Jaap de Roode

Tomato hornworms (*Manduca sexta*) are often attacked by Braconid wasps (*Cotesia*). Wasp larvae eat their way out of the caterpillar and subsequently spin cocoons on the outside of the caterpillar. After the wasps emerge from their cocoons, the caterpillar may survive for a while, but will ultimately die before realizing its own metamorphosis.

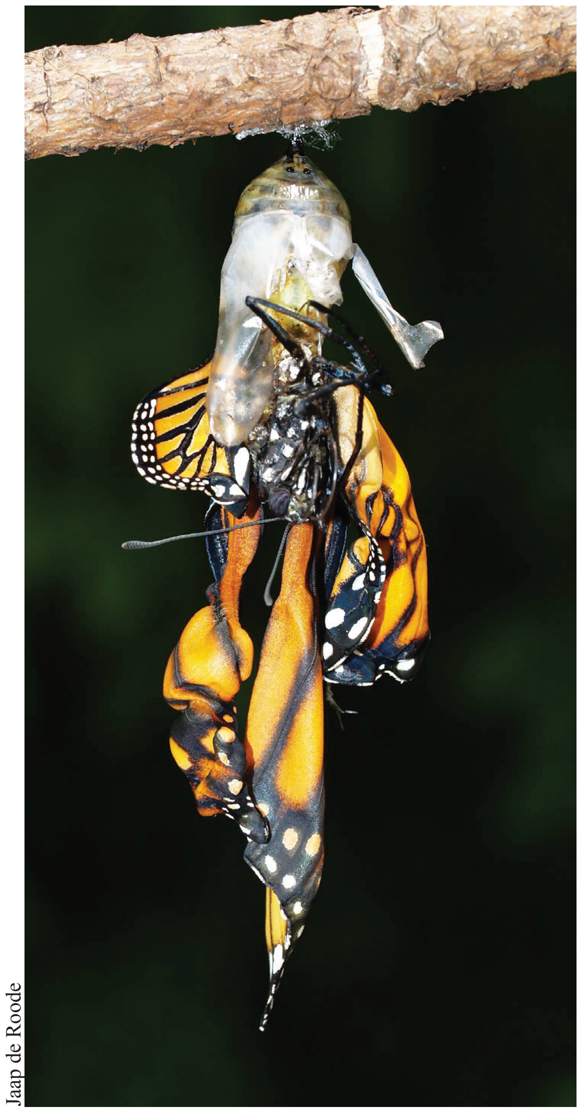
When Butterflies get Bugs:
The ABCs of Lepidopteran Disease

by Sonia Altizer and Jaap de Roode

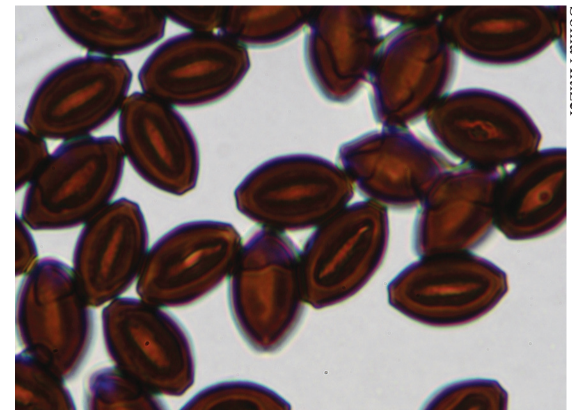
Introduction:
On a cool morning in late winter, sunlight creeps into a high altitude forest in Central Mexico, illuminating the bodies of millions of Monarchs draped over the limbs of oyamel fir trees. As temperatures warm, sunlight wraps around motionless clusters of densely packed butterflies suspended high in the forest canopy. Monarchs open and close their wings as their flight muscles warm in the sun. Suddenly, thousands of butterflies take flight in a bright orange explosion, their jewel colored wings stirring the air under a clear blue sky. A small group of tourists gasp with delight and spectacular disbelief. Soon the air is filled with Monarchs, some landing on the heads and shoulders of visitors standing near the colony's edge.

