

# The Parasitic Wasp's Secret Weapon

*Parasitic wasps must develop inside living caterpillars. They survive this hostile environment by smuggling in a virus that suppresses their host's immune system*

by Nancy E. Beckage

**T**his caterpillar will never become a moth. It lurks deep in the foliage of a tasty tomato plant, hidden from predators, but its enemy has found it anyway. In search of a nanny for her offspring, the parasitic wasp has homed in on the distinctive scent of her lepidopteran victim and its lunch. Now the tiny wasp injects a clutch of eggs through the caterpillar's tough cuticle and into its body cavity, where her larvae will thrive by feeding on their living nursery. At a critical moment in development, the wasp larvae will burst through the flanks of the caterpillar to spin their cocoons on its surface. These wasps eventually depart as adults, metamorphosis complete, but their host is now destined to die as a caterpillar.

If this were a one-on-one interspecies scuffle, the caterpillar might stand a chance—it has an immune system capable of engulfing and killing invading wasp eggs before they can do permanent harm. The wasp, however, does not come to this encounter alone. In addition to her eggs, she injects hordes of virus particles. These viral warriors rapidly defeat the caterpillar's immune response, tipping the balance of power in favor of the wasp progeny. The caterpillar, doubly parasitized, slowly ceases feeding, fails to pupate and dies a premature death.

Host-parasite relationships such as those involving the wasp, the virus and the doomed caterpillar are among the most complex in nature. The wasp is an endoparasite—it must develop inside its host. If the caterpillar dies before the wasp larvae are properly fed, the wasps

will die as well. Yet the caterpillar cannot be allowed to gain the upper hand using its immune defenses. Much of the responsibility for maintaining this delicate balance falls to the wasp's viral accomplice. Like the wasp, many parasites of insect hosts have evolved associations with bacteria and viruses that help them perform their often deadly deeds.

## Microbial Weapons

**A** simple example of such a partnership occurs in certain parasitic worms that carry a virulent bacterium in their digestive tracts. These worms regurgitate the bacteria into their insect hosts, killing the hosts within days of infection. The rapidly dividing bacteria are an immediate food source for the developing worms, and they provide further sustenance by secreting digestive enzymes that soon turn the host cadaver into a nutrient-rich soup. In return, the bacteria benefit by using the worms as vehicles for invasion of new hosts. These interacting partners are strictly independent organisms—they do not share genes.

In contrast, the interaction between endoparasitic wasps and the virus they exploit is more intimate. Not only are the fates of the partners intertwined, but their genetic material is also permanently mingled. And the relationship goes further—the wasp and the virus possess related genes. All this raises a thought-provoking question: Are the wasp and the virus two entities or one?

The first hint that endoparasitic wasps might have unusual weapons in their arsenal came in 1965. George Salt of

