers of some African acacias contained an ant repellent, they tested for the presence of a repellent in the flowers of *A. hindsii*. They tested for the presence of a repellent by rubbing several acacia tissues on the bark of
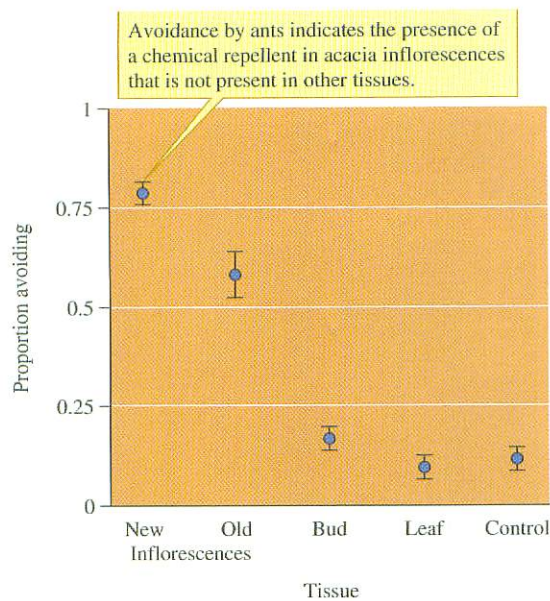
Avoidance by ants indicates the presence of a chemical repellent in acacia inflorescences that is not present in other tissues.

Figure 15.11   Proportion of ants avoiding control areas and areas rubbed with tissues of new and old inflorescences, buds, and leaves of the swollen thorn acacia, *Acacia hindsii;* values are means ± one standard error (data from Raine, Willmer, and Stone 2002).

branches actively patrolled by ants. The tissues tested were new inflorescences, old inflorescences, leaves, and buds. Each of these tissues were rubbed within 3 cm squares marked on the bark of patrolled branches using water-based markers. As a control, Raine, Willmer, and Stone marked one set of squares but did not rub any plant tissues on them. Once experimental and control squares had been established, the researchers watched patrolling ants, noting whether they entered experimental and control squares or avoided crossing them.

Figure 15.11 shows the results of the repellent experiment. Raine, Willmer, and Stone found that new inflorescences were strongly repellent to patrolling ants and that older inflorescences, though repellent, were rejected less often. Meanwhile, leaf and bud rubbings were rejected no more frequently than were control squares. In summary, protection and pollination mutualisms do not come into conflict on *A. hindsii* because of spatial separation of inflorescences and resources used by guarding ants, and because *A. hindsii* inflorescences lack a potential ant attractant (nectar) and instead contain a chemical repellent.

While tropical plant protection mutualisms are most often cited, there are many examples of mutualism between plants and ants in the temperate zone. A particularly well-studied interaction is that between ants and the aspen sunflower, *Helianthella quinquenervis.*

A Temperate Plant Protection Mutualism

Aspen sunflowers live in wet mountain meadows of the Rocky Mountains from Chihuahua, Mexico, to southern

Idaho, at elevations as low as 1,600 m in the northern part of its range to 4,000 m in the south. Ants are attracted to aspen sunflowers because they produce nectar at **extrafloral nectaries,** nectar-producing structures outside of the flowers. In the case of aspen sunflowers the extrafloral nectaries are associated with structures called *involucral bracts,* modified leaves that first enclose the flower head prior to flowering and then surround the base of the flower after it opens. Some early researchers hypothesized that extrafloral nectaries function to attract ants, while others suggested that they are primarily excretory organs.

The extrafloral nectar produced by aspen sunflowers is rich in sucrose and contains high concentrations of a wide variety of amino acids. So, like the swollen thorn acacias studied by Janzen the aspen sunflower provides food to ants. In contrast to swollen thorn acacias, however, this sunflower does not provide living places. This contrast is general across temperate ant-plant mutualisms, which involve food as an attractant but no living quarters.

David Inouye and Orley Taylor (1979) recorded five species of ants on aspen sunflowers, including *Formica obscuripes, F. fusca, F. integroides planipilis, Tapinoma sessile,* and *Myrmica* sp. These ants are not obligately associated with aspen sunflowers and can be found tending aphids on other species of plants or even collecting flower nectar on some plants. However, Inouye and Taylor never observed them collecting nectar from aspen sunflower blossoms nor tending aphids on this plant. Apparently, the extrafloral nectar produced by the aspen sunflower is a sufficient attractant. Ants find the plant so attractive that Inouye and Taylor observed up to 40 ants on a single flower stalk.

While the ants visiting the extrafloral nectaries of *Helianthella* clearly derive benefit, it is not obvious that the plant receives any benefits from the association. What benefits might this sunflower gain by having ants roaming around its flowers and flower buds? Inouye and Taylor proposed that the ants may protect the sunflower's developing seeds from seed predators. In the central Rocky Mountains, the seeds of aspen sunflowers are attacked by a variety of seed predators, including the larvae of two species of flies in the family Tephritidae, a fly in the family Agromyzidae, and a phycitid moth. These seed predators damaged over 90% of the seeds produced by some of the flowers at one of Inouye and Taylor's study sites.

The high densities of ants that can occur on a single aspen sunflower certainly have the potential to deter seed predators, but these same ants might also interfere with pollination. This potential for interference is not realized, however, because seed predators generally attack aspen sunflowers before they are fully mature and before they have formed ray florets, the "petals" of sunflowers, daisies, etc. Prior to opening of the flower bud, when tephridid and agromyzid flies try to oviposit on the bud, ants visiting the extrafloral nectaries patrol the whole surface of the flower bud in large numbers. Later, the fully formed ray florets, which act as attractants for pollinators (mainly bumblebees), form a screen between the involucral bracts and the
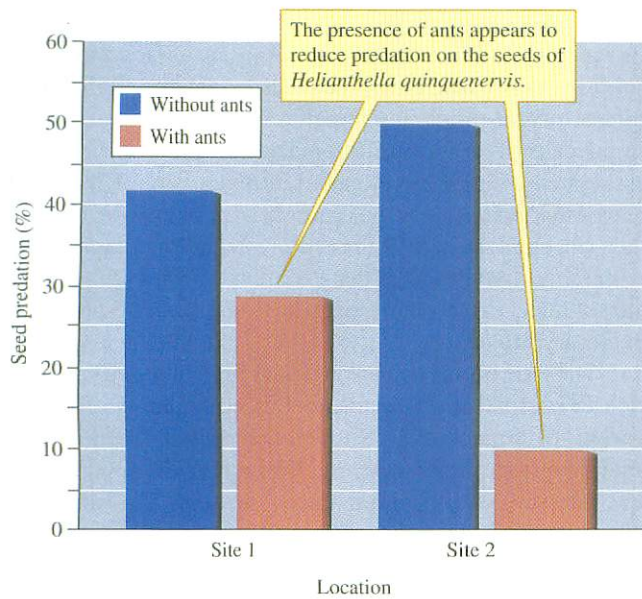
Figure 15.12 Predation on the seeds of aspen sunflower with and without ants (data from Inouye and Taylor 1979).

Figure 15.13 Effect of excluding ants on rates of seed predation on aspen sunflowers (data from Inouye and Taylor 1979).

flower head, reducing the potential for ants to interfere with pollinators.

The question asked by Inouye and Taylor was whether or not the presence of ants on aspen sunflowers reduces the rate of attack by seed predators. They addressed this question in several ways. First, they compared rates of attack by seed predators on flowers tended by ants with rates of attack on flowers where ants were naturally absent. This comparison showed that flowers without ants suffered two to four times as much seed predation (fig. 15.12). The researchers also found that the average number of ants per flower stalk decreased with distance from an ant nest and that the plants with fewer ants suffered higher rates of seed damage by seed predators.

Next, Inouye and Taylor performed an experiment in which they prevented ants from moving onto some plants by applying a sticky barrier to the base of flower stalks. They used adjacent plants as controls. The results of Inouye and Taylor's experiment demonstrated that (fig. 15.13), exclusion of ants from flowers resulted in significantly higher rates of seed predation.

As in the tropical swollen thorn acacia-ant mutualism, ants associated with aspen sunflowers provide protection while receiving substantial benefits in the form of food. Unlike the tropical system, the association between aspen sunflowers and ants incorporates a significant degree of flexibility. This flexibility may be a hallmark of many temperate mutualisms.

