How Parasitic Wasps Find Their Hosts

Besides recognizing odors from their caterpillar hosts, wasps also learn to identify compounds released by the plant on which the caterpillars feed

by James H. Tumlinson, W. Joe Lewis and Louise E. M. Vet

ide-and-seek is not simply a game for children. Animals _ "play" it too, and for most the stakes are life or death. For parasitic wasps, the goal is propagation. Females ready to lay eggs must inject them into a host, often the caterpillar of a moth or butterfly. The caterpillar becomes paralyzed, and the eggs inside develop into wasp larvae. The growing larvae feed on the host, thus killing it. The larvae then form cocoons on or near the caterpillar body and pupate. Needless to say, it is in the best interest of the caterpillar to remain hidden under leafy cover. Secrecy would not seem to present much of a problem in a large, lush crop field.

How, then, do wasps detect their victims within the complex environment, particularly when the caterpillars evade their attackers by hiding, moving or otherwise remaining concealed? Random searching alone certainly seems an implausible way to achieve such a literally vital goal; field studies sug-

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gest that a wasp might spend its entire lifetime searching for a single host if the wasp had to find it by chance. For the past 10 years, our research groups have been collaborating in pursuit of the answer. We think we have found it. Furthermore, the basic principles we have discovered seem to apply to other kinds of parasitic wasps, including species that parasitize eggs or other insect stages of development.

Briefly the answer is this: the wasps home in on unique volatile compounds that they can detect over substantial distances. These chemicals come from the caterpillar's feces and, perhaps more surprising, from the plants on which the caterpillar feeds. Our guest led us into two areas that had been relatively unexplored with respect to these animals. The first was the ability of wasps to learn. They decipher many indirect and frequently changing cues that indicate the location of hosts. This ability represents a level of sophistication previously unexpected in these insects. The second area is the interaction between the wasps and the plants. When attacked by a caterpillar, a plant produces signals that wasps home in on. Research suggests that investigators can exploit the learning abilities of wasps and the wasp-plant conspiracy to control caterpillar pests in the field.

ur findings about the complicated interactions among the parasites, hosts and plants began with the assumption that a specific set of odors from the hosts would guide the wasps to their victims. This supposition was rooted in many studies that indicated parasitic wasps respond to certain chemical cues. Early efforts identified and synthesized several kinds of chemicals that can act as guides. Such compounds belong to a class of chemical mediators called kairomones. These

substances, which come predominantly from the oral secretions and feces of the caterpillar, act at close range as trail odors or searching stimulants.

These kinds of kairomones, however, could not be the whole story. Because they are nonvolatile, they do not disburse well in the atmosphere. Thus, they cannot be detected over long distances. If wasps had to rely solely on short-range cues from the hosts, their success in finding caterpillars would only be slightly better than chance.

In this regard, S. Bradleigh Vinson and his co-workers at Texas A&M University uncovered a clue. They found that parasitic wasps are also attracted to volatile chemicals. Such compounds can be detected over several meters and could provide the beacon wasps need. Only after work done in one of our laboratories in Wageningen, the Netherlands, clearly demonstrated that wasps can exploit volatile chemicals to find hosts did we have the first indication of the importance of learning in foraging.

To prove it, we used a device called an airflow olfactometer, which was essentially a square container. The olfactometer can provide up to four different odor fields (one from each corner) to a wasp placed in the center. We found that *Leptopilina clavipes* wasps responded to the odor of decaying mushrooms, which are likely to harbor its host, fungus-eating *Drosophila* fruit fly larvae. We also demonstrated that wasps can develop preferences for odors from other sources, such as fermenting fruit, if they have been reared on hosts that have fed on that food and have laid eggs in those hosts

Although experiments with the airflow olfactometer showed that wasps respond to volatile signals, they did not indicate how the wasps actually used the odors in the field. The wasps did not fly in the olfactometer, and the device was too small to mimic natural conditions realistically.

We therefore decided to use wind tunnels to study the responses of flying wasps to volatile chemicals. Such tunnels are essentially long containers that insulate the interior from extraneous odors and air currents. Investigators have used such devices to analyze the responses of moths to pheromones and other attractants. One can also use them to test other insects, such as fruit flies [see "The Amateur Scientist," page 144]. A small fan blows the scent from one end of the tunnel to the other. We

placed caterpillar-infested plants at one end and released the wasps at the other. We reasoned that the wasps would find their hosts by flying upwind into an odor plume originating from the host.

Our initial results were disconcerting. Females of the parasitic wasp *Microplitis croceipes* responded poorly to odors of their hosts, corn earworm larvae, which were feeding on leaves of cowpeas. Yvonne Drost and Oliver Zanen, working in our laboratory in Tifton, Ga., discovered that only about 10 percent of laboratory-reared female wasps

responded to odors from the larvae-infested plants.