Jaap de Roode

Jaap de Roode



Jaap de Roode



Sonia Altizer

Top left: A healthy Monarch pupa.

Top right: Under natural conditions, *Ophryocystis elektroscirrha* rarely kills Monarchs before they turn into free-flying adults. However, captive conditions can result in contamination and a build-up of many parasite spores. When caterpillars ingest large numbers of these spores, they often die as pupas

Left: Heavily infected adults may become stuck to their chrysalis when they are trying to eclose. In such cases, neither the Monarch nor its parasites will be able to reproduce.

Above: The parasite is spread to the next generation of caterpillars when females scatter parasite spores (shown at 400x magnification) onto their eggs.

Richard Bartz

Adult braconid wasps are tiny, approximately 1/16 inch in length (actual size of wasp is shown by white line), and females have a long, slender ovipositor protruding from the abdomen."

What the tourists cannot see, however, is that thousands of these Monarchs are infected by a debilitating protozoan parasite called *Ophryocystis elektroscirrha* (page 17). Everywhere the infected Monarchs land they scatter hundreds of parasite spores — on the ground, on tree limbs, and even on people's clothing, cameras and daypacks — leaving microscopic souvenirs clinging in hidden places. In April when the Monarchs return to their breeding grounds in the southern United States, some of the infected Monarchs will join them, spreading the parasites to a new generation of caterpillars.

Most of us are well aware of the risks pathogens pose for human health, in part because of high profile news stories describing outbreaks of emerging diseases like SARS, influenza, Ebola, and West Nile virus. But even the most enthusiastic butterfliers don't often realize that butterflies can get sick, too. This is probably because in the wild, butterflies and caterpillars that die from disease can quickly disintegrate and disappear, leaving little trace of infection. In other cases, insects harboring pathogens might show only subtle outward signs of infection, and disease-causing microbes can be hard to detect without viewing squashed bits of insects under a microscope and using advanced diagnostic guides.

But the fact remains that butterflies, like most other animal species, can be infected by many parasites ranging from viruses and bacteria to parasitic worms and mites. Technically speaking, a parasite is any organism that lives within and obtains nutrients from another organism, called the host — usually to the host's detriment. There are hundreds of parasite species that can spread among butterflies or caterpillars, with major impacts on their development and survival. Examples in this article will illustrate some of the ways that parasites can affect the lives of free-living and captive lepidopterans, and how current human activities might be altering the spread of butterfly parasites in some unexpected ways.

Attack of the Parasitoids

In the science fiction movie *Alien*, larval creatures entered human bodies where they fed, grew, and eventually emerged, ultimately killing their human host. One of the most apparent types of parasites that attack butterflies and moths has a life cycle that follows this same script. Termed parasitoids, these parasitic flies and wasps are actually a hybrid between a predator and a parasite. Tiny as they might be, they pack a powerful punch, almost always killing their host after successful infection. And on the scale of things that are totally disgusting yet simultaneously fascinating, parasitoids rank pretty high up on the ladder.

As one example of a parasitoid group, miniscule wasps in the Braconid family comprise over 1700 North American species. The wasps most frequently target caterpillars, but they can also parasitize the larvae, eggs and pupae of other insects. Adult female wasps have a needle-like ovipositor and can lay 20 or more eggs inside a single insect host. Larvae of the wasps feed on the living caterpillar's tissues for over a week. The wasps can either pupate inside the host, or in the case of braconid wasps parasitizing the tomato hornworm (see photo, page 16), form dozens of white cocoons peppering the caterpillar's back. A tiny wasp will eventually emerge from each cocoon, leaving the caterpillar behind to die.

Parasitoids can employ incredible stealth in locating their butterfly prey, as illustrated by two closely related egg parasitoids (*Trichogramma* sp) of Cabbage Whites and European large whites (*Pieris brassicae*). Tiny female parasitoids (smaller than a dandelion seed) hitch a ride on the backs of mated female

E. Sternberg

Parasitoid flies kill many Monarchs

Left: Adult tachinid flies lay eggs on the outside of Monarch caterpillars, after which the maggot burrows its way into the caterpillar.