Why does the relationship between ants and aspen sunflowers remain facultative? In other words, why hasn't there been strong selection for the kind of obligate relationship, such as that between bullhorn acacia and the ant *P. ferruginea*? Continuing studies by David Inouye provide clues. He estimated the abundance of aspen sunflowers on two study plots for more than two decades. This long-term study shows that every few years the flower heads of aspen sunflowers are
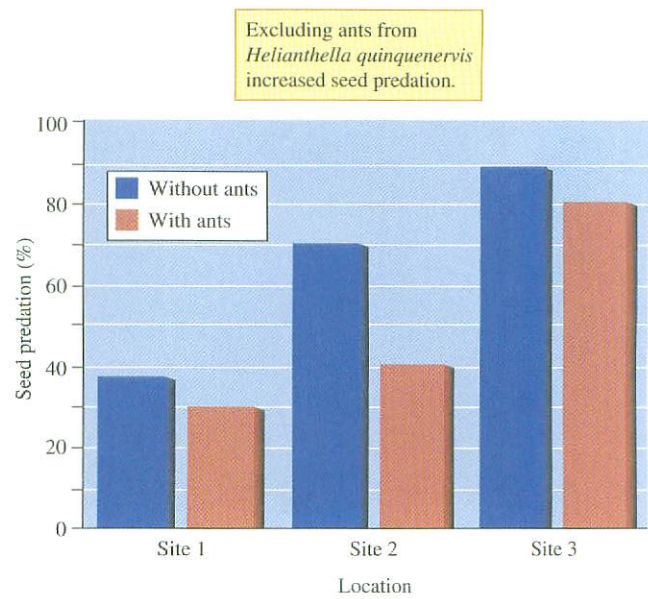
killed by late frosts. From 1974 to 1995, aspen sunflowers produced few or no flower heads in 1976, 1981, 1985, 1989, and 1992 (fig. 15.14). An ant species with an obligate mutualistic relationship with the aspen sunflower and that relied entirely on it as a source of nectar would not survive long. Inouye points out that paradoxically the frosts are beneficial to aspen sunflowers in the long run because they reduce populations of seed predators such as tephritid flies, which have no place to lay their eggs when hard frost kills the flower heads. In the coevolutionary relationships between the aspen sunflower and its predators, the physical environment plays a significant role. In temperate climates generally, the physical environment seems to play as large a role as biological relationships in determining ecological patterns and processes.

If we venture into tropical seas and probe their inhabitants, we soon uncover a wide variety of mutualistic relationships at least as rich as those we examined between terrestrial plants and their partners. The most striking marine counterparts to the mutualisms of terrestrial plants are those centered around reef-building corals.

Concept 15.1 Review

1. Why did Johnson create her inocula by mixing sterilized and unsterilized soils from the fertilized and unfertilized study areas?
2. Why did Johnson's control consist of a sterilized mixture of soils from the fertilized and unfertilized study areas?
3. In Inouye and Taylor's study why wasn't the comparison of seed predation on plants naturally with and without ants sufficient to demonstrate the influence of ants on rates of seed predation?
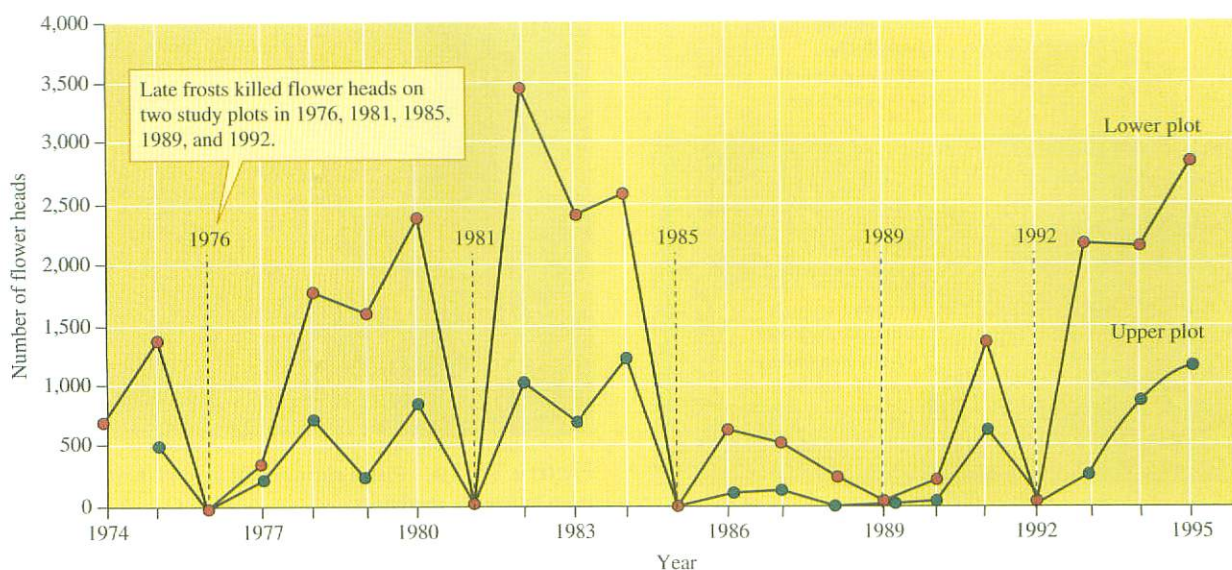
Figure 15.14 Annual variation in numbers of flower heads produced by aspen sunflowers on two plots at the Rocky Mountain Biological Station (data courtesy of David W. Inouye).

15.2 Coral Mutualisms

Reef-building corals depend upon mutualistic relationships with algae and animals. Because of the importance of mutualism in the lives of reef-building corals, it appears that the ecological integrity of coral reefs depends upon mutualism. Coral reefs show exceptional productivity and diversity. Recent estimates put the number of species occurring on coral reefs at approximately 0.5 million, and coral reef productivity is among the highest of any natural ecosystem. As we saw in chapter 3, the paradox is that this overwhelming diversity and exceptional productivity occurs in an ecosystem surrounded by nutrient-poor tropical oceans. The key to explaining this paradox lies with mutualism; in this case, between reef-building corals and unicellular algae called zooxanthellae, members of the phylum Dinoflagellata. Most of these organisms are free-living unicellular marine and freshwater photoautotrophs.

Zooxanthellae and Corals

The association between corals and zooxanthellae is functionally similar to the relationship between plants and mycorrhizal fungi. Zooxanthellae live within coral tissues at densities averaging approximately 1 million cells per square centimeter of coral surface. Like plants, zooxanthellae receive nutrients from their animal partner. In return, like mycorrhizal fungi, the coral receives organic compounds synthesized by zooxanthellae during photosynthesis.

One of the most fundamental discoveries concerning the relationship between corals and zooxanthellae is that the release of organic compounds by zooxanthellae is controlled by the coral partner. Corals induce zooxanthellae to release organic compounds with "signal" compounds that alter the permeability of the zooxanthellae cell membrane. Zooxan-

thellae grown in isolation from corals release very little organic material into their environment. However, when exposed to extracts of coral tissue, zooxanthellae immediately increase the rate at which they release organic compounds. This response appears to be a specific chemically mediated communication between corals and zooxanthellae. Zooxanthellae do not respond to extracts of other animal tissues, and coral extracts do not induce leaking of organic molecules by any other algae that have been studied.

Corals not only control the secretion of organic compounds by zooxanthellae, they also control the rate of zooxanthellae population growth and population density. In corals, zooxanthellae populations grow at rates 1/10 to 1/100 the rates observed when they are cultured separately from corals. Corals exert control over zooxanthellae population density through their influence on organic matter secretion. Normally, unicellular algae show **balanced growth,** growth in which all cell constituents, such as nitrogen, carbon, and DNA, increase at the same rate. However, zooxanthellae living in coral tissues show unbalanced growth, producing fixed carbon at a much higher rate than other cell constituents. Moreover, the coral stimulates the zooxanthellae to secrete 90% to 99% of this fixed carbon, which the coral uses for its own respiration. Fixed carbon secreted and diverted for use by the coral could otherwise be used to produce new zooxanthellae, which would increase population growth.