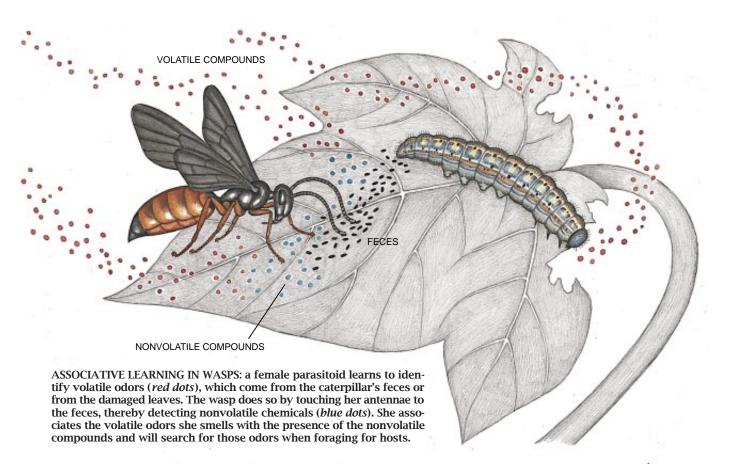
This curious outcome was rooted in the fact that the *M. croceipes* wasps had been reared on hosts maintained on a laboratory diet (generally of ground beans supplemented with vitamins). Even in their larval stages, the wasps had never come in contact with hosts in natural conditions, host products or cowpea plants. Only after we allowed the female wasps to contact the corn earworm larvae did they respond to the odors in the wind tunnel.

This result was a major break-



FEMALE PARASITIC WASP stings a plant-feeding caterpillar, in the process laying eggs in her victim. By studying how the

wasp tracks down her host, researchers believe they can use the wasps to help control caterpillar pests.



through in our investigations of the foraging behaviors of these wasps. It alerted us to the fact that experience played a more important role in the exploitation of odors than we had realized. Even more surprising, the experience need not include direct physical contact with a host. After touching its antennae to the feces of the caterpillar for about 30 seconds, wasps could later locate the larvae.

Subsequent tests in the wind tunnel showed that wasps are attracted to the hosts' feces. Indeed, Fred J. Eller, working in our laboratory in Gainesville, Fla., found that the feces odor enabled the wasps to determine the plant on which the host has fed. He discovered that females familiar with the feces of hosts fed one plant would fly to the odors of those feces more often than to the odors from hosts feeding on a different plant species.

xactly what in the feces attracted the wasps? The volatile compounds in them seemed to be the obvious candidate. We thought the wasps might have an innate ability keyed to the smell of the hosts' feces. To confirm that hypothesis, we extracted the volatile compounds from the feces with hexane, a liquid solvent derived from petroleum. In flight-tunnel tests, the volatile extracts attracted the wasps as strongly as did the host-infested plants.

Yet more thorough experimentation soon presented us with a puzzle. We tested females who had never been exposed to caterpillar feces. Rather these wasps had contact only with the volatile extract. To our surprise, these females responded poorly. In fact, they did no better than wasps that had not touched anything.

We decided, then, to examine the remaining, nonvolatile components of the feces. We removed those compounds with water and tested to see whether they would attract the wasps. The females were only slightly attracted to the material extracted by water. Yet after touching these compounds with their antennae, the wasps responded to the volatiles extracted with hexane.

These results, taken together, indicate that a crucial component lies in the water-soluble, nonvolatile compounds. This component is specific to each type of host. The wasps must associate the volatile chemicals with the nonvolatile ones.

To determine conclusively that wasps can learn volatile chemicals by association, we experimented with completely novel odors. For example, we allowed the wasps to smell vanilla extract as they touched the water extract. Sure enough, they subsequently flew to the smell of vanilla; they "thought" that hosts were nearby. This ability to link a wide variety of odors to their hosts greatly broadens the range of chemicals parasitic wasps can use to locate their prev.

Hans Alborn, working in our Gainesville laboratory, showed that the chemicals in the water-soluble extract are not related to diet. He demonstrated that the chemicals that the wasps recognize could be obtained from feces from corn earworm larvae raised on artificial diets. The food could even be filter paper. That the nonvolatile chemical does not depend on the host's food contrasts sharply with the volatile compounds, which are specific to diet. Current studies are trying to identify the nonvolatile component.

