CHAPTER 2

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The Arms Race

There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved.

—Charles Darwin, *On the Origin of Species*, final sentence

A monarch butterfly is perched on a milkweed flower, ready to take a sip of nectar. What a site of harmony in nature (fig. 2.1). But this butterfly is not a pollinator of milkweed. Instead, the butterfly is hoping to find a mate and then to have children that will devour the milkweed plant. Through evolution, the butterfly has adapted to exploit the plant, but there is nothing in it for the milkweed. And through a process called coevolution, the milkweed does not invite the monarch, but rather tries to ward it off. Their battle has been intense, so much so that back in Darwin's day, scientists used monarchs and milkweeds to advance new ways of looking at nature. Out of these insights, new disciplines of science emerged, including what we now call chemical ecology. What scientists eventually discovered was that the monarch-milkweed relationship initiates an extraordinary arms race.

EVOLUTION AND COEVOLUTION

All organisms on the planet, from ants and bacteria to cats and dogs, to elephants, figs, and giant saguaros, descended from a single, universal common

FIGURE 2.1. A male monarch butterfly perched on the flowers of common milkweed in the author's front yard.

ancestor. Darwin got it right when, in the last paragraph of his 1859 opus, *On the Origin of Species*, he wrote about this common ancestor and the subsequent and sustained evolution of diverse forms. This represents what is termed "macroevolution," that which involves the generation (and loss) of distinct species. Such evolution is evident all around us, and many biologists spend their time interpreting the features of life in the context of evolution, because that is the only context in which biology makes sense.

The mechanism of Darwinian evolution is what we term "microevolution." Microevolution is a change in the frequency of alternate forms of

genes (or alleles) within a population of a single species, where these genes code for traits presumably important to the organism's form and function. Through natural selection, the frequency of particular genes in a population changes. Think about the four human blood types (A, B, AB, and O), which are determined by a single gene. In some human populations, natural selection has favored a higher representation of particular forms of that gene, typically because different blood types are more or less resistant to particular diseases.

In their simplest forms, micro- and macroevolution are beautiful, powerful, and commonplace at the same time. Polar bears' white fur helps to camouflage them in the arctic, while their underlying black skin absorbs heat. Plants living in deserts have evolved many ways to conserve water, and animals that live in caves commonly lose their eyesight over evolutionary time. But evolution occurs not only with respect to the physical and chemical attributes of the environment (such as moisture, light, and temperature), but also in relation to the "biotic environment," the other organisms with which a species interacts. Take, for example, the evolutionary origin of the revolutionary drug penicillin.

Fungi that eat dead and decaying things, like the *Penicillium* molds that gave us penicillin, evolved to produce antibiotics to defend the resource they are eating from other tiny consumers, like bacteria. Each of the hundreds of species in the genus *Penicillium* produces distinct chemicals, most of which have been shaped by natural selection. Natural selection can be likened to a filter. As long as there is heritable variation in a population, natural selection can cause evolutionary change, because those individuals with advantageous traits are the ones who survive and successfully reproduce. Natural selection hones traits within populations of a species, and as populations diverge, macroevolution takes hold, allowing for speciation. In the case of *Penicillium*, diverse antibacterial toxins have evolved as a defense among the many species in this genus of fungi.

Coevolution takes evolution in response to the biotic environment to the next level (fig. 2.2). When organisms interact, like the competing molds and
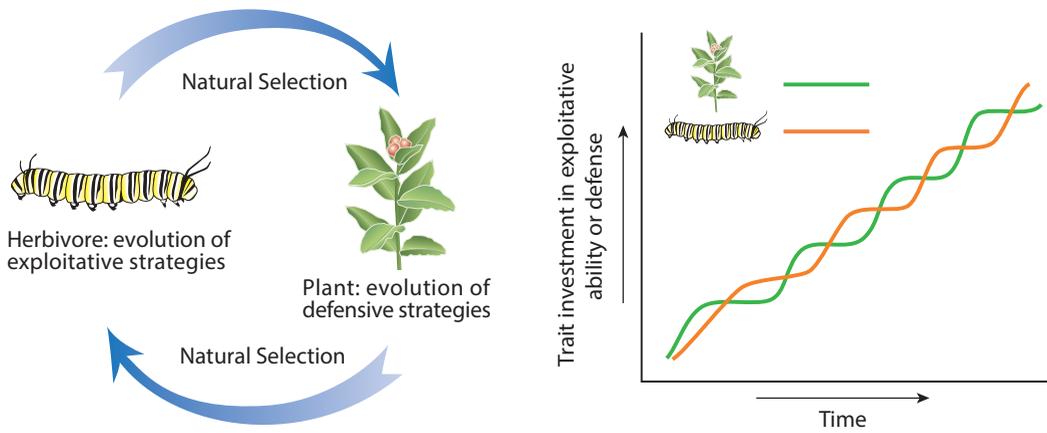
FIGURE 2.2. Two ways to envision coevolution. *Left*, reciprocal natural selection is illustrated as a continuous cycle. *Right*, the same process over time results in an "arms race," with increased investment by the plant in defensive traits and by the insect in traits that exploit the plant. Note that there are other possible outcomes of coevolution not discussed here.