What benefits do the zooxanthellae get out of their relationship with corals? The main benefit appears to be access to higher levels of nutrients, especially nitrogen. Corals feed on zooplankton, which gives them a means of capturing nutrients, especially nitrogen and phosphorus. When corals metabolize the protein in their zooplankton prey, they excrete ammonium as a waste product. L. Muscatine and C. D'Elia (1978) showed that coral species such as *Tubastrea aurea* that do not
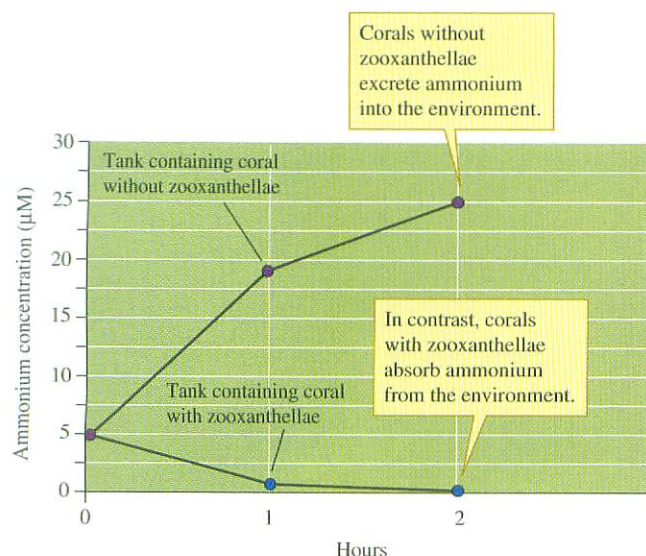
Figure 15.15 Zooxanthellae, corals, and ammonium flux (data from Muscatine and D'Elia 1978).

Figure 15.16 Pistol shrimp will defend their home coral from attacking predators.

harbor zooxanthellae continuously excrete ammonium into their environment, while corals such as *Pocillopora damicornis* do not excrete measurable amounts of ammonia (fig. 15.15). What happens to the ammonium produced by *Pocillopora* during metabolism of the protein in their zooplankton prey? Muscatine and D'Elia suggested that this ammonium is immediately taken up by zooxanthellae as the coral excretes it. In addition to internal recycling of the ammonium produced by their coral partner, zooxanthellae also actively absorb ammonium from seawater. By absorbing nutrients from the surrounding medium and leaking very little back into the environment, corals and their zooxanthellae gradually accumulate substantial quantities of nitrogen. So, as in tropical rain forest, large quantities of nutrients on coral reefs accumulate and are retained in living biomass.

A Coral Protection Mutualism

The ant-acacia mutualism that we reviewed previously has a striking parallel on coral reefs. Corals in the genera *Pocillopora* and *Acropora* host a variety of crabs in the family Xanthidae, mainly *Trapezia* spp. and *Tretralia* spp. as well as a species of pistol shrimp, *Alpheus lottini*. In this mutualistic relationship (fig. 15.16), the crustaceans protect the coral from a wide variety of predators while the coral provides its crustacean partners with shelter and food.

Peter Glynn (1983) surveyed the coral-crustacean mutualism and found that the eastern, central, and western areas of the Pacific Ocean contain 13 species of corals that are protected by crustacean mutualists, including 17 species of crabs and 1 species of shrimp, all of which are found only on corals in what is apparently an obligate mutualism. These crustaceans protect the corals from a variety of sea stars that prey on corals but especially from attacks by the crown-of-thorns sea star, *Acanthaster planci*. At the approach of the sea star, the crabs become highly disturbed and then attack by pinching and

clipping the sea star's spines and tube feet, grasping it and jerking it up and down and resisting its retreat. The mutualistic shrimp also attacks the sea star by snipping spines and tube feet and making loud snapping sounds with an enlarged pincer specialized for the purpose. The loud popping sounds, which have given shrimp in the genus *Alpheus* the name "pistol shrimp," are so intense they stun small fish.

Glynn used field and laboratory experiments to test whether this aggression by crustaceans is effective at repelling attacks by predatory sea stars. He conducted a field experiment at 8 to 12 m depth on a reef in Guam, where he removed the crustaceans from an experimental group of corals and gave sea stars a choice between these and an equal number of corals that retained their crustacean partners. Over a period of 2 days the sea stars attacked the unprotected corals at a much higher frequency (fig. 15.17). Glynn obtained similar results in a laboratory study of the corals and crustacean mutualists of Panama, in which sea stars attacked 85% of the unprotected colonies. These results show that the crustacean mutualists of corals substantially improve the chances that a coral will avoid attack by sea stars.

Observations by Glynn and also John Stimson (1990) suggest that mutualistic crabs also protect corals from other less conspicuous attackers. Glynn observed that the presence of crabs seems to enhance the condition of coral tissues. Stimson found that when he removed crabs, corals showed tissue death in the deep axils of their branches and that these areas were soon invaded by algae, sponges, and tunicates. It appears that, in addition to protecting corals from the attacks of large predators, the activities of crabs promote the health and integrity of coral tissues. If this is a mutualistic relationship, what do the crabs receive in return for their investment?

Like swollen thorn acacias, corals provide their crustacean mutualists with shelter and food. The corals harboring crabs and pistol shrimp have a tightly branched growth form that offers shelter, and the crustaceans feed on the mucus produced by the corals. *Trapezia* spp., the most common crabs guarding pocilloporid corals, stimulate mucus flow from
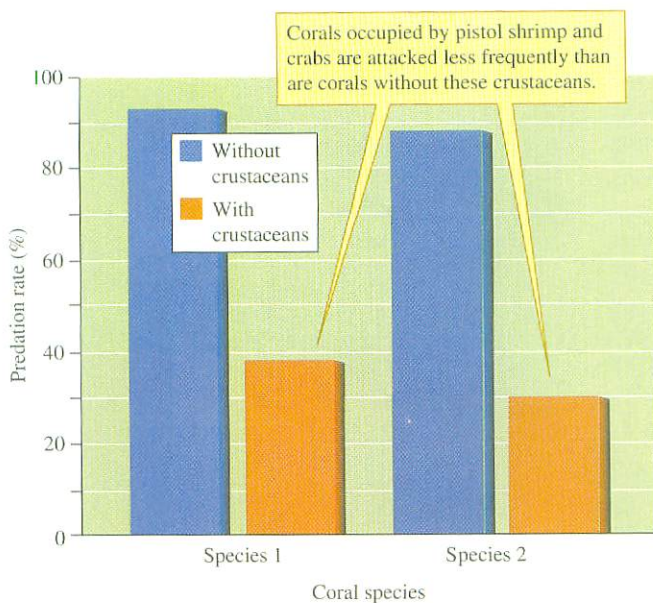
Figure 15.17 callout: Corals occupied by pistol shrimp and crabs are attacked less frequently than are corals without these crustaceans.

Figure 15.17 Attacks on corals with and without pistol shrimp and crabs (data from Glynn 1983).

Figure 15.18 callout: The presence of crabs appears to stimulate increased fat body production by *Pocillopora*.

Figure 15.18 Fat body production by the coral *Pocillopora damicornis* in the presence and absence of crabs (data from Stimson 1990).

corals by inserting their legs into coral polyps, a behavior not reported for any other crabs. Corals contain large quantities of lipids that constitute 30% to 40% of the dry weight of their tissues, much of which they release with mucus. This release may constitute up to 40% of the daily photosynthetic production by zooxanthellae.

The pocilloporid corals that host crustaceans concentrate some of this lipid into fat bodies that are 300 to 500 μm in length. Glynn suggested that the fat bodies produced by pocilloporid corals hosting protective crabs may be a part of their mutualistic relationship. Stimson tested this hypothesis by determining whether commensal crabs influence the production of fat bodies by coral polyps. He conducted his experiments at the Hawaii Institute of Marine Biology on Coconut Island in Kaneohe Bay, Oahu, Hawaii. He collected colonies of *Pocillopora* 8 to 10 cm in diameter from the midbay region of Kaneohe Bay, placed them in buckets of seawater, and took them back to the marine laboratory on Coconut Island. There, he divided the corals into experimental and control groups. He then removed crabs and pistol shrimp from the experimental coral colonies by "teasing" them out with a small wire. Corals with and without crabs were then kept separately in outdoor tanks supplied with flowing seawater.