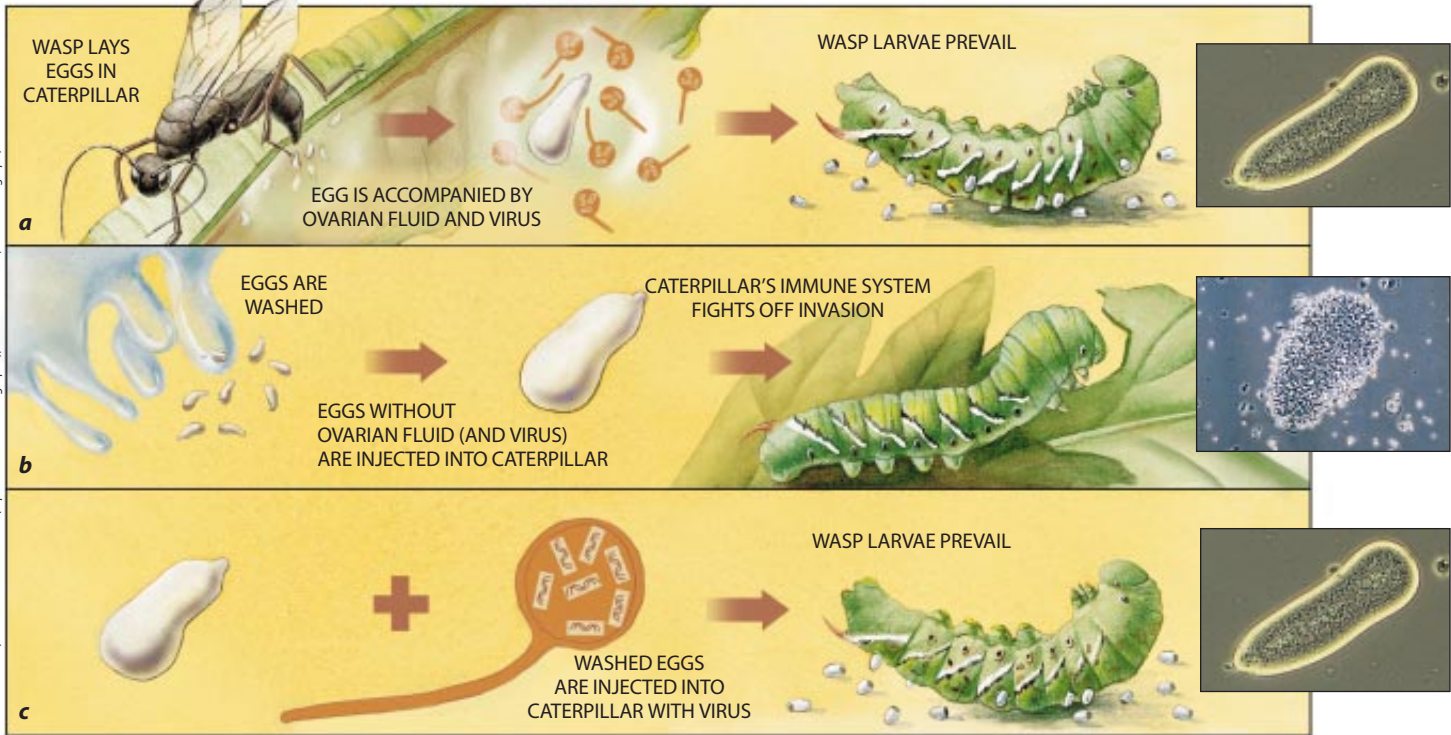
the University of Cambridge suspected that female *Venturia* wasps inject substances required for successful development of their progeny into host larvae along with eggs during the process called oviposition. In particular, Salt noted that the ovary of the female wasp harbors substances that prevent destruction of wasp eggs by the caterpillar's immune cells.

Normally, injected wasp eggs float freely in the bloodlike fluid, called the hemolymph, that fills the body cavity of the caterpillar. When Salt washed the wasp eggs prior to injection, however, they provoked a rapid immune response. These eggs, stripped of an unidentified factor, were quickly attacked by the host's immune cells and ultimately killed. In 1973 electron micrographs taken by

**WASP AND CATERPILLAR** are the David and Goliath of the insect world. The huge caterpillar's immune system threatens the wasp's eggs, which must mature inside a living host. But the tiny wasp prevails using a deadly weapon: a virus.







**BATTLE OF THE INSECTS** rages between the caterpillar and the wasp. Wasp eggs escape attack by the caterpillar's immune system thanks to a virus injected into the caterpillar by the wasp along with her eggs (a). The virus disables the caterpillar's immune cells, allowing the free-floating eggs (top micrograph) to develop into normal wasp larvae. The fate of the caterpillar is

not so fortunate. In the laboratory, however, wasp eggs that have been washed lack the protective virus (b) and are rapidly engulfed by the caterpillar's immune cells (center micrograph); no wasps survive this encounter. Injecting pure virus along with the washed eggs (c) allows the wasp eggs (bottom micrograph) to develop into larvae and emerge from the caterpillar.

Susan Rotherham, also at Cambridge, offered a clue to the identity of the protective substance. These images showed that the surface of a *Venturia* egg becomes impregnated with viruslike particles as it passes through the wasp's oviduct during oviposition.