bacteria, or the mutually beneficial relationship between flowering plants and pollinating bees, or the cat-and-mouse game of predators and prey, coevolution is possible. Coevolution is the reciprocal adaptation that occurs as species interact. The term "adaptation" means that the frequencies of particular beneficial genes in a population will increase. In coevolution, the changes are imposed by the interaction partner, say the cat imposing natural selection on the mouse. The evolutionary response to this selection may be that mice are better camouflaged or faster runners, as the more apparent or slower individuals will be removed from the population by predators. Critically, what makes coevolution different from everyday evolution is that coevolution involves reciprocity. That is, following adaptation by the mouse, say with faster and faster running speeds, natural selection is now imposed on the cat, causing the evolution of a more acute ability to sniff out or chase down mice.

The birth of coevolution as a concept, studied as the warfare between plants and herbivores, is credited most prominently to Ernst Stahl, who summarized his studies of snails and plants in 1888, only a few years after Darwin passed away. Stahl developed a theory of coevolution, and his framework set the stage

for unraveling some of the mysteries of monarchs and milkweeds. His critical experiment was to take plant species that appeared to be avoided by snails in the field, to show that in the laboratory snails refused to eat these plants, and then to remove chemicals from the leaves using an extraction procedure (imagine soaking the leaves in alcohol overnight) and showing that those leaves were now palatable to the same snails. He interpreted his results in light of "reciprocal adaptation," a very early reference to what we now call arms race coevolution. In particular, Stahl noticed that some snails were generalist feeders, and their ability to eat plants was enhanced by removal (extraction) of the plant's chemical defenses. Alternatively, other snail species limited their diet to one or a few plant species—call them "specialist herbivores"—and these snails were observed to prefer plants in their intact state. It stood to reason that specialists were engaged in an arms race with the plants, eventually overcoming the plants' defenses, and even using these "defenses" for their own purposes.

MONARCHS, I DON'T REALLY NEED YOU

How species involved in interactions evolve and coevolve depends acutely on the nature of the interaction. If two species are strictly antagonists, then an arms race may ensue. Sometimes, however, species play dual roles, positive under some circumstances and negative in others. In this case, say if the monarch were both a beneficial pollinator and an herbivorous pest of the milkweed, perhaps the plant would not mount defenses. The benefits of butterfly pollination to milkweed could outweigh the costs of caterpillar herbivory. But here is where I must dispel a widely held myth about monarch butterflies. Milkweeds do not need monarchs, because the butterflies are simply no good as pollinators. Monarchs are strictly pests.

Unlike many other coevolutionary relationships, that between monarchs and milkweeds is not symbiotic. Although many definitions of symbiosis exist, nearly all require the relationship to be close, and many definitions require the relationship to be mutually beneficial. From the monarch's perspective, the

relationship is intimate and beneficial. However, from the milkweed's perspective, it is neither. The importance of this distinction will be increasingly apparent as we move forward, but for now let's focus on the fact that monarchs frequently can be found collecting nectar from milkweed flowers. They drink nectar, but they are typically ineffective at pollinating the flowers. Without understanding this issue, we cannot understand the nature of the monarch-milkweed arms race.

First, a bit of the birds and the bees—the reproductive biology of milkweeds. All milkweeds are perennial (they typically live and reproduce for many years). Despite their disappearance aboveground in the winter, the bulk of their biomass lives underground in the soil, stays dormant in the winter, and has plenty of stored reserves to power new shoots in the spring. Additionally, some, although certainly not all, milkweeds are clonal. That is, they send stems foraging underground and pop up new shoots when needed. This cloning of stems is in part responsible for common milkweed's weediness. In addition, milkweeds reproduce sexually. They produce flowers, attract pollinators, send pollen to other plants, and receive pollen to fertilize ovaries and initiate the production of seeds (fig. 2.3). Most milkweeds do not accept their own ("self") pollen; they need to receive pollen from an independent milkweed plant in order to successfully make a fruit full of seeds. Milkweeds cover a lot of bases with this way of life. They live many years with many opportunities for reproduction, which after all, is the main goal of all life. Where conditions are good and a particular plant is successful, milkweeds clone themselves locally. But, as a means to colonize new habitats, and to mix genes with mates, milkweeds engage in pollination and sex.