Bottom left: After about a week, the maggots will have grown large enough to eat their way out. This particularly unlucky caterpillar harbored no fewer than 3 tachinid fly maggots.

Bottom right: A maggot will then form a small brown pupa.

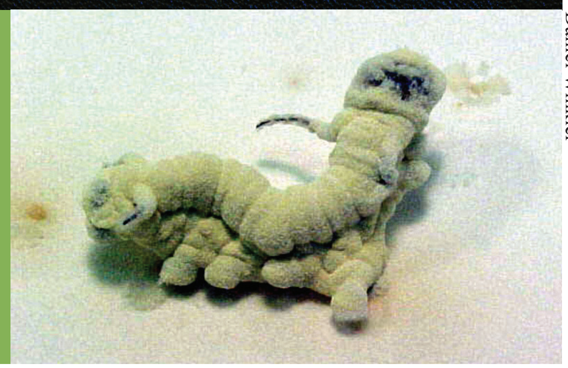
Jaap de Roode

Sonia Altizer

Sonia Altizer

Fungal pathogens of Lepidoptera.
Above: Moth caterpillars killed by *Cordyceps* fungus sprout long stroma (finger-like projections) from their head capsules from which fungal spores are released.

Right: A Monarch caterpillar cadaver infected with *Beauvaria* shows the characteristic growth of white fungal mycelium and conidia.



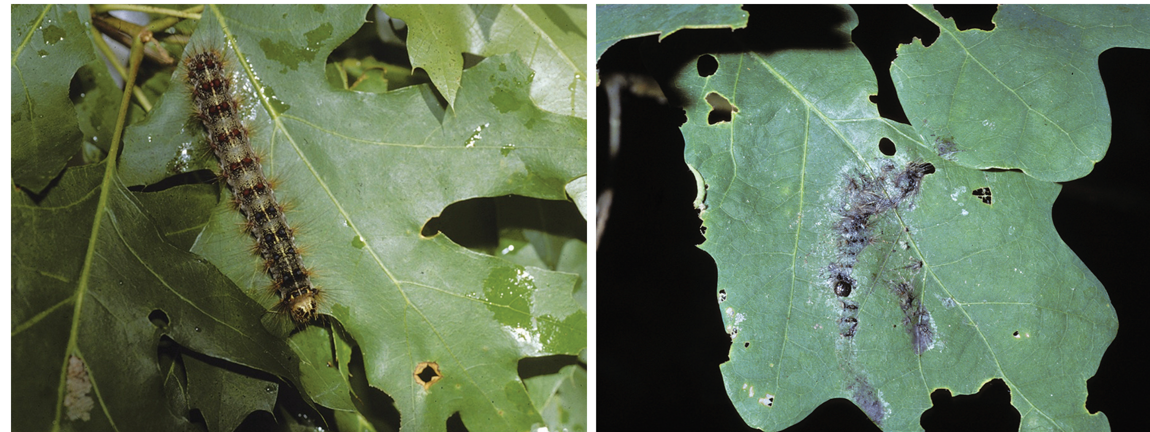
Daniel Winkler

butterflies. After the butterfly lays a clutch of eggs, the parasitoid jumps off and lays its own eggs on or near the host's. Amazingly, these parasitoids hunt down female Cabbage Whites using an anti-aphrodisiac pheromone that male butterflies transfer to females upon mating. This pheromone signals other males to keep away from mated female butterflies, but the same scent lures wasps to freshly mated females.

Given that parasitoids are common and highly lethal, they can have major impacts on caterpillar survival in the wild. For example, Monarchs in the United states are frequently attacked by the generalist parasitoid *Lespesia archippivora*, a tachinid fly that has been reported to infect caterpillars from 14 different families (see page 19). One study of fly parasitism across North America found that up to 30% of Monarchs die from parasitoid attack in some regions and years, with up to 10 fly maggots emerging from a single Monarch pupa. These high rates of parasitism suggest that tachinid flies could be a major factor regulating wild Monarch populations.