Nearly a decade later Donald B. Stoltz of Dalhousie University conducted an extensive taxonomic survey of parasitic wasps in collaboration with S. Bradleigh Vinson of Texas A&M University. They showed that viruslike particles are invariably found in certain wasp species that develop as internal parasites of lepidopteran hosts. Moreover, they observed that these viruses replicate exclusively in the ovarian tissue of female wasps. During oviposition, the wasp injects thousands of virions into the caterpillar along with one or more eggs.

It seemed reasonable to suspect that these viruses are the ovary-derived substances that accompany wasp eggs into the host and squelch the host's immune response. The evidence was purely circumstantial until 1981, when Stoltz, Vinson and their co-workers finally confirmed that this job can be performed by

purified virus. But how exactly do the viruses—today called polydnviruses (pronounced “puh-LID-nah-viruses”)—disable the caterpillar immune system?

### Immune Deficiency

To answer this question, my colleagues and I study the parasitic wasp *Cotesia congregata*, which can lay hundreds of eggs in each caterpillar. These eggs hatch into larvae that dine on the host's hemolymph fluid instead of consuming its tissue, thereby allowing the infected caterpillar to survive well past emergence of the wasp progeny. *Manduca sexta*, the tobacco hornworm, serves as our model host. Anyone who grows tomatoes has probably had a run-in with these giant leaf-green caterpillars, which forage on tomato, tobacco and jimsonweed and often grow to the size of a man's little finger. Tobacco hornworms are convenient to work with in the laboratory: obtaining blood samples from these enormous caterpillars is easier than obtaining samples from mice.

We have observed one immediate consequence of the parasitism by *Cotesia*

wasps: certain cells, known as hemocytes, circulating in the caterpillar's blood undergo rapid physical transformation. Graduate student Mark D. Lavine has seen these effects within a few hours of oviposition. Affected hemocytes “round up,” failing to adhere to substrates such as glass or parasite eggs. They also undergo extensive blebbing, or pinching off of bits of their membrane and cell contents. The damaged hemocytes clump together and are removed from circulation. Overall, this transformation bears a striking resemblance to the cell suicide, or apoptosis, that occurs in mammalian cells [see “Cell Suicide in Health and Disease,” by Richard C. Duke, David M. Ojcius and John Ding-E Young; *SCIENTIFIC AMERICAN*, December 1996].

Granulocytes and plasmatocytes are among the caterpillar hemocytes most affected by parasitism; Michael R. Strand of the University of Wisconsin has shown that granulocytes in particular die by apoptosis. These are exactly the host immune cells that respond to foreign objects, including *Cotesia* eggs. In a normal immune response, granulocytes first release granules that coat the

invading egg. Plasmotocytes then adhere to the egg surface in multiple layers, forming a thick capsule that eventually kills the egg inside. Selective removal of the granulocytes and the plasmotocytes from circulation disables the caterpillar's first line of defense against the endoparasite. Similar phenomena occur during human immunodeficiency virus (HIV) infection in humans. In that case, the virus targets lymphocytes, causing the clumping and apoptotic death of the cells. Opportunistic infectious agents are then free to ravage the victim, much like the wasp progeny that overtake their unfortunate caterpillar host.

When we inject unparasitized tobacco hornworm larvae with purified polydnavirus, caterpillar hemocytes undergo changes in appearance and behavior analogous to those we observe in normal parasitism. But if we chemically inactivate the virus prior to injection, the hemocytes remain unaltered. This result suggests that virus capable of directing manufacture of viral proteins is required for immune suppression.

Graduate student Steven H. Harwood has shown that polydnaviral proteins do indeed appear rapidly in the caterpillar host. We detect the first evidence that polydnaviral genes are active within 30 minutes of oviposition. By this time viral particles have spread throughout the host, entering cells, including hemocytes. In our tobacco hornworm system, we have also shown that at least one polydnavirus-encoded protein is produced inside host hemocytes following parasitism. This protein is called EP1, for "early protein 1."