Most important, the reproductive cycle of milkweeds does not include a role for monarchs. Although frequent visitors and drinkers of milkweed's nectar, monarchs are ineffective extractors and deliverers of pollinia (or pollen sacs). Milkweed flowers do not offer up loose pollen grains the way 90 percent of plants do. In fact, two groups of flowering plants, orchids and the subfamily that includes milkweeds (Asclepiadoideae) have evolved pollen packages called
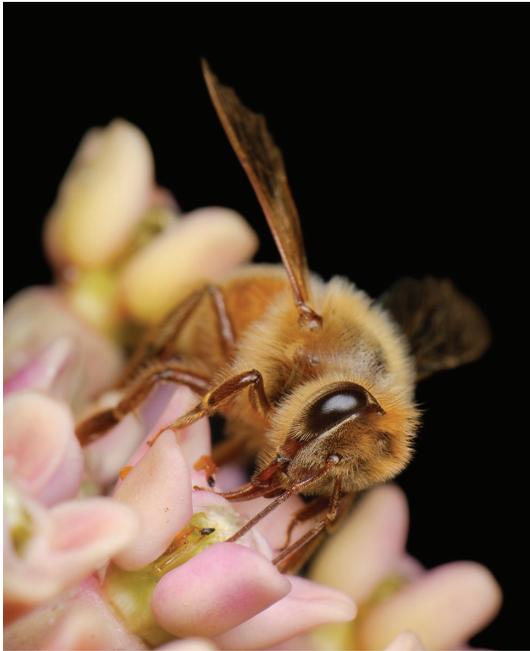
a   b

FIGURE 2.3. Pollination of milkweeds. When bees and other large insects in the order Hymenoptera visit milkweed flowers to drink nectar, (a) their legs slide near the flower's slit, and the bee's leg hairs often grab the top of the (b) wishbone-shaped pair of pollinia. (c) A successful removal of the pollinia results in its becoming attached to the bee's leg. Later, when the bee climbs over other flowers, a pollinium incidentally gets inserted into a slit, allowing

"pollinia," each with hundreds of tiny pollen grains. Milkweeds do not have loose pollen grains that can be collected by bees, rubbed onto insect abdomens, stuck to a butterfly proboscis (the tubular drinking mouthpart), or blown in the wind. So, for pollination to occur, the pollinia must be extracted from the milkweed flower and then inserted into the flower's female slit (or "stigmatic groove" in botanical terms). As my colleague Steven Broyles from the State University of New York at Cortland says, monarchs, with their long legs, simply don't contact the business-end of the milkweed flower.

In other plant-insect associations, the same insect species may serve as a pollinator and also as herbivore of the plant. Although this is most famously known from the yucca plant and yucca moths, and figs and fig wasps, many plant-

c

d

for fertilization of the ovules by germinating pollen grains. (d) Monarchs, however (shown here on *Asclepias tuberosa*), are not good pollinators of milkweed. Because of their large size and way of sitting on flowers, monarchs uncommonly come into contact with the pollinia and slit.

insect relationships follow suit. For example, the cabbage white butterfly (*Pieris rapae*), perhaps the most abundant butterfly in the world, not only pollinates mustards (relatives of cabbage in the family Brassicaceae), but also lays eggs on the plants after pollinating. Their larvae are voracious herbivores of mustard leaves and flowers. In such relationships, there is a conflict for the plant: how to attract and effectively use a pollinator without suffering from that pollinator's young consuming the plant. No such conflict exists in the milkweed-monarch matrix, so the plant can focus its energies first and foremost on defending against monarchs as pests.

This nonpollinating aspect of monarchs is not widely appreciated. Although monarchs may successfully pollinate some plant species (perhaps in the sun-

flower family, Asteraceae), this phenomenon has not been well-studied, and they are surely unimportant compared with the myriad other flower visitors. Nonetheless, in a recent presidential memorandum (June 20, 2014), "Creating a Federal Strategy to Promote the Health of Honey Bees and Other Pollinators," Barack Obama singled out monarchs as the only species other than the honeybee (*Apis melifera*) to be named as an important pollinator. Because the monarch is *not*, however, a good pollinator, the arms race proceeds, with plants evolving defenses to reduce attack by monarchs and insect adaptations to overcome these defenses.