After 24 days, Stimson compared the number of fat bodies on corals with and without crabs. He also compared these experimental results with the density of fat bodies on *Pocillopora* in Kaneohe Bay that naturally hosted or lacked mutualistic crabs. The results of these experiments and field observations show clearly that *Pocillopora* increases its production of fat bodies in the presence of crabs both in the laboratory and in the field (fig. 15.18). Stimson also examined the digestive tract of crabs inhabiting corals and found that they contained large quantities of lipids. At the same time, no
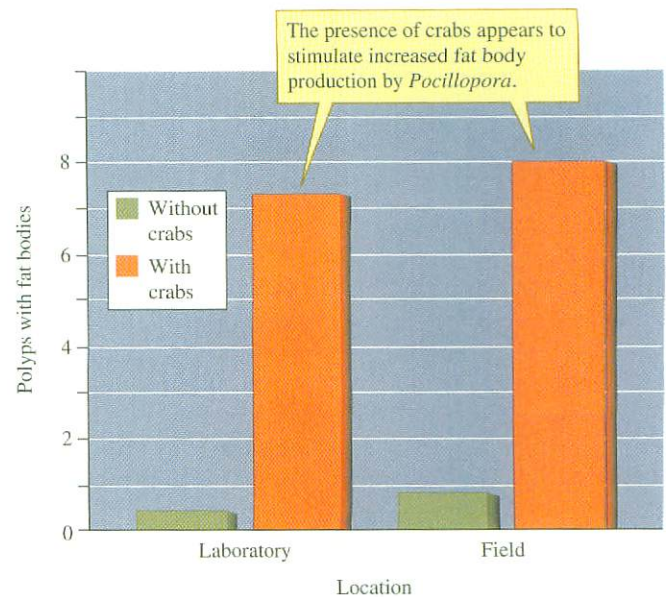
significant reductions in either the reproductive rate or growth rates of corals supporting crabs were found. Stimson concluded that the relationship between corals and crabs is a true mutualism, with both partners receiving substantial benefit.

The *extent* of benefit may be the essential factor driving the evolution of mutualisms. In the following section, we review theoretical analyses of how the relative benefits and costs of an association influence the evolution of mutualistic relationships.

Concept 15.2 Review

1. If reef-building corals are placed in the dark, they will expel the zooxanthellae in their tissues. What does this suggest concerning controls on the relationship between corals and zooxanthellae?
2. In terms of costs and benefits, why might corals expel their zooxanthellae when placed in the dark?

15.3 Evolution of Mutualism

Theory predicts that mutualism will evolve where the benefits of mutualism exceed the costs. We have reviewed several complex mutualisms both on land and in marine environments. There are many others (fig. 15.19), every one a fascinating example of the intricacies of nature. Ecologists not only study the present biology of those mutualisms but also seek to understand the conditions leading to their evolution and persistence. Theoretical analyses point to the relative costs and benefits of a possible relationship as a key factor in the evolution of mutualism.

Modeling of mutualism has generally taken one of two approaches. The earliest attempts involved modifications of

Investigating the Evidence 15

Confidence Intervals

In chapter 14 we reviewed how to calculate the standard error, $s_{\bar{X}}$, which is an estimate of variation among means of samples drawn from a population. Here, we will use the standard error to calculate a **confidence interval.** A confidence interval is a range of values within which the true population mean occurs with a particular probability. That probability, which is called the **level of confidence,** is calculated as one minus the significance level, α, which is generally 0.05:

$$\text{Level of confidence} = 1 - \alpha$$
$$\text{Level of confidence} = 1 - 0.05 = 0.95$$

Using this level of confidence produces what is called a 95% confidence interval that is calculated as follows:

$$\text{Confidence interval for } \mu = \bar{X} \pm s_{\bar{X}} \times t$$

where

μ = true population mean
$\bar{X}$ = sample mean
$s_{\bar{X}}$ = standard error
t = value from a Student's t table

A Student's t table, available in most statistics textbooks, summarizes the values of a statistical distribution known as the Student's t distribution. The value of t we use for calculating a confidence interval is determined by the degrees of freedom $(n-1)$ and the significance level, which in this case is $\alpha = 0.05$.

Let's calculate a 95% confidence interval using the body length measurements for the sample of loach minnows, *Tiaroga cobitis*, that we used to calculate a standard error in chapter 14 (see p. 330).

In chapter 6 (p. 151), we calculated mean of this sample as:

$$\bar{X} = 56.2 \text{ mm}$$

And, in chapter 14 we calculated the standard error of the sample as:

$$s_{\bar{X}} = 1.96 \text{ mm}$$

This sample of body lengths included measurements of 10 fish $(n = 10)$ and so the degrees of freedom for this sample $(n-1)$ is 9. Using a significance level of 0.05 and degrees of freedom of 9, we find that the critical value of t from a Student's t table is 2.26 (table A.1, p. 554, of Appendix A). Therefore, the 95% confidence interval calculated from this sample is:

$$\text{Confidence interval for } \mu = \bar{X} \pm s_{\bar{X}} \times t$$
$$= 56.2 \text{ mm} \pm 1.96 \text{ mm} \times 2.26$$
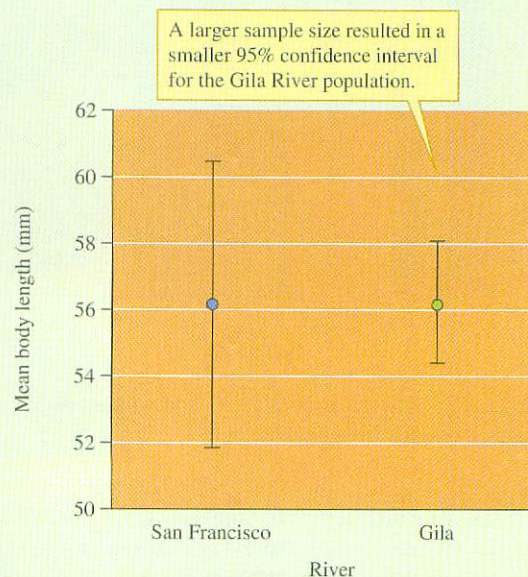$$= 56.2 \text{ mm} \pm 4.43 \text{ mm}$$

Figure 1   Average body length of loach minnows and 95% confidence intervals calculated from samples collected in the San Francisco River $(n = 10)$ and Gila River $(n = 50)$.

With this confidence interval, we can say that there is a 95% probability that the true mean body length in this population of loach minnows is somewhere between 60.63 mm (56.2 mm + 4.43 mm) and 51.77 mm (56.2 mm − 4.43 mm).

This is shown graphically in figure 1, along with the mean and 95% confidence interval for the sample of loach minnows from the Gila River that we first considered in chapter 14 (p. 330). Notice that the 95% confidence interval for the Gila River sample is much smaller. This smaller confidence interval is the result of the larger sample size from the Gila River $(n = 50)$, which produced a smaller standard error $(s_{\bar{X}} = 0.88)$ and a smaller critical t value (2.01), since the degrees of freedom is 49. As a consequence of having a larger sample, our estimate of the true population mean has been narrowed to a much smaller range for the Gila River population of loach minnows.
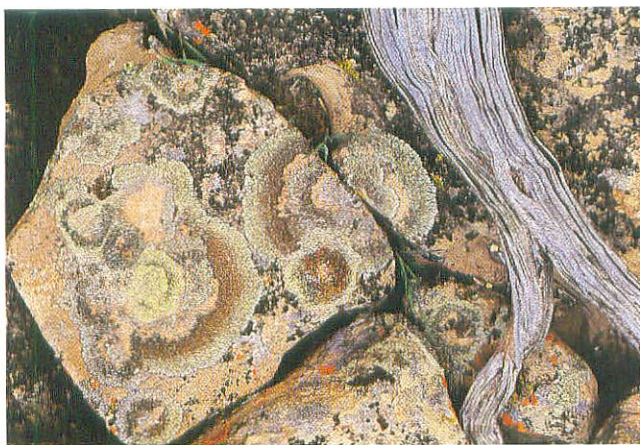
CRITIQUING THE EVIDENCE 15

1. What is the 95% confidence interval for the Gila River sample of loach minnows?
2. What value of t would you use from table A.1 for calculating a 95% confidence interval, if your sample size was 18 and your significance level was $\alpha = 0.05$?
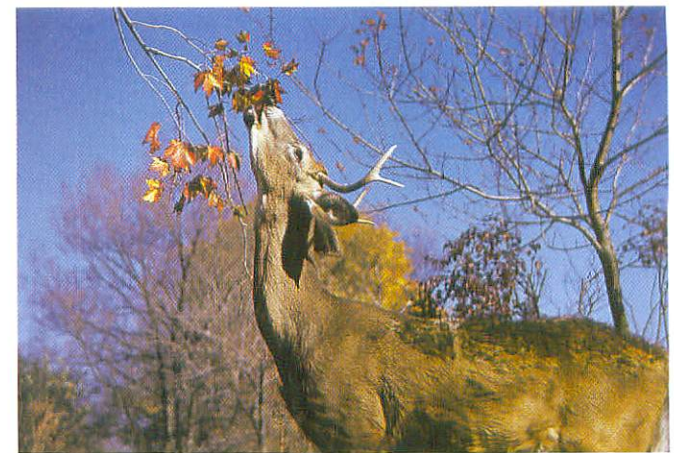
(a)

(c)

(b)

(d)

Figure 15.19 A diversity of mutualisms: (*a*) cleaner wrasse and sea bass; (*b*) lichens are an association between a fungus and cyanobacteria; (*c*) soybeans fix molecular nitrogen through their association with bacteria within nodules on their roots; (*d*) deer access the energy stored in plant tissues through the activities of a community of mutualistic microorganisms living in their gut.

the Lotka-Volterra equations to represent the population dynamics of mutualism. The alternative approach has been to model mutualistic interactions using cost-benefit analysis to explore the conditions under which mutualisms can evolve and persist. In chapters 13 and 14, where we discussed models of competition and predation, we focused on the population dynamic approach to modeling species interactions. Here, we concentrate on cost-benefit analyses of mutualism.