We took great care to establish that EP1 is in fact a polydnaviral protein. EP1 production can be induced in tobacco hornworm larvae by injection of polydnavirus alone, suggesting that the EP1 gene resides in the genome (the characteristic set of genes) of the virus; alternatively it could reside in the genome of the host and merely be activated by the virus. We deduced part of the sequence of the gene encoding the EP1 protein and then searched for this sequence in various organisms. We turned up no such gene in *Manduca* but instead found the EP1 gene in the polydnavirus genome. Intriguingly, manufacture of this polydnaviral-encoded protein correlates temporally with the most dramatic effects of parasitism on host hemocytes.

We detected high levels of the EP1

protein inside hemocytes one day after oviposition, when these cells were quite disabled. We continued to find evidence of EP1 in the caterpillar for six days; hemocyte function returned to normal on the eighth day—but too late for the caterpillar to kill the wasp larvae.

Researchers led by Otto Schmidt of the University of Adelaide have found a similar correlation in a different host-parasite pair: hemocytes first become damaged during the brief period when viral protein is produced—then immune response rallies in two or three days. We speculate that hemocyte damage occurs as long as such viral proteins persist; once viral protein levels drop, damaged hemocytes would recover or be replaced, replenishing the functional supply.

One consequence of this timing is that the host immune response resumes in full force before developing wasps are ready to leave the caterpillar. Unlike vulnerable eggs or young larvae, however, older wasp larvae seem able to withstand active immune cells on their own. Polydnavirus provides a long but temporary reprieve from immune attack, allowing the wasps to become mature enough to protect themselves.

Bruce A. Webb of the University of Kentucky, who works with parasitized tobacco budworms, has discovered how the wasps fill one final chink in their immune suppression armor. Although the cellular immune response by the caterpillar is essentially immediate, there is a lag before polydnaviral proteins are available to alter the behavior of host

hemocytes. Webb has shown that immediate but short-term protection against immune cells is conferred by ovarian protein molecules injected directly into the host by the wasp. The job of long-term protection then falls to the polydnavirus through sustained viral protein production in caterpillar cells.

### Arresting Development

Another important aspect of the *Cotesia-Manduca*-polydnavirus tripartite relationship (and the one that first piqued my interest in the field) is the way the parasite manipulates the development of the host. A growing internal parasite benefits by extending the interval over which its host remains a feeding larva. For this reason, many endoparasites develop strategies to delay host metamorphosis. I was particularly interested by the case of the tobacco hornworm parasitized by *Cotesia*, because this host remains developmentally stunted long after the wasp progeny leave the body cavity; the caterpillars often linger two weeks before dying.

Developmental arrest in lepidopteran hosts is mediated through the endocrine system. I studied endocrine disruption caused by parasitism as a graduate student in Lynn M. Riddiford's laboratory at the University of Washington. There I observed that the concentration of a key hormone regulating metamorphosis is disturbed after parasitism of tobacco hornworms by *C. congregata*. The level of juvenile hormone (JH) is

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## Enlisting Insects in the War on Weeds

Wasps and viruses are not the only organisms capable of hardball tactics: humans are past masters. And lately scientists have turned caterpillars and their parasites into lethal weapons against weeds. Their worthy opponent is kudzu, a fast-growing, pernicious climber that carpets seven million acres in the southern U.S.

Entomologist David Orr and his colleagues at North Carolina State University have recently deployed soybean looper caterpillars to combat kudzu. In field tests the loopers defoliate the weed, and Orr believes the plants' efforts to replace lost leaves will slowly exhaust their enormous root systems (a single plant can have roots that weigh as much as 300 pounds).

Because soybean loopers eat crops as well as kudzu, each caterpillar enters the field equipped with a safety mechanism to prevent its escape—parasitic wasps that execute the caterpillar as it spins its cocoon, ensuring that no moths will emerge to fly away and reproduce. An added benefit is that the parasitized loopers eat more kudzu: the wasps extend both the feeding interval of the caterpillars and their appetite.

It is not clear how the eggs of this wasp, *Copidosoma truncatellum*, escape attack by the immune system of the caterpillar; the wasp does not carry polydnaviruses. Several wasps that do carry polydnavirus have also been used in biological-control strategies, though, often as weapons against populations of pest insects, including destructive fruit flies, moths and aphids.