EAT AND DON'T BE EATEN

The arms race signifies a battle between species, although not one where they are simply stockpiling weapons to destroy each other. In the arms race between plants and herbivores, plants evolutionarily accumulate defenses while the herbivore evolves means to circumvent these defenses. For milkweeds, the plant has evolved several forms of armament, including potent toxic chemicals, and monarchs have evolved the physiological means to tolerate these chemicals. As coevolution proceeds, escalation in defense and offense reciprocally ensues (see fig. 2.2).

But life isn't so simple. Every organism has its need for food and also has its own enemies to contend with. A major issue for most animals, really any organism, be it vegetable, animal, or germ, is to eat and avoid being eaten. These factors, along with finding mates and surviving abiotic stresses (such as extreme temperature, weather, and ultraviolet light) make up a large fraction of what we might call the ecology of an organism. Insects are no different: cope with the environment, eat, and don't be eaten. For insect herbivores, the food is decidedly vegetable, but what plant species to eat, what specific plant tissues to focus on, and having a sufficient supply, especially one that is not highly sought after by competitors, are important factors. Ditto for not being eaten. Insect herbivores may hide or avoid predators by crypsis (blending into the

Butterflies and Birds.

From Country Life in America.

Of all the "children of the air" that gladden a June day, the monarch butterfly is one of the most noticeable. Its wings shimmer like gold alloyed with copper as it pursues its lazy flight in the sunshine. The male monarch is a true dandy and carries on each hind wing a black sachet bag containing a strong perfume, most attractive to the other sex. The monarch is immune from bird enemies; the callow birdling that takes a bite from it wipes his beak in disgust and forever after connects the noisome taste with orange wings. A too hasty conclusion of which the Viceroy butterfly takes advantage and, by donning the monarch's uniform, escapes scatheless, although any bird might find it a beaksome morsel.

FIGURE 2.4. Newspaper article about mimicry from the *Washington Post*, June 8, 1902.

environment), or they may simply flee when they sense the risk of predation. However, some animals, like the monarch butterfly, eat in the open, do not blend in with their plant, and seem to advertise themselves through highly contrasting coloration. As we will see in later chapters, the coloration of monarchs is linked to their toxic diet. This is where the monarch-milkweed arms race took a turn, with monarchs taking advantage of milkweed's defensive poisons.

As early as the late nineteenth century, it was known that monarch butterflies were unpalatable, living with immunity from most predators, especially birds. A newspaper account from 1902 accurately describes not only their toxicity, but how bird predators learn to avoid the monarch's coloration and how the unrelated, and less toxic, viceroy butterfly benefits from coloration similar to that of monarchs (fig. 2.4). This phenomenon, termed Batesian mimicry, after the English naturalist Henry Walter Bates of the same era, will be discussed in chapter 6. What was less widely accepted at the time, but still studied among scientists today, is the origin of the butterfly's distastefulness.

YOU MAKE ME PUKE

In the late nineteenth century, a Belgian scientist, Léo Errera, was studying plant poisons called alkaloids, widely known for their pharmacological effects. Caffeine, capsaicin, morphine, and nicotine are all alkaloids, to name a few. In 1887, he made conclusions about their functional role in plants: "Most alkaloid-containing plants are avoided by browsing animals. A few grams of alkaloids are equally efficient protective means as the most forceful thorns." But what if in the course of a coevolutionary arms race, specialized herbivores not only overcame this toxicity, but evolved a means to put these chemicals to work for themselves? This hypothesis is termed "sequestration" and suggests that at a late stage in the arms race, some herbivores might be taking in and storing toxic compounds from their host plants. Sequestration is at the intersection of "eat" and "avoid being eaten," because through the herbivore's leafy diet, a sequestered toxin is used to avoid being eaten by predators.

If the plant produces toxins that are ultimately sequestered by herbivores for their own defense, then perhaps natural selection will favor reduced production of plant toxins. E. B. Poulton, a British evolutionary biologist, issued a call in 1914 for chemists to collaborate with biologists to solve the mystery of what makes monarch butterflies distasteful. Was it indeed the consumption of toxic substances from their milkweed host plants? And what are those substances? It took some sixty years to answer this question. Leading the charge was a group of talented scientists, including the Nobel Prize–winning Swiss chemist Tadeus Reichstein, the British naturalist Miriam Rothschild, and the young American lepidopterist Lincoln Brower. Not only did these scientists identify monarchs and milkweeds as one of the premier species pairs through which to study coevolution, but they also helped to establish chemical ecology as a discipline in its own right.