Viruses and Fungi and Protozoa… Oh My

Although invisible to the naked eye, microbial pathogens, including viruses, bacteria, and protozoa, can commonly infect butterflies. These pathogens most often attack caterpillars (as opposed to other life stages), and routes of invasion tend to be through the accidental ingestion of capsules or spores. One of the most common and lethal groups of caterpillar infections are caused by baculoviruses,



Alison Hunter (2)

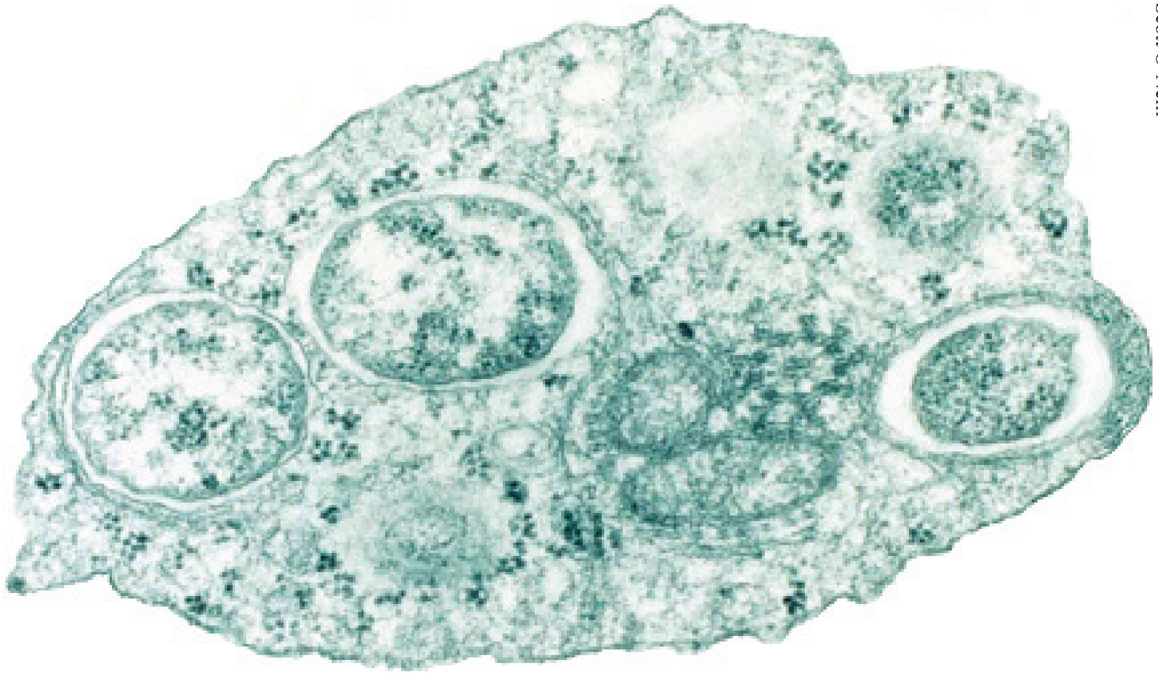
Baculoviruses infect gypsy moth caterpillars.
Left: A healthy gypsy moth caterpillar ingests viral particles (NPV) when feeding on contaminated leaf material.

Right: Within a few days after exposure, the caterpillar dies and rapidly disintegrates, leaving behind a puddle of viral particles and fragments of the caterpillar's cuticle which disseminate viruses to the surrounding leaves.

also called nuclear polyhedrosis viruses (NPVs). These pathogens win the distinction of behaving like the Ebola virus of the insect world (see photos, this page). Once a caterpillar unknowingly ingests NPV capsules, the virus replicates massively inside host cell nuclei, killing the host rapidly by turning it into a sac of jelly, from which millions of viral occlusion bodies (protective capsules containing virus particles) then leak out onto surrounding vegetation. In part owing to their rapid lethality and host specificity (most NPVs infect a narrow range of Lepidoptera), preparations of these viruses have been used as effective biocontrol agents against several caterpillar pests, including gypsy moths in North America, velvet bean caterpillars (*Anticarsia gemmatalis*) in Brazil, and African armyworms (*Spodoptera exempta*) in Tanzania.