Kathleen Keeler (1981, 1985) developed models to represent the relative costs and benefits of several types of mutualistic interactions. Among them are two of the mutualistic interactions we discussed in chapter 15: ant-plant protection mutualisms and mycorrhizae. Keeler's approach requires that we consider a population polymorphic for mutualism containing three kinds of individuals: (1) *successful mutualists,* which give and receive measurable benefits to another organism; (2) *unsuccessful mutualists,* which give benefits to another organism but, for some reason, do not receive any benefit in return; and (3) *nonmutualists,* neither giving nor receiving benefit from a mutualistic partner. The

bottom line in Keeler's approach is that for a population to be mutualistic, the fitness of successful mutualists must be greater than the fitness of either unsuccessful mutualists or nonmutualists. In addition, the combined fitness of successful and unsuccessful mutualists must exceed that of the fitness of nonmutualists. If these conditions are not met, Keeler proposed that natural selection will eventually eliminate the mutualistic interaction from the population.

In general, we can expect mutualism to evolve and persist in a population when and where mutualistic individuals have higher fitness than nonmutualistic individuals.

Keeler represented the fitness of nonmutualists as:

$$w_{nm} = \text{fitness of nonmutualists}$$

(Fitness has been traditionally represented by the symbol *w* and though it might be clearer to use another symbol, such as *f,* the traditional symbol is used here.) Keeler represents the fitness of mutualists as:

$$w_m = pw_{ms} + qw_{mu} \qquad (1)$$

where:

> p = the proportion of the population consisting of successful mutualists
>
> w_{ms} = the fitness of successful mutualists
>
> q = the proportion of the population consisting of unsuccessful mutualists
>
> w_{mu} = the fitness of unsuccessful mutualists.

We can represent Keeler's conditions for the evolution and persistence of mutualism as:

$$pw_{ms} + qw_{mu} > w_{nm} \tag{2}$$

or

$$w_m > w_{nm} \tag{3}$$

Keeler predicts that mutualism will persist when the combined fitness of successful and unsuccessful mutualists exceeds the fitness of nonmutualists. Why do we have to combine the fitness of successful and unsuccessful mutualists? Remember that both confer benefit to their partner, but only the successful mutualists receive benefit in return.

The analysis is more convenient if we think of these relationships in terms of **selection coefficients,** the relative selective costs associated with being either a successful mutualist, an unsuccessful mutualist, or a nonmutualist:

$$s = 1 - w \quad \text{and} \quad w = (1 - s).$$

Using selective coefficients, Keeler expressed the selective cost of being a successful mutualist, an unsuccessful mutualist, or a nonmutualist as:

$$s_{ms} = (H)(1 - A)(1 - D) + I_A + I_D \tag{4}$$
$$s_{mu} = (H)(1 - D) + I_A + I_D \tag{5}$$
$$s_{nm} = H(1 - D) + I_D \tag{6}$$

where:

H = the proportion of the plant tissue damaged in the absence of any defenses

D = the amount of protection given to the plant tissues by defenses other than ants (e.g., chemical defenses); so, $1 - D$ is the amount of tissue damage that would occur in spite of these alternative defenses

A = the amount of herbivory prevented by ants (so, again, $1 - A$ is the amount of herbivory that occurs in spite of ants)

I_a = the investment by the plant in benefits extended to the ants

I_D = investment in defenses other than ants

Using these selective coefficients we can express Keeler's conditions for evolution and persistence of the ant-plant mutualism as:

$$p(1 - s_{ms}) - q(1 - s_{mu}) > 1 - s_{nm}$$

into which Keeler substituted the relationships given in equations (4), (5), and (6). By simplifying the resulting equation, she produced the following expression of benefits relative to costs:

$$p[H(1 - D)A] > I_A$$

Facultative Ant-Plant Protection Mutualisms

Keeler applied her cost-benefit model to facultative mutualisms involving plants with extrafloral nectaries and ants that feed at the nectaries and provide protection to the plant in return. These are mutualisms like that between *Helianthella quinquenervis* and ants, which we discussed earlier in the section Concept (15.1) on plant mutualism. Her model is not appropriate for obligate mutualisms like that between swollen thorn acacias and their mutualistic ants. In addition, Keeler wrote her model from the perspective of the plant side of the mutualism. Let's step through the general model and connect each of the terms with the ecology of facultative plant-ant protection mutualisms.

In this model, w_{ms} is the fitness of a plant that produces extrafloral nectaries and that successfully attracts ants effective at guarding it, while w_{mu} is the fitness of a plant that produces extrafloral nectaries but that has not attracted enough ants to mount a successful defense. You may remember that Inouye and Taylor found that *Helianthella* far away from ant nests attracted few ants. These plants would correspond to Keeler's unsuccessful mutualists. In addition, Keeler includes the fitness of nonmutualistic plants, w_{nm}, which would be the fitness of individuals of a plant such as *Helianthella* that does not produce extrafloral nectaries.

Keeler's model represents potential benefits to the host plant as:

$$p[H(1 - D)A]$$

where:

> p = the proportion of the plant population attracting sufficient ants to mount a defense

Keeler's model represents the plant's costs of mutualism as:

$$I_A = n[m + d(a + c + h)]$$

where:

n = the number of extrafloral nectaries per plant

m = the energy content of nectary structures

d = the period of time during which the nectaries are active

a = costs of producing amino acids in nectar

c = costs of producing the carbohydrates in nectar

h = costs of providing water for nectar

Again, Keeler's hypothesis is that for mutualism to persist, benefits must exceed costs. In terms of her model:

$$p[H(1 - D)A] > I_A$$

This model proposes that for a facultative ant-plant mutualism to evolve and persist, the proportion of the plant's

energy budget that ants save from destruction by herbivores must exceed the proportion of the plant's energy budget that is invested in extrafloral nectaries and nectar.

The details of Keeler's model offer insights into what conditions may produce higher benefits than costs. First, and most obviously, I_A, the proportion of the plant's energy budget that is invested in extrafloral nectaries and nectar should be low. This means that plants living on a tight energy budget, for example, plants living in a shady forest understory, should be less likely to invest in attracting ants than those living in full sun. Higher benefits result from (1) a high probability of attracting ants, that is, high p; (2) a high potential for herbivory, H; (3) low effectiveness of alternative defenses, low D, and (4) highly effective ant defense, high A.

The task for ecologists is to determine how well these requirements of the model match values of these variables in nature.

Concept 15.3 Review

1. Suppose you discover a mutant form of *Helianthella quinquenervis* that does not produce extrafloral nectaries. What does Keller's theory predict concerning the relative fitness of these mutant plants and the typical ones that produce extrafloral nectaries?
2. According to Keller's theory, under what general conditions would the mutant *Helianthella quinquenervis*, lacking extrafloral nectaries, increase in frequency in a population and displace the typical plants that produce extrafloral nectaries?

Applications

Mutualism and Humans

Mutualism has been important in the lives and livelihood of humans for a long time. Historically, much of agriculture has depended upon mutualistic associations between species and much of agricultural management has been aimed at enhancing mutualisms, such as nitrogen fixation, mycorrhizae, and pollination to improve crop production. Agriculture itself has been viewed as a mutualistic relationship between humans and crop and livestock species. However, there may be some qualitative differences between agriculture as it has been generally practiced and mutualisms among other species. How much of agriculture is pure exploitation and how much is truly mutualistic remains an open question.