The story begins with Dame Miriam Rothschild, a naturalist, one of the founders of the field of chemical ecology, and someone who had a knack for bringing people together. She was an eccentric force of nature, known for her

love of fleas, her "delightfully disheveled garden," and her flamboyant purple dresses, typically coupled with a kerchief around her hair and high-top sneakers. She had no formal education, and yet she had a keen sense of the biology of organisms and for frontier questions in science. Rothschild had been studying parasites and butterflies, among other things, and had been interested in their toxicity and mimicry. In the early 1960s, she encouraged a graduate student at Oxford, John Parsons, to pursue the toxicity of insects feeding on milkweeds. Their choice of a toxic food and their advertisement with bright colors had suggested to Rothschild, as it had to naturalists before her, that sequestration (the accumulation of toxins from the host plant) was likely.

Indeed, the toxic properties of milkweed were reminiscent of another plant, foxglove (*Digitalis*, from which the well-known drug takes its name), recognized to be emetic (vomit-inducing) for centuries. We now know that both milkweeds and foxglove contain compounds called cardenolides, and in the next chapter I will examine the essential chemical aspects of cardenolides in order to understand the milkweed-monarch arms race. Rothschild was interested in the hypothesis that similar chemical toxins in milkweed were finding their way into monarchs, making them distasteful and vomitous to bird predators. Although Parsons was not able to trace cardenolides moving from milkweeds to monarchs (could the monarchs be making them independently?), he did show the digitalis-like properties of several milkweed-feeding insects in a series of papers from 1963 to 1965. The last of these studies showed that monarch butterflies contained such a substance in the chrysalis and adults. Parsons purified the compounds and showed that they had toxic effects on frog hearts, guinea pig intestines, the blood pressure of cats, and enzymatic activity of human blood cells, and that they also caused starlings to vomit.

Next enter Tadeus Reichstein, a Swiss steroid chemist, fascinated by both animals and plants. In 1950, he was awarded the Nobel Prize in physiology or medicine (with E. C. Kendall and P. S. Hench), for work that resulted in the discovery of cortisone, one of the most important stress hormones in animals. Only one year later, in 1951, he described cardenolides from close relatives of

milkweeds (the same class of steroidal compounds that give *Digitalis* its kick). And a decade later, in 1964, Rothschild wrote a letter to Reichstein, requesting his assistance in isolating cardenolides from monarchs. At the time, there were no known steroids derived from insects, and accordingly this was a fateful challenge for the renowned chemist. Rothschild and Reichstein engaged in a collaboration that would last more than a decade, and their first preliminary results were reported by Rothschild at a conference in 1966.

Lincoln Brower, a graduate student at Yale University, received his doctorate in 1957, working on the evolution of swallowtail butterflies. He quickly became obsessed with the hypothesis of mimicry among butterflies and the idea of sequestration, the notion that butterflies could accumulate toxic chemicals from host plants, thereby using these for their own benefit. In fact, his laboratory at Amherst College did much of the rearing for Parsons's, Reichstein's, and Rothschild's work, generating kilograms of monarchs (literally thousands of butterflies) for chemical analyses conducted in Europe. Not having access to the right equipment, and lacking abilities in chemistry himself, Brower and his colleagues took an unimaginably novel approach for their own studies of sequestration. Brower took thousands of monarch eggs and attempted to rear them on cabbage, which he assumed to be a benign host plant without toxins. If monarchs were gaining their toxicity from milkweeds, a cabbage-reared monarch would not be distasteful. His critical assay was not for the presence or absence of a chemical, but the behavior of a bird. In other words, Brower addressed the ecological consequences of the monarch's diet: would birds vomit if they ate monarch adults reared on milkweed (fig. 2.5), but not if they were reared on cabbage? He reasoned that if sequestration of plant poisons was important, birds would feed without nausea on cabbage-reared monarchs.

The reason this approach seems unimaginable is that, in nature, monarchs eat only milkweed. Period. If you asked me, after studying monarch caterpillars for more than a decade, could I ever get them to eat cabbage, my answer would be a resounding no, and without a second thought. So, how did he do it? Over five butterfly generations, Brower reared monarch caterpillars bit by bit,

FIGURE 2.5. Lincoln Brower's famous images of a blue jay barfing after feeding on a monarch butterfly. This highly repeatable assay of monarch toxicity usually concludes within twelve minutes.

only as long as they survived on cabbage. Hatched from thousands of eggs, nearly all the caterpillars were destined for a quick death by starvation, since they simply did not eat the unfamiliar food. In the first few generations, the larval survival rate was very low, with most of the caterpillars that did attempt to feed dying midway through development. Caterpillar death was not likely due to cabbage being deficient in some essential nutrient, but it could have been the result of cabbage's own defense compounds (mustard oils, or gluco-sinolates), or perhaps more likely because monarchs simply did not recognize cabbage as food. Whatever the case might have been, Brower persisted, switching them back to milkweed when they were near death. After this persistent set of rearings, in the fifth generation, Brower had nurtured a few caterpillars to adulthood that had fed only on cabbage and never on milkweed.