Fungi outnumber all other butterfly pathogens by a factor of about 4:1. Instead of causing the animals to disintegrate into goo, fungal pathogens cause a light and fluffy growth over the outside of the animal, giving it a marshmallow-like appearance (see photo, middle of opposite page). That white fluff is a mix of thread-like fungal hyphae and powder-like spores called conidia. The fungal spores disperse in a puff of dust that showers the vegetation, where they wait to be contacted by another caterpillar. Once a spore germinates on the surface of its insect host, the thread-like hyphae can penetrate the host's skin and replicate inside the animal's body cavity.

Some of the most bizarre-looking fungal pathogens are in the genus *Cordyceps*. Caterpillars infected by *Cordyceps* die after their entire body cavity is filled by the fungal hyphae; the fungus then sprouts finger-like projections from the cadavers (opposite page top) to help disperse its spores. In traditional Chinese medicine, *Cordyceps* has been used for medicinal purposes for almost 1500 years, with the goal of maintaining healthy heart function, energy, positive outlook, immune health and even sexual stamina. Current consumption in China and other countries has driven down the abundance of wild-collected *Cordyceps* and led to increasing cultivation of this fungus by humans. It is interesting that a pathogen highly lethal to caterpillars is viewed as promoting the health of humans!



Scott O'Neill

Transmission electron micrograph of Wolbachia bacteria developing within an insect cell.

***Wolbachia*: Harmless Symbiont or Sexual Sabotage?**

Bacteria in the genus *Wolbachia* are some of the most common microbes infecting Lepidoptera and other insects. These maternally-transmitted bacteria don't kill their hosts directly, but can manipulate insect reproduction. Depending on the host species affected, *Wolbachia* can destroy male embryos, turn males into females, or cause mating incompatibility between males and females with unrelated strains. These manipulations give the bacteria a distinct advantage in spreading through host populations, and also cause problems for some butterfly species. In African populations of the White-barred Acraea (*Acraea encedon*), for example, 70-100% of the butterflies are infected with *Wolbachia*. At sites with high infection rates, the proportion of males is exceedingly low, most females will die before mating, and females have taken to mobbing the few remaining males. Extreme loss of males has also occurred in Polynesian populations of the butterfly *Hypolimnas bolina*. In an interesting twist of events, between 2004-2006, scientists discovered a dramatic recovery of males on some of the islands, indicating that these butterflies had evolved resistance to *Wolbachia*'s male-killing properties.

Given the widespread nature of these endosymbiotic bacteria and their ancient associations with insects and other invertebrates, it is tempting to assume that *Wolbachia* do not pose a significant risk for wild insect populations. However, these parasites can reduce host reproduction during the invasion phase, and can also lower host genetic diversity due to extreme sex ratio bias.

This risk was recently evaluated for 'Karner' Melissa Blues. *Wolbachia* that were closely related to strains from the non-endangered western populations of Melissa Blues were found to be common in the western range of 'Karner' Melissa Blues, suggesting that a recent transmission event occurred between the two sub-species. A computer analysis showed that *Wolbachia*-infected 'Karner' Melissa Blue populations faced a 2-4 fold higher probability of extinction than uninfected populations due to the impacts of this pathogen during the invasion phase.

Why do Parasites Cause Disease or Kill their Hosts?

Because of the disease they cause and the killing they do, parasites are not often seen as friendly creatures. But from the parasite's point of view, all that matters in life is to produce offspring that can infect new hosts - even if this requires killing its current host. In this regard, it makes sense for baculoviruses to turn caterpillars into the sticky goo that contains millions of virus offspring. This is because the virus particles that ooze onto leaf material can be ingested by new caterpillars, causing the next generation of infections. And parasitoids can usually only grow big enough to form a cocoon by eating so much caterpillar tissue or blood that the caterpillar will die before completing its own metamorphosis.