—Mia Schmiedeskamp

dramatically elevated in parasitized hosts, never descending to the low level needed for pupation. The high levels of JH are probably caused by a lack of sufficient juvenile hormone esterase, an enzyme that clears JH from the organism. Parasitism apparently leads to sustained low esterase levels, in a manner that prevents pupation even after departure of the wasps.

It turns out that developmental arrest is largely executed by polydnavirus, although the presence of the wasp itself is needed to obtain full arrest. When we inject unparasitized larvae with low doses of polydnavirus, they fail to pupate normally. The amount of virus required to retard development appears to be less than that required to suppress the immune response. In fact, postdoctoral fellow Mitch Dushay showed that eggs that have been washed prior to injection retain trace amounts of virus particles or viral proteins that do not suffice to prevent encapsulation by immune cells but may cause developmental failure of the host. Polydnavirus may also contribute to developmental effects even after departure of the wasps from the host. We speculate that the virus remains as a latent infection in the caterpillar—perhaps mediating lasting effects on development.

Polydnavirus is clearly responsible for manipulating a number of developmental and immune programs of the caterpillar host in a manner that is beneficial to the wasp. In many ways, the virus is essential to successful parasitism. The amazing strength of this relationship between wasp and polydnavirus becomes even clearer when one considers the genetics of the partners.

### Permanent Partners

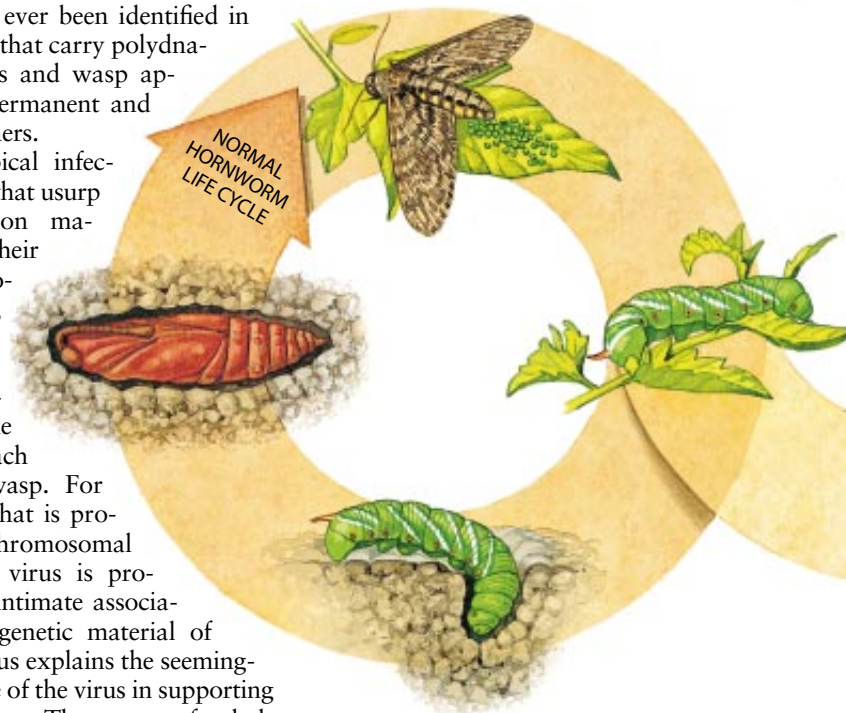
The size and complexity of polydnaviral genomes greatly exceeds that of other DNA viruses: each polydnavirus comprises up to 28 separate circles of double-stranded DNA (thus their name, from “polydisperse DNA viruses”). In 1986 Jo-Ann G. W. Fleming and Max D. Summers of Texas A&M discovered that the extremely complex polydnavirus genome is integrated into the genome of both male and female wasps. This viral DNA is thought to be scattered throughout the wasp chromosomes. Wasp inheritance of the virus appears to be strictly Mendelian—the viral sequences are copied and passed to successive generations as part of the

wasp chromosomes. No virus-free individuals have ever been identified in wasp species that carry polydnaviruses. Virus and wasp appear to be permanent and integral partners.