Working with wild-caught blue jays, and assaying their frequency of vomiting, he had an elegant experimental design. The birds were fed one of four foods: (1) monarch caterpillars reared on tropical milkweed (*Asclepias curassavica*), (2) monarch caterpillars reared on cabbage, (3) monarch caterpillars reared on a tropical milkweed vine called *Gonolobus*, or (4) mealworms, a most tasty food for birds. The milkweed-fed monarchs served as a positive control, scientific parlance for a treatment that should elicit an expected result: a barfing blue jay. Mealworms served as the negative control, since there was no expectation of vomiting. Brower's critical result was that cabbage-fed monarchs did not elicit vomiting. And furthermore, *Gonolobus* ended up being the exception that proved the rule. Brower had fully expected monarchs, which do feed on *Gonolobus* in nature, to be toxic when feeding on this milkweed vine. But, alas, the birds did not vomit. Much to his excitement, when Brower sent *Gonolobus* leaves to Reichstein in Switzerland, the results came back negative—no cardenolides. This was the confirmation that he needed to implicate plant toxins, which were now shown to be variable in host plants, as agents of the monarch's toxicity. Among Brower's four treatments, only when monarchs were fed a milkweed with cardenolides did they elicit a vomiting response from blue jays. The results were published in 1967.

In 1968, Reichstein, Parsons, and Rothschild published a study that was the last important step in nailing down proof of the monarch's sequestration. In fact, monarchs had two concentrated cardenolides, calactin and calotropin, in their adult bodies, and these same cardenolides were found in their milkweed host. The authors reasoned, "The fact that the cardioactive toxin of the monarch butterfly is of the cardenolide type . . . supports the suggestion that it is derived from the food plant and stored either unchanged or with only minor metabolic transformation." This paper, building on the three previous studies, sealed the deal on the paradigm of sequestration.

Later on, as the sequestration paradigm was cemented, some tension occasionally arose between Rothschild and Brower. They continued to work independently on monarchs and their sequestered toxins for decades, and perhaps both worried about their respective legacies. Who would be remembered as the discoverer of monarch sequestration? A few points about this scientific tension are worth explaining. First, it was Parsons and Reichstein (not Rothschild or Brower) who did the early heavy lifting in terms of physiology and chemistry, inspired and aided by Rothschild and Brower. They could not have done this work themselves. Second, Rothschild and Brower were themselves collaborative and reciprocally inspiring. In each of their respective early publications, the acknowledgments section is very telling. In most scientific publications, acknowledgments are provided at the end—usually crediting colleagues who contributed substantially and generously, but not enough to warrant being a coauthor of the study. The spirit of the acknowledgments in these four key studies is one of excitement, collaboration, and sharing. Letters, live butterflies, dried leaves, ideas, and inspiring words were being shipped across countries and oceans, with nearly all names appearing in all four papers' bylines or acknowledgments. That is what made this science move forward and, more generally, what makes science great fun. Despite the tension between them in later years, Rothschild and Brower share the legacy of being pioneers of chemical ecology and hugely important in the development of knowledge about monarchs and milkweeds.

THE DARWINIAN DEMON

The fact that monarchs sequester cardenolides from their milkweed host plants does not mean that these herbivores have won the arms race. As we will learn, the plants have responded and will continue to do so. Nonetheless, their co-evolutionary escalation cannot go on forever. It does have limits. All evolutionary adaptations are constrained by a trade-off between energy investment in traits and growth or reproduction, a concept known to biologists as resource allocation. Investment in a beneficial behavior, physical feature, or internal physiology comes at a cost, because that energy cannot be used for some other function. The trade-off concept can be visualized as a Y-shaped tube, whereby all the energy that flows in from the bottom must flow to one of two (or a few) pathways. This Y-tube model has been very influential, probably because it is simple and intuitive. With a limited pool of resources, and nearly all adaptations taking energy, trade-offs must occur (fig. 2.6). Simply put, an organism cannot maximally grow, defend against predators, attract pollinators, and fend off competitors all at once.