But not all parasites kill their hosts. The protozoan *Ophryocystis elektroscirrha*, for example, spreads to new hosts when female Monarchs lay eggs onto which the parasite spores are scattered. These parasites are eaten by caterpillars hatching from these eggs. Because this parasite needs its host to reach adulthood and lay eggs, killing the host prematurely would be a bad idea, and this parasite rarely kills Monarch caterpillars or pupas under natural conditions. This is not to say that *O. elektroscirrha* does not damage Monarchs. Indeed, the parasite needs to produce millions of offspring to successfully transmit spores to Monarch eggs, and this reproduction means that infected adult Monarchs don't live as long or fly as well as uninfected butterflies. Moreover, in captivity, the parasite can often build up to high numbers (unless careful measures are taken to prevent transmission) and thereby cause damaging infections in captive-bred Monarchs.

To facilitate their own transmission, many parasites have evolved the ability to manipulate their hosts' behavior.

A practical guide to preventing disease when rearing butterflies at home

Many people enjoy bringing caterpillars from their gardens indoors to pupate and emerge as adult butterflies. But occasionally, these caterpillars harbor infectious parasites including bacteria, viruses or protozoa.

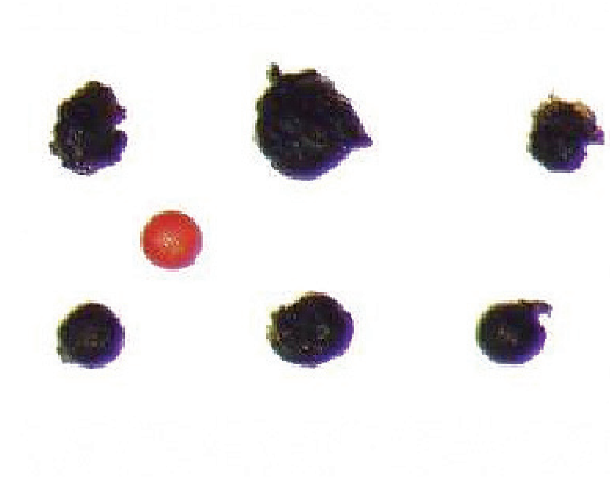
Sick caterpillars might appear lethargic, pale, and may stop eating or fail to molt properly — although some animals harboring low levels of infection could appear identical to healthy caterpillars.

Importantly, many parasite species can produce millions of propagules under the right conditions, and it often takes only one of these to cause a new infection. In nature, the chances of parasite propagules reaching new caterpillars are low, but captive conditions often facilitate the spread of parasites: parasites can easily build up in rearing chambers and thereby cause unwanted infection of caterpillars reared side-by-side with an infected animal.

Because some parasite spores can live for many months after a butterfly is reared and released, a single infected caterpillar could continue to infect multiple generations of butterflies.

One remedy to deal with such contaminations is to regularly sterilize (with 10% bleach solution) rearing chambers, tools and gloves that are used for rearing caterpillars or housing pupas and butterflies. It is also wise to quickly remove any sick-looking caterpillars and individuals to avoid the maturation of parasites and their subsequent spread.

Sonia Altizer



E. Sternberg

Butterfly immune defenses

Above left: Butterflies have hemocytes (immune cells) in their blood; shown here are plasmatocytes, one type of immune cell that helps to encapsulate foreign material.

Above right: To measure encapsulation activity, sephadex beads (shown unencapsulated as red bead) were implanted into a Monarch caterpillar. The next day, beads were extracted, showing the deposition of black pigments onto them. Caterpillars can use such pigments to encapsulate parasitoid larvae and other types of parasites. See the article on page 27 to learn how many wasps avoid this defense.

Baculoviruses and fungal pathogens can cause summit disease, a syndrome that makes caterpillars climb to high vegetation. Here the pathogens will kill the host and produce spores or capsules that can be carried far and wide by the wind to infect new caterpillars. One of the most bizarre examples of host manipulation comes from a braconid wasp parasitoid of the geometrid moth *Thyrinteina leucocerae*. In this case, most parasitoids eat their way out of the caterpillar when they are ready to form a cocoon, which they do on the vegetation outside of the caterpillar. However, a number of wasps stay behind inside the caterpillar and apparently manipulate it to become a bodyguard of the cocoons: the infected caterpillar fends off predators of the wasp cocoons with violent head-swings and finally dies when the wasps have emerged from their cocoons and no longer need the caterpillar.