Unlike typical infectious viruses that usurp the replication machinery of their hosts to reproduce wildly, polydnavirus reproductive success is affected by the survival of each and every wasp. For every wasp that is produced, a chromosomal copy of the virus is produced. This intimate association of the genetic material of wasp and virus explains the seemingly selfless role of the virus in supporting wasp parasitism. The success of polydnavirus depends on efficient reproduction of the wasp, which in turn depends on an essential host-parasite relationship. Any role the virus plays in ensuring the success of the parasite also ensures the success of viral transfer to the next generation.

Because viral transmission from wasp to wasp takes place through inheritance of a virus integrated into the chromosome, there must be some other rationale for the mass production of virus in the wasp’s ovaries. In fact, the packaged viruses produced at this step seem to be useless for the typical viral mission of infection with intent to replicate—but they are masters of host manipulation. Viruses are expert at spreading throughout a host and entering host cells. Parasitic wasps appear to have harnessed this talent to target useful viral protein production to caterpillar cells, allowing for insider manipulation of their host’s biology.

The integration of polydnaviruses into wasp chromosomes prompts questions about the origin of the virus. A typical answer might be that the viruses originated as independent pathogens of the caterpillar hosts, or of the wasps themselves, and later combined with the wasp DNA. A much more intriguing possibility is suggested by the apparently permanent and exclusive association of viral DNA with wasp DNA. Perhaps there was never a separate viral entity. Instead wasps may have ac-



quired the ability to copy and package a subset of useful genes selectively from their own genomes, for shipment into caterpillar cells. Work by Webb and Summers on wasp venom proteins may fit with this last hypothesis.

These researchers have found that some wasp venom genes are similar to polydnaviral genes. Moreover, antivenom antibodies raised in the laboratory also recognize viral proteins that are important for manipulation of the caterpillar. It seems, then, that wasp venom genes and polydnaviral genes may be evolutionarily related. This result is especially exciting because certain wasp venom proteins are known to play a supporting role in manipulating caterpillar physiology.

In one evolutionary scenario, initially independent polydnaviruses may have picked up useful venom genes from the wasp genome. In another scheme, the wasp may have found an incredibly efficient way to utilize its own venom proteins, by copying their genes, packaging them and routing them to caterpillar cells where they can maintain a sustained effect. Either scenario results in increased fitness of both the wasp and virus; either way the genetic boundaries between the wasp and virus are obscured.

Whatever the origins of the polydnaviruses, their associations with wasps and caterpillars offer rich opportunities for the study of evolutionary biology.