There is, however, at least one human mutualism that fits comfortably among the earlier discussions in chapter 15, a mutualism involving communication between humans and a wild species with clear benefit to both. This mutualism joins the traditional honey gatherers of Africa with the greater honeyguide, *Indicator indicator* (fig. 15.20). Honey gathering has long been an important aspect of African cultures,

Figure 15.20   The greater honeyguide, *Indicator indicator.*

important enough that there are scenes of honey gathering in rock art painted over 20,000 years ago (Isack and Reyer 1989). No one knows how long humans have gathered honey in Africa, but it is difficult to imagine the earliest hominids resisting such sweet temptation. Whenever honey gathering began, humans have apparently had a capable and energetic partner in their searches.

The Honeyguide

Honeyguides belong to the family Indicatoridae in the order Piciformes, an order that also includes the woodpeckers. The family Indicatoridae includes a total of 17 species, 15 of which are native to Africa. Honeyguides have the unusual habit of feeding on waxes of various sorts—most feed on beeswax and insects. Of the 17 species of honeyguides, only the greater honeyguide, *I. indicator,* is known to guide humans and a few other mammals to bees' nests.

The greater honeyguide is found throughout much of sub-Saharan Africa. It avoids only dense forests and very open grasslands and desert, and its distribution corresponds broadly with the distributions of tropical savanna and tropical dry forest. Like all of the honeyguides, the greater honeyguide is a brood parasite that, like cuckoos, lays its eggs

in the nests of other birds. This way of life is reflected in the early morphology of nestling honeyguides, which retain "bill hooks" on their upper and lower bills for the first 14 days of life that they use to lacerate and kill their nest mates. However, nests sometimes contain two honeyguide nestlings, so apparently there is some mechanism by which nestlings of the same species can coexist. After the deaths of their nest mates, honeyguide nestlings receive all the food brought by their foster parents, which continue to feed young honeyguides until they are completely independent, approximately 7 to 10 days after leaving the nest.

Greater honeyguides are capable of completely independent life without mutualistic interactions with humans, so we would classify their mutualism as facultative. Living independently, honeyguides feed on beeswax, and on the adults, larvae, pupae, and eggs of bees. They also feed on a wide variety of other insects. Greater honeyguides show highly opportunistic feeding behavior and sometimes join flocks of other bird species foraging on the insects stirred up by large mammals. The most distinguishing feature of the greater honeyguide, however, is its habit of guiding humans and ratels, or honey badgers, to bees' nests.

Guiding Behavior

The first written report of the guiding behavior of *I. indicator* was authored in 1569 by João Dos Santos, a missionary in the part of East Africa that is now Mozambique. Dos Santos first noticed honeyguides because they would enter the mission church to feed upon the bits of beeswax on candlesticks. He went on to describe their guiding behavior by saying that when the birds find a beehive, they search for people and attempt to lead them to the hive. He noted that the local people eagerly followed the birds because of their fondness for honey, and he observed that the honeyguide profits by gaining access to the wax and dead bees left after humans raid the hive. Dos Santos's report of this behavior was confirmed by other European visitors to almost all parts of Africa for the next four centuries. However, it wasn't until the middle of the twentieth century that the mutualism of honeyguides with humans was examined scientifically. The foundation work of these studies was that of H. Friedmann (1955), who reviewed and organized the observations of others, including those of Dos Santos, and who conducted his own extensive research on the honeyguides of Africa.

Friedmann's report of some of the African legends surrounding the greater honeyguide suggests that a wide variety of African cultures prescribed rewarding the bird for its guiding behavior and that native Africans recognized the need for reciprocity in their interactions with honeyguides. One proverb reported by Friedmann was, "If you do not leave anything for the guide [*I. Indicator*], it will not lead you at all in the future." Another proverb stated more ominously, "If you *do not leave anything for the* guide, it will lead you to a dangerous animal the next time." Friedmann also observed that many African cultures forbid killing a honeyguide and once

"inflicted severe penalties" for doing so. These observations suggest long association between humans and honeyguides and that the association has been consciously mutualistic on the human side of the balance sheet.

The mutualistic association between humans and honeyguides may have developed from an earlier association between the bird and the ratel, or honey badger, *Mellivora capensis*. The honey badger is a powerful animal, well equipped with strong claws and powerful muscles to rip open bees' nests, that readily follows honeyguides to bees' nests. The honey badger, though secretive, has been observed often following honeyguides while vocalizing. African honey gatherers also vocalize to attract honeyguides, and Friedmann reported that some of their vocalizations imitate the calls of honey badgers.

The most detailed and quantitative study of this mutualism to date is that of H. Isack of the National Museum of Kenya and H.-U. Reyer of the University of Zurich (Isack and Reyer 1989), who studied the details of the interaction of the greater honeyguide with the Boran people of northern Kenya. The Boran regularly follow honeyguides and have developed a penetrating whistle that they use to attract them. The whistle can be heard over 1 km away, and Isack and Reyer found that it doubles the rate at which Boran honey gatherers encounter honeyguides. If they are successful in attracting a honeyguide, the average amount of time it takes to find a bees' nest is 3.2 hours. Without the aid of a honeyguide the average search time per bees' nest is about 8.9 hours. This is an underestimate of the true time, however, since Isack and Reyer did not include days in which no bees' nests were found in their analysis. The benefit of the association to the bird seems apparent from Isack and Reyer's analysis, since they report that 96% of the nests to which the Boran were guided would have been inaccessible to the birds without human help.

The greater honeyguide attracts the attention of a human by flying close and calling as it does so. Following this initial attention-getting behavior the bird will fly off in a particular direction and disappears for up to 1 minute. After reappearing, the bird again perches in a conspicuous spot and calls to the following humans. As the honey gatherers follow, they whistle, bang on wood, and talk loudly in order to "keep the bird interested." When the honey gatherers approach the perch from which the honeyguide is calling, the bird again flies off, calling and displaying its white tail feathers as it does so, only to reappear at another conspicuous perch a short time later. This sequence of leading, following, and leading is repeated until the bird and the following honey gatherers arrive at the bees' nest.

Isack, who is a Boran, interviewed Boran honey gatherers to determine what information they obtained from honeyguides. The main purpose of the study was to test assertions by the honey gatherers that the bird informs them of (1) the direction to the bees' nest, (2) the distance to the nest, and (3) when they arrive at the location of the nest. The data gathered by Isack and Reyer support all three assertions.
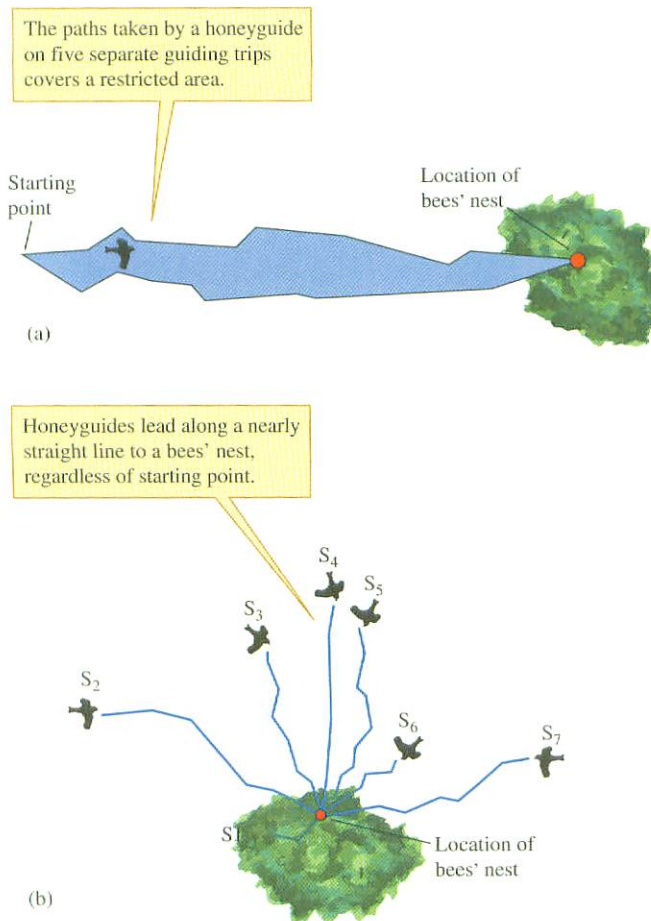
The paths taken by a honeyguide on five separate guiding trips covers a restricted area.

Starting point

Location of bees' nest

(a)

Honeyguides lead along a nearly straight line to a bees' nest, regardless of starting point.

S_4 S_5
S_3
S_2
S_6 S_7
$S1$ Location of bees' nest

(b)

Figure 15.21   Paths taken by honeyguides leading people to bees' nests (data from Isack and Reyer 1989).