Butterflies Fight Back

Caterpillars and butterflies can warn or trick predators by using camouflage, false body parts and warning coloration, but how do they fight off microbial diseases? When people get sick, our immune systems mobilize rapidly to fight off infection, and humans can engage in behaviors like hand washing and covering our mouths during a sneeze or cough to limit disease transmission. Like people, butterflies and other insects can deploy several lines of defense to protect themselves against infectious agents. In terms of immune defenses, antimicrobial proteins float around in insect blood, and attach to (and mark or attack) extracellular pathogens. Insects also have immune cells called hemocytes that operate similar to our white blood cells (see photo, top left). Some of these hemocytes can engulf (or phagocytize) microbial parasites directly. Parasites that are too large to be engulfed by immune cells, such as parasitoid eggs, can be encapsulated by aggregations of hemocytes and through melanization, which involves the deposition of melanin pigment, effectively producing a dark capsule around foreign material (see photo, opposite page, top right).

Insects can also employ physical and behavioral defenses to avoid parasites. Gypsy moth caterpillars, for example, can actively avoid feeding on leaves contaminated with virus particles and hence avoid infection. There is also evidence that arctiid moth caterpillars can increase their body temperature by basking in the sun, and this may help them fight their parasitoid flies.

Much like humans have mastered the art of herbal medicine, moths and butterflies also seem able to use nature as an apothecary against their bugs. African armyworms, for example, can increase their dietary intake of proteins, which they use to fuel their immune response against bacterial pathogens. And woolly bear caterpillars can extract toxic chemicals from their host plants to fight their parasitoid flies. Recent studies in our own labs have also shown that Monarch caterpillars that feed on certain milkweed species containing high levels of cardinolides, such as tropical milkweed (*Asclepias currasavica*), experience less severe infections by the parasite *Ophryocystis elektroscirrha*. Interestingly, female butterflies appear able to take advantage of these curative plants by preferentially laying their eggs on them to reduce the disease symptoms of their offspring.

Butterfly Diseases in a Changing World

Humans are currently changing the climate and habitats of butterflies in ways that might affect their interactions with parasites and other natural enemies. In general, at least three types of human activities might impact butterfly-parasite interactions: habitat destruction, climate warming, and releases of farmed butterflies into the environment.

First, global climate change may impact butterfly diseases by affecting pathogen development, survival rates of hosts and parasites, disease transmission processes, and host susceptibility. Because harsh winters can cause the die offs of pathogens that might hang out in the environment, a question raised in recent years is "will a warmer world be a sicker world?" With less seasonality and warmer temperatures, will pathogens survive better and be transmitted over longer periods of time?

> "global climate change may impact butterfly diseases"

Moreover, habitat destruction and fragmentation could crowd some insect populations into smaller habitat patches, potentially increasing disease transmission, and exposure to pesticides or other environmental stressors could increase susceptibility to infection. In terms of habitat changes, for many butterfly species, humans have converted habitats in open meadows or prairies or coastal areas into suburban/urban developed areas or monoculture crop systems. This is especially true with the increasing popularity of roundup ready crops that are genetically engineered to be herbicide tolerant, so that agricultural weeds that butterflies might otherwise use for food or shelter are much more effectively eliminated. Butterflies that are crowded into remnant habitats could encounter more competition for resources — and possibly higher spread of parasites.

As a final issue, the growing popularity of raising and releasing insects such as ladybird beetles, bees, and butterflies has resulted in the rising commercial sales of many insect species. Butterflies in particular are sold for educational activities or for release at weddings and other special events. In the United States alone, as of 1998, more than 60 commercial butterfly farms were in operation, with an estimated tens of millions of dollars spent annually on butterfly sales. Introducing large numbers of captive raised insects into the wild raises concerns for the spread of pathogens, especially because rearing animals at high densities in commercial operations could increase their exposure to some diseases.