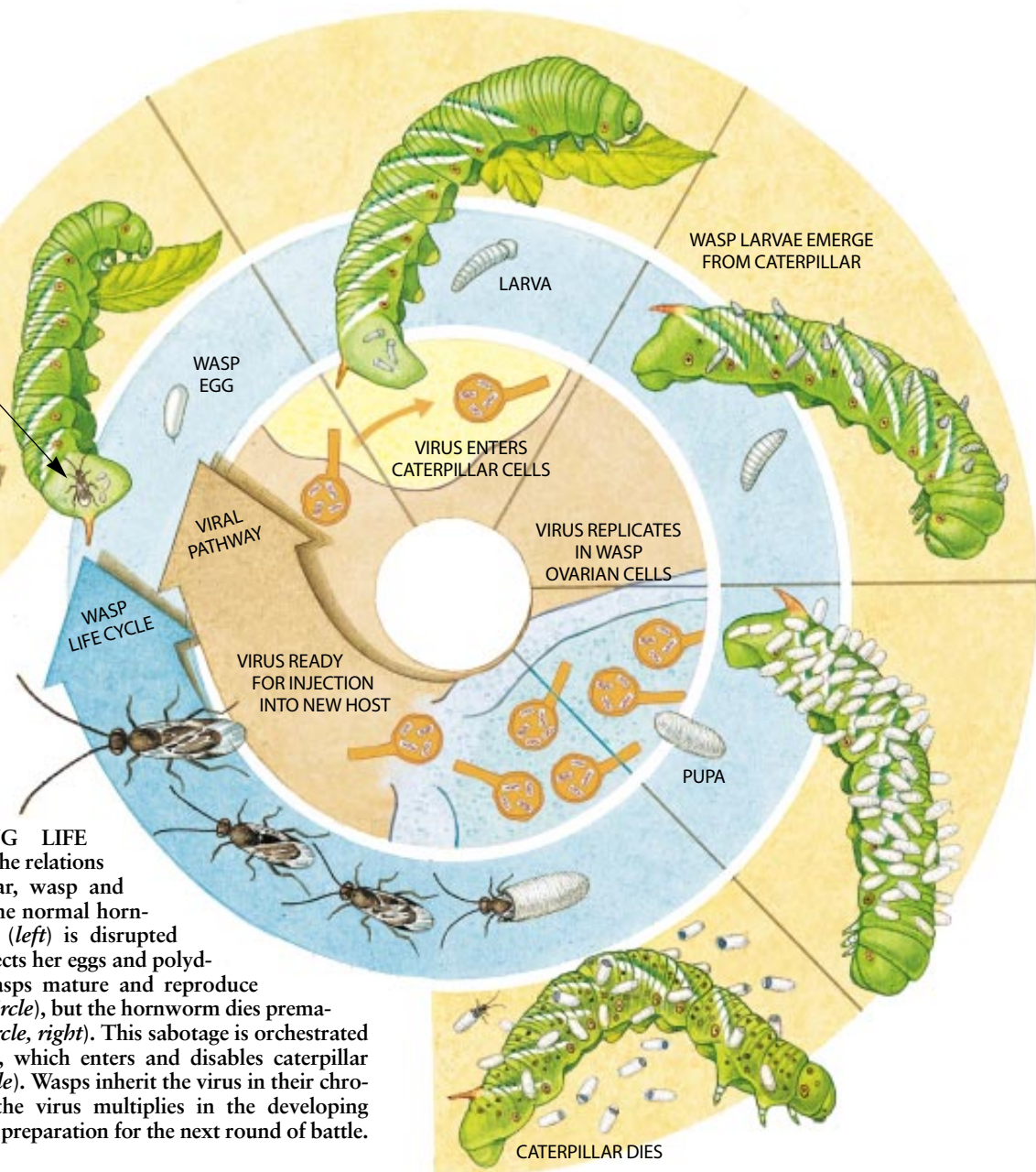
WASP INJECTS EGGS AND POLYDNAVIRUS INTO CATERPILLAR

WASP LARVAE EMERGE FROM CATERPILLAR

ALTERED HORNWORM LIFE CYCLE

WASP LIFE CYCLE

**INTERTWINING LIFE CYCLES** reveal the relations among caterpillar, wasp and polydnavirus. The normal hornworm life cycle (left) is disrupted when a wasp injects her eggs and polydnavirus. The wasps mature and reproduce normally (blue circle), but the hornworm dies prematurely (yellow circle, right). This sabotage is orchestrated by polydnavirus, which enters and disables caterpillar cells (brown circle). Wasps inherit the virus in their chromosomes, and the virus multiplies in the developing wasp's ovaries in preparation for the next round of battle.



ROBERTO OSTI

The vicious tactics of the wasp and its viral accomplice against their hijacked caterpillar host argue against the tenuous hypothesis that the most highly evolved parasites exhibit only minimal virulence to their hosts [see "The Evolution of Virulence," by Paul W. Ewald;

SCIENTIFIC AMERICAN, April 1993]. Endoparasitic wasps invariably kill their hosts. The event is exquisitely timed and coordinated, however, to ensure the success of the wasp. In beautiful contrast to these hardball tactics, the mutually advantageous relationship

between wasp and virus is so intimate that it blurs interspecies genetic boundaries. The complex question of why the caterpillar does not become a moth should keep a raft of scientists from evolutionary biologists to endocrinologists busy for years to come.

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*Further Reading*

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