Honeyguide stays in sight longer if it begins guiding far from a bees' nest.

First disappearance (s)

Distance to nest (m)

As the distance to a bees' nest decreases, the distances between stops declines.

Distance between stops (m)

Distance to nest (m)

As the nest is approached, a honeyguide perches lower and lower to the ground.

Perching height (m)

Stops since encounter

Figure 15.22   Changes in behavior of the honeyguide as it nears a bees' nest (data from Isack and Reyer 1989).

Honey gatherers reported that the bird indicated direction to the bees' nest on the basis of the direction of its guiding flights. One method used by Isack and Reyer to test how well flight direction indicated direction was to induce honeyguides to guide them from the same starting point to the same known bees' nest on five different occasions. Figure 15.21*a* shows the highly restricted area covered by these five different guiding trips. Another approach was to induce the bird to guide them to a bees' nest from seven different starting points (fig. 15.21*b*). The result was a consistent tendency by the bird to lead directly to the site of the bees' nest.

The Boran honey gatherers said that three variables decrease as distance to the nest decreases: (1) the time the bird stays out of sight during its first disappearance following the initial encounter, (2) the distance between stops made by the bird on the way to the bees' nest, and (3) the height of the perch on the way to the nest. Data gathered by Isack and Reyer support all three statements (fig. 15.22).

The honey gatherers also report that they can determine when they arrive in the vicinity of a bees' nest by changes in the honeyguide's behavior and vocalizations (fig. 15.23).
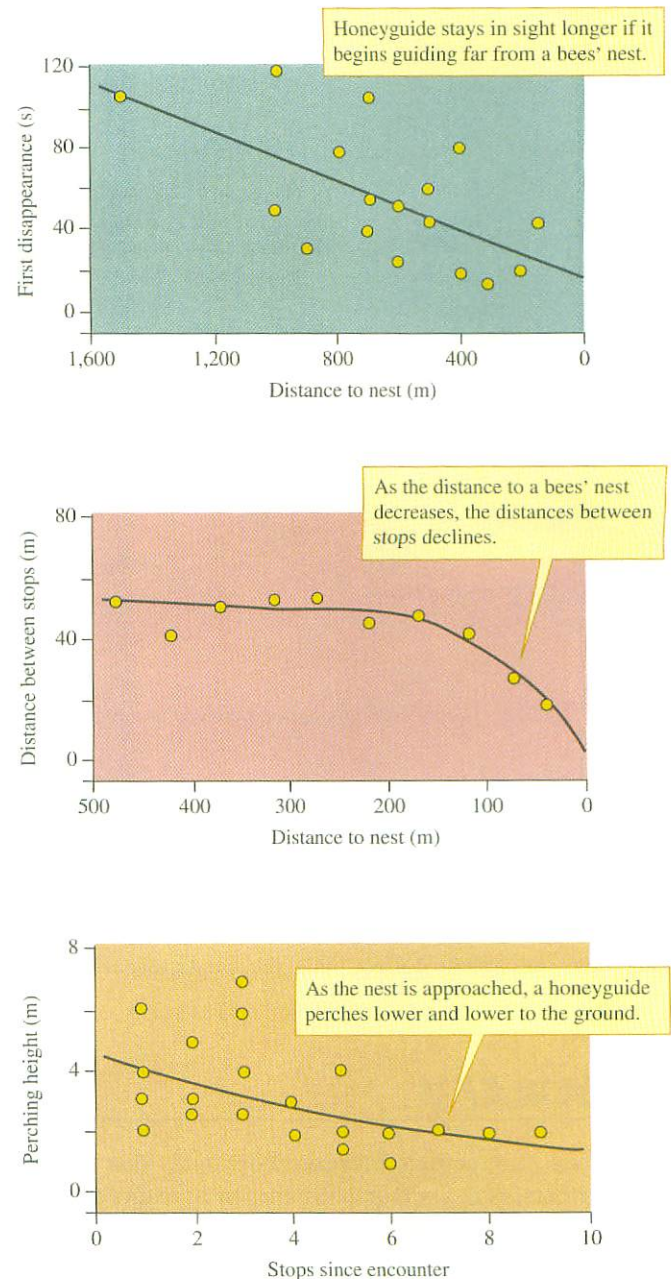
Isack and Reyer observed several of these changes. While on the path to a bees' nest a honeyguide emits a distinctive guiding call and will answer human calls by increasing the frequency of the guiding call. On arriving at a nest, the honeyguide perches close to the nest and gives off a special "indication" call. After a few indication calls, it remains silent and does not answer to human sounds. If approached by a honey gatherer, a honeyguide flies in a circle around the nest location before perching again nearby.

Isack and Reyer observe that their data do not allow them to test other statements by the Boran honey gatherers,
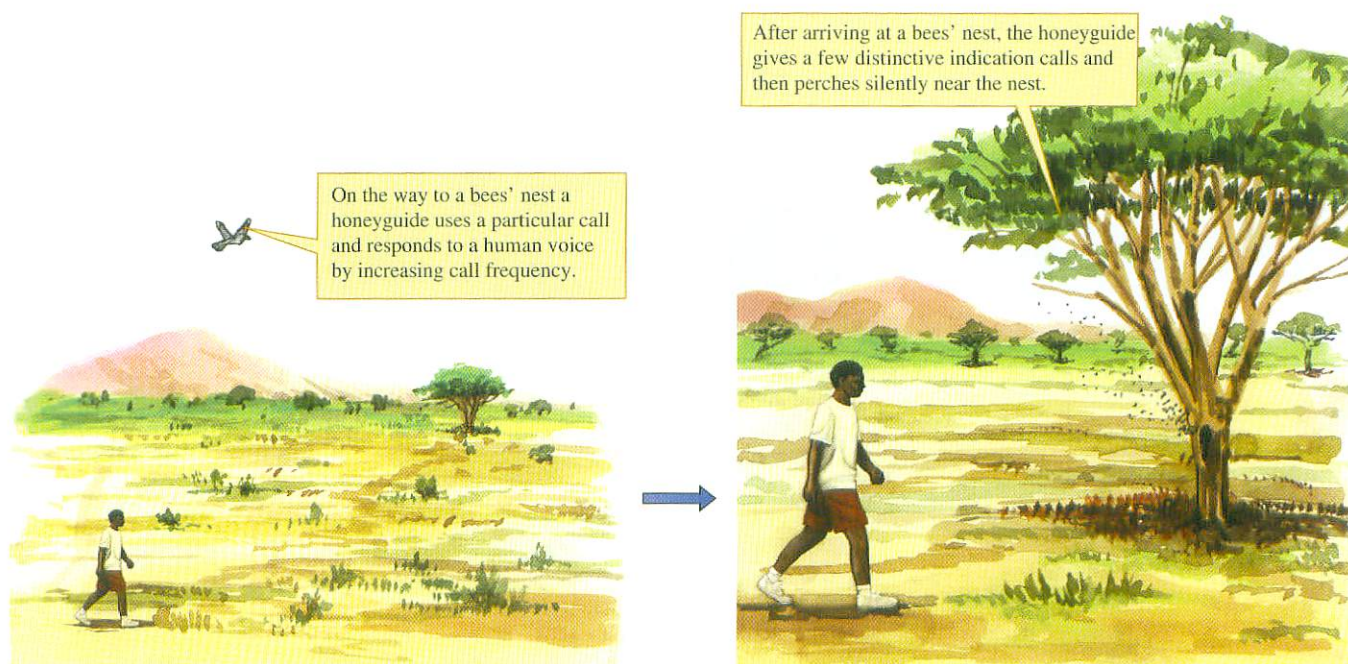
On the way to a bees' nest a honeyguide uses a particular call and responds to a human voice by increasing call frequency.

After arriving at a bees' nest, the honeyguide gives a few distinctive indication calls and then perches silently near the nest.

Figure 15.23   Vocal communication between honeyguides and humans.

including that when bees' nests are very far away (over 2 km) the honeyguide will "deceive" the gatherers about the real distance to the nest by stopping at shorter intervals. Isack and Reyer add, however, that they have no reason to doubt these other statements, since all others have been supported by the data they were able to collect. What these data reveal is a rich mutualistic interaction between wild birds and humans. The results of Isack and Reyer's study caused Robert May (1989) to wonder how much important ecological knowledge may reside with the dwindling groups of native people living in the tropical regions of the world, regions about which the field of ecology has so little information.