Although the USDA regulates the interstate shipment of live butterflies, existing permits do not track the number of butterflies transported nor do they require the butterflies to be screened for disease. Both NABA and the Xerces Society have proposed a ban on the environmental release of commercially reared butterflies, with the spread of parasites and diseases listed as one major concern. Given the growing popularity of butterfly releases, lack of required screening for parasites, and potential for cross-species transmission in operations where multiple butterfly species are reared together, the time is ripe to reexamine regulations on commercial growers who sell live butterflies for release into the environment.

It is important to note that before scientists can predict how butterfly pathogens will respond to future environmental changes, we need baseline information on how common butterfly pathogens are in wild populations — and how butterfly diseases are affected by factors like climate and habitat characteristics. Parasites and infectious diseases comprise a major fraction of the biodiversity on Earth, with half or more of all species being parasitic in nature. At the same time, biologists have uncovered only a miniscule percentage of the diversity of infectious organisms from natural host communities, and nowhere is this gap more extreme than for pathogens from the vast majority of butterflies. Not only is describing butterfly pathogens important for inventories of biodiversity, but baseline data on the distribution and prevalence of insect pathogens are essential to detecting future changes in prevalence or novel pathogen introductions that might result from anthropogenic change. This is especially true for butterfly populations subject to habitat loss and exposure to environmental stressors that could make species more susceptible to disease-mediated declines or extinction.

> "The time is ripe to reexamine regulations on commercial growers who sell live butterflies for release into the environment."

For Further Reading

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Polydnaviruses and Parasitoids:
The Ultimate Virus-Host Relationship

by Don Stoltz

In the article "When Butterflies get Bugs" by Sonia Altizer and Jaap de Roode, on page 16 of this issue of *American Butterflies*, you learned about the many diseases of butterflies. It might be a surprise for most people to learn that for every lepidopteran species, there exists one or more species of parasitic wasp (parasitoid) which attacks it. Here, we take a look at a very unusual relationship between viruses belonging to the family, *Polydnaviridae*, and certain species of wasp belonging to the well-known wasp families, *Braconidae* and *Ichneumonidae*.

Those of you who keep a garden, and avoid the use of chemical pesticides, are likely to have observed Cabbage White caterpillars festooned with small white cocoons — these will have been spun by larvae of the braconid parasitoid, *Cotesia rubeculae*. Wasps in the genus *Cotesia* belong to a lineage (termed the microgastrine lineage), comprising more than 10,000 species worldwide. As far as we can tell, every species within this very large and successful lineage of wasps carries a polydnavirus. Polydnaviruses are also associated with at least two ichneumonid subfamilies. Polydnaviruses carried by braconid wasps are called bracoviruses while those carried by ichneumonids are called ichnoviruses. In terms of morphology, the bracoviruses and the ichnoviruses are very different (see photos, pages 28 and 29), however their life cycles are essentially identical and remarkable!

Here is a somewhat simplified description of the entwined life histories of the wasp and the virus. Much of this story was worked out over the past 40 years in my laboratory and in the laboratories of other scientists who became interested in this fascinating system.

When a braconid wasp encounters the right kind of caterpillar, it tries to inject its eggs into the caterpillar using a specialized structure called an ovipositor (many of you will have experienced the effects of a certain modification of the ovipositor, namely the stinger!). If it succeeds, it also injects some fluid, and this fluid contains polydnavirus particles. The recognition that virus particles were injected along with the eggs was made in the mid 1970s in my laboratory.

In a strange twist, however, the virus particles that have been examined don't seem to contain any viral DNA; rather they contain wasp DNA. Once the polydnavirus particles are within the caterpillar, the wasp genes contained within them spring into action and prevent the caterpillar's immune system from destroying the wasp eggs. But, the DNA released into the caterpillar cells from the polydnavirus particles doesn't replicate. In the 1990s, the DNA packaged into certain polydnavirus particles was completely sequenced. One of the results of this work was the realization that the genes required to make progeny virus particles were not packaged into said particles! So, the polydnaviruses are in the unique position of being unlike any other kind of virus, to the point that some questioned whether they should legitimately be called viruses.

Inevitably, the debate centered on the question of origins, for which there were two possibilities: one, certain wasps had evolved