Darwin and his predecessors recognized the importance of these counter-weights in biology, as early crop and animal breeders realized that no organism could be bred to have all of the desirable traits. Indeed, because each trait is costly and trades off with allocation to other traits, breeders often develop specialized varieties that favor some traits at the expense of others. In the first chapter of *On the Origin of Species*, Darwin wrote: "As Goethe expressed it, 'in order to spend on one side, nature is forced to economise on the other side.' I think this holds true to a certain extent with our domestic productions: if nourishment flows to one part or organ in excess, it rarely flows, at least in excess, to another part; thus it is difficult to get a cow to give much milk and to fatten readily. The same varieties of the cabbage do not yield abundant and nutritious foliage and a copious supply of oil-bearing seeds."

Monarchs and milkweeds are subject to the same rules, and trade-offs are important in their evolution. We find high levels of genetic variation in toxin
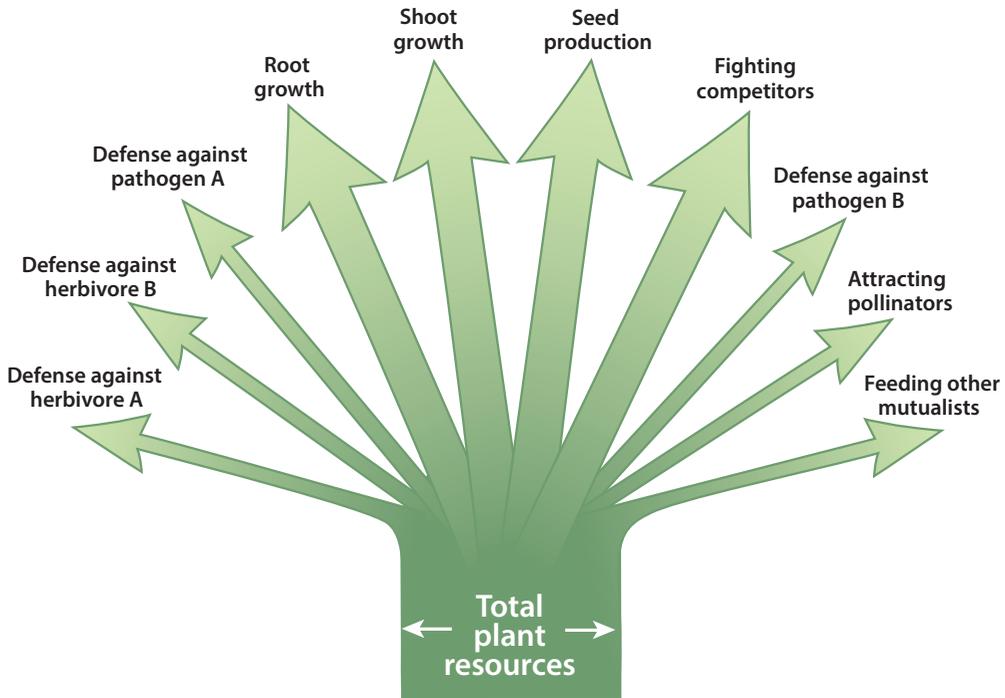
FIGURE 2.6. This diagram shows the allocation of limiting resources. Such diagrams are often drawn with simply two arrows, with energy flowing one way or another, say to growth or defense in a plant. It is now widely understood that even for a subset of the energy budget of an organism, there are many competing demands.

production among populations of common milkweed. The production of milk-weed's major toxins (cardenolides) counterbalances with plant growth (fig. 2.7). Even though cardenolides are costly to make, under some conditions or in some years (for example, when soil nutrients are plentiful and when herbi-vore pressure is high), natural selection favors producing more. Nonetheless, under other conditions, the costs outweigh benefits, and plants are favored to produce minimal amounts of toxin.

Yet, in addition to the energetic drain caused by the production of a toxin (or any other trait), there may also be ecological offsets. Ecological trade-offs are experienced only under certain conditions and are not due to resource