Summary

Mutualism, interactions between individuals that benefit both partners, is a common phenomenon in nature that has apparently made important contributions to the evolutionary history of life and continues to make key contributions to the ecological integrity of the biosphere. Mutualisms can be divided into those that are *facultative,* where species can live without their mutualistic partners, and *obligate,* where species are so dependent on the mutualistic relationship that they cannot live without their mutualistic partners.

Plants benefit from mutualistic partnerships with a wide variety of bacteria, fungi, and animals.   Mutualism provides benefits to plants ranging from nitrogen fixation and enhanced nutrient and water uptake to pollination and seed dispersal. Ninety percent of terrestrial plants form mutualistic relationships with mycorrhizal fungi, which make substantial contributions to plant performance. Mycorrhizae, which are mostly either vesicular-arbuscular mycorrhizae or ectomycorrhizae, are important in increasing plant access to water, nitrogen, phosphorus, and other nutrients. In return for these nutrients, mycorrhizae receive energy-rich root exudates. Experiments have shown that the mutualistic balance sheet between plants and mycorrhizal fungi can be altered by the availability of nutrients. Plant-ant protection mutualisms are found in both tropical and temperate environments. In tropical environments, many plants provide ants with food and shelter in exchange for protection from a variety of natural enemies. In temperate environments, mutualistic plants provide ants with food but not shelter in trade for protection.

Reef-building corals depend upon mutualistic relationships with algae and animals.   The coral-centered mutualisms of tropical seas show striking parallels with terrestrial plant-centered mutualisms. Mutualistic algae called zooxanthellae provide reef-building corals with their principal energy source; in exchange for this energy, corals provide zooxanthellae with nutrients, especially nitrogen, a scarce resource in tropical seas. The mutualism between corals and zooxanthellae appears to be largely under the control of the coral partner, which chemically solicits the release of organic compounds from zooxanthellae and controls

zooxanthellae population growth. Crabs and shrimp protect some coral species from coral predators in exchange for food and shelter.

Theory predicts that mutualism will evolve where the benefits of mutualism exceed the costs.   Keeler built a cost-benefit model for the evolution and persistence of facultative plant-ant protection mutualisms in which the benefits of the mutualism to the plant are represented in terms of the proportion of the plant's energy budget that ants protect from damage by herbivores. The model assesses the costs of the mutualism to the plant in terms of the proportion of the plant's energy budget invested in extrafloral nectaries and the water, carbohydrates, and amino acids contained in the nectar. The model predicts that the mutualism will be favored where there are high densities of ants and potential herbivores and where the effectiveness of alternative defenses are low.

Humans have developed a variety of mutualistic relationships with other species, but one of the most spectacular is that between the greater honeyguide and the traditional honey gatherers of Africa. In this apparently ancient mutualism, humans and honeyguides engage in elaborate communication and cooperation with clear benefit to both partners. The mutualism offers the human side a higher rate of discovery of bees' nests, while the honeyguide gains access to nests that it could not raid without human help. Careful observations have documented that the honeyguide informs the honey gatherers of the direction and distance to bees' nests as well as of their arrival at the nest.

Key Terms

arbuscular mycorrhizal fungi (AMF) 349	confidence interval 360	facultative mutualism 348	obligate mutualism 348
arbuscule 349	ectomycorrhizae (ECM) 349	hyphae 349	selection coefficient 362
balanced growth 357	extrafloral nectary 355	level of confidence 360	vesicle 349
		mutualism 348	

Review Questions

1. List and briefly describe mutualistic relationships that seem to contribute to the ecological integrity of the biosphere.

2. What contributions do mycorrhizal fungi make to their plant partners? What do plants contribute in return for the services of mycorrhizal fungi? How did Hardie (1985) demonstrate that mycorrhizae improve the water balance of red clover? How do mycorrhizae improve the capacity of plants to take up water from their environment?

3. Outline the experiments of Johnson (1993), which she designed to test the possibility that artificial fertilizers may select for less mutualistic mycorrhizal fungi. What evidence does Johnson present in support of her hypothesis?

4. Explain how mycorrhizal fungi may have evolved from ancestors that were originally parasites of plant roots. Do any of Johnson's results (1993) indicate that present-day mycorrhizal fungi may act like parasites? Be specific.

5. Janzen (1985) encouraged ecologists to take a more experimental approach to the study of mutualistic relationships. Outline the details of Janzen's own experiments on the mutualistic relationship between swollen thorn acacias and ants.

6. Inouye and Taylor's study (1979) of the relationship between ants and the aspen sunflower, *Helianthella quinquenervis,* provides a reasonable representative of temperate ant-plant protection mutualisms. Compare this mutualism with that of the tropical mutualism between swollen thorn acacias and ants.

7. How are the coral-centered mutualisms similar to the plant-centered mutualisms we discussed in chapter 15? How are they different? The exchanges between mutualistic partners in both systems revolve around energy, nutrients, and protection. Is this an accident of the cases discussed or are these key factors in the lives of organisms?

8. Outline the benefits and costs identified by Keeler's (1981, 1985) cost-benefit model for facultative ant-plant mutualism. From what perspective does Keeler's model view this mutualism? From the perspective of plant or ant? What would be some of the costs and benefits to consider if the model was built from the perspective of the other partner?

9. How could you change the Lotka-Volterra model of competition we discussed in chapter 13 into a model of mutualism? Would the resulting model be a cost-benefit model or a population dynamic model?

10. Outline how the honeyguide-human mutualism could have evolved from an earlier mutualism between honeyguides and honey badgers. In many parts of Africa today, people have begun to abandon traditional honey gathering in favor of keeping domestic bees and have also begun to substitute refined sugars bought at the market for the honey of wild bees. Explain how, under these circumstances, natural selection might eliminate guiding behavior in populations of the greater honeyguide. (In areas where honey gathering is no longer practiced, the greater honeyguide no longer guides people to bees' nests.)

Suggested Readings

Allen, M. F. 1991. *The Ecology of Mycorrhizae*. Cambridge, England: Cambridge University Press.

A thorough, concise, and readable overview of the ecology of mycorrhizae.

Becker, J. H. A. and A. S. Grutter. 2005. Client fish ectoparasite loads and cleaner shrimp *Urocaridella* sp. c hunger levels affect cleaning behaviour. *Animal Behaviour* 70:991–96.

This study, which delves into the behavioral details underlying a cleaning symbiosis on the Great Barrier Reef, finds coherence between the predictions of optimal foraging theory and theories of mutualism.

Bever, J. D., P. A. Schultz, A. Pringle, and J. B. Morton. 2001. Arbuscular mycorrhizal fungi: more diverse than meets the eye and the ecological tale of why. *BioScience* 51:923–31.

Interesting account of diversity among arbuscular mycorrhizal fungi.

Bronstein, J. L. 1994. Our current understanding of mutualism. *The Quarterly Review of Biology* 69:31–51.

Bronstein reviews studies of mutualism set within the perspective of approaches used to study competition and predation. She identifies several key research questions to guide future studies of mutualism.

Buscot, F., J. C. Munch, J. Y. Charcosset, M. Gardes, U. Nehls, and R. Hampp. 2000. Recent advances in exploring physiology and biodiversity of ectomycorrhizas highlight the functioning of these symbioses in ecosystems. *FEMS Microbiology Reviews* 24:601–24.

This paper provides a microbiological perspective on the connection between the physiology and biodiversity of ectomycorrhizae to ecosystem function.

Eckardt, W. and K. Zuberbühler. 2004. Cooperation and competition in two forest monkeys. *Behavioral Ecology* 15:400–411.

This fascinating study shows how two species cooperate, despite a near complete overlap in niches, apparently due to the mutual need for defense against predators.

Hoeksema, J. D. and E. M. Bruna. 2000. Pursuing the big questions about interspecific mutualism: a review of theoretical approaches. *Oecologia* 125:321–30.

An overview of the fundamental questions regarding the evolution of interspecific mutualisms.

Huntzinger, M., R. Karban, T. P. Young, and T. M. Palmer. 2004. Relaxation of induced indirect defenses of acacias following exclusion of mammalian herbivores. *Ecology* 85:609–14.

The researchers found that following exclusion of herbivores swollen thorn acacias reduce their allocation to defensive ant mutualists.

Mueller, U. G., T. R. Schultz, C. R. Currie, R. M. M. Adams, and D. Malloch. 2001. The origin of the attine ant-fungus mutualism. *Quarterly Review of Biology* 76:169–97.

Fascinating consideration of the origin of the mutualism between leafcutter ants and their dietary fungi.