FIGURE 2.7. As common milkweed plants produce more cardenolides, they have slower growth rates. In this graph, each dot represents a genetic family of plants (each family having the same maternal and paternal parent plant), and the shading is the 95 percent confidence interval. Note the *x* axis, which shows that these genetic families exhibit two-fold variation in leaf cardenolide production. The families that produce more cardenolides have proportionally reduced growth rates, demonstrating a cost of producing defense chemicals.
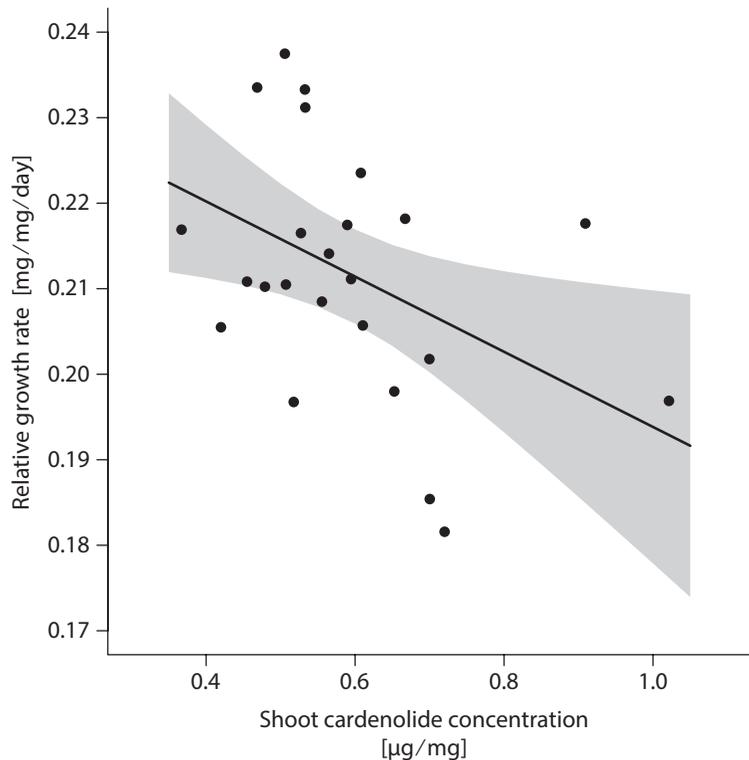
limitation itself. Take, for example, a trait like the mating calls of crickets, which not only bear the cost of production, but also the ecological trade-off of attracting enemies. The chirps of a cricket are information released in the environment, and they may be used by any organism. Professor Marlene Zuk, from the University of Minnesota, found that cricket songs used in mating were also specifically attractive to a parasitic fly. This is just the sort of trade-off that can affect the evolution of an organism's traits, and perhaps even coevolution. Ecological trade-offs work alongside energetic costs and represent conflicts of interest.

In coevolutionary interactions, when one species is imposing natural selection on the other, and the second species is adapting and reciprocally imposing

selection on the first, trade-offs can limit the speed and intensity of an arms race. One can just imagine the arms race grinding to a halt, or ending in stalemate as it were, as the respective species' armament is simply becoming too burdensome, weighty, or expensive. In other words, species may reach a tipping point in either the energetic expenditure or ecological cost for some particular defense that has too high a price for too little benefit. In the case of monarchs and milkweeds, there are many trade-offs on both sides, with not only energetic drains, but also ecological costs associated with offensive and defensive traits.

The discovery that milkweeds do not need monarchs for pollination, but that monarchs need milkweed, both for food and defense, helps us to understand evolution and coevolution through the lens of trade-offs. Although the cardenolides of milkweed provide defense against insects, they also provide protection to one of its major pests, the monarch butterfly. As the milkweed has evolved more toxic cardenolides, monarchs themselves face an ecological trade-off, that of the negative effects of ingesting cardenolides and the positive protective effects of sequestering them as a defense against their own predators. The balancing of such conflicting interests represents a general dilemma faced by all organisms, one that ultimately limits the extent of adaptation. It simply may not be possible for an organism to do it all—and certainly not all at once.

Because of trade-offs, most species tend to harbor a substantial amount of genetically based variation in traits. Under some conditions, for example, when resources are plentiful or when a major predator is present, the most costly defensive trait may be favored. Under other ecological conditions, however, other traits will be bolstered. Because the combinations of environmental conditions are endless, and conditions vary across space and time, most species live as multiple populations, in a patchwork being pushed and pulled by natural selection in different ways. This is how and why species maintain a lot of genetically based variation in traits. Think of this view of evolution as a giant pinball machine, with the ball being flung in all sorts of directions across the

landscape. The ball represents the traits of a species and is flung in different directions by the forces of natural selection, which vary in space and time. Over the long haul, when we zoom out, the result is the maintenance of variation within a species. As long as the far-flung individuals are at least occasionally favored by natural selection, and are also exchanging genes with (mating with) other individuals of the species, the species will continue to harbor variation. Such genetic variation is the raw material for natural selection to work on. And when species are engaged in an intense arms race, spectacular outcomes are possible.

Coevolutionary warfare between monarchs and milkweeds has made use of extraordinary chemical compounds. The cardenolides introduced in this chapter are at the center of their struggle. Although the chemistry of milkweed defense and monarch sequestration is not unique, it is extreme, and it serves as an important model to generally understand how chemical compounds can be a focal point in coevolution. The persistence of their ecological and evolutionary interaction has led to plants that produce heart-stopping toxins and butterflies that have derived molecular solutions to this poisonous challenge.