Although chemical cues are the primary sources of information that guide parasitic wasps, the shapes, colors and patterns of the area around the caterpillar also play a role. Felix L. Wäckers, working in our Tifton laboratory, studied how female *M. croceipes* wasps use such visual cues in a wind tunnel. Using odor, he taught wasps to associate a caterpillar with various color patches. such as solid orange or black-and-white squares, attached to plant leaves. When the odor was removed, the wasps responded to the color design alone.

Wäckers also showed that the females quickly developed preferences for combinations of odor and visual stimuli. Odor and visual effects proved to be additive—that is, the learned preference for the combinations was stronger than either the odor or visual cue alone.

This sophisticated ability to learn visual and chemical cues and to use them in combination equips the wasps with a powerful system for detecting and tracking their prey. With the acquired information, they can readily locate likely sites and concentrate their search on the most profitable plant parts.

uring the wasp's hunt for caterpillars, the plant does not remain a passive observer. In fact, its reaction to an attacking caterpillar may be the most surprising element in this predator-prev system. When chewed on, plants actively produce and release volatile compounds from both damaged and undamaged tissues. This response suggests a defensive mechanism designed to repel invaders: studies have shown that many of the volatiles released by damaged plants are toxic to insects. The wasps, however, are not repelled by these odors. Instead, they exploit the smells to find their hosts.

The first indication that plants enlist the help of natural enemies to fight off herbivorous attackers came in 1988. Marcel Dicke and Maurice W. Sabelis, colleagues of one of us (Vet), and their co-workers studied predatory mites that prey on plant-feeding spider mites. They found that when herbivorous spider mites feed on lima bean leaves, the plant releases a blend of volatiles that attracts predatory mites. The blend is specific for both the plant species and the species of spider mite that feeds on

it. The predatory mites can discern these differences and thereby recognize their prev.

The volatile chemicals released by caterpillar-infested plants have proved to be potent attractors of wasps. In fact, recent studies have shown that wasps are more sensitive to the chemicals from plants than they are to those from the host and host feces. Ted C. J. Turlings, working in our Gainesville laboratory on Cotesia marginiventris, a parasitoid of moth larvae, found in wind-tunnel tests that corn plants damaged by hosts were the most important source of volatile attractants. Removing the hosts and host products, including feces, only slightly diminished the attractiveness of the plants. Neither hosts nor feces were as attractive as the plants alone.

One might suspect that the "greenleafy" odor reminiscent of freshly cut grass attracts the wasps. Yet such signals are intermittent; they emanate only as a caterpillar feeds. Once it stops eating and moves away, the damaged section of the leaf no longer emits the freshly cut scent.

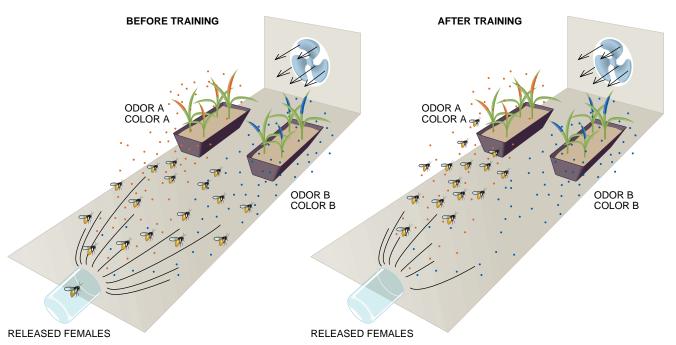
More detailed analysis, however, revealed that plants produce a more consistent set of volatile attractants. These compounds—hydrocarbons called terpenes and sesquiterpenes—are produced several hours after the attack and persist for several hours, perhaps days. The delay in forming the terpenes and sesquiterpenes indicates that the physiology and biochemistry of plants

actively change in response to herbivore damage. The mechanisms behind this change are unknown.

Chemicals from the caterpillar are essential if the plant is to release the volatile compounds. When we mimicked caterpillar damage on leaves with a razor blade, the plants did not emit large amounts of the volatile compounds. But if we applied oral secretions from the caterpillar to the wounds, the plants would release terpenes and sesquiterpenes several hours later. Caterpillar regurgitant on undamaged leaves did not induce such emission. Wind-tunnel tests revealed that oral secretions placed on artificially damaged leaves made the plants as attractive to the wasps as the plants with real caterpillar damage.

Turlings also found that the response of the plant to the oral secretions is systemic—that is, the entire plant releases volatile compounds when one or more leaves are attacked by caterpillars. Dicke had earlier found a similar effect, in which intact leaves of a plant injured by a spider mite attracted predatory mites. This systemic effect is significant in that it makes the plant under attack stand out from its neighbors.

by the plant has evolved to attract the natural enemies of the herbivores or whether the insect carnivores only exploit a plant defense mechanism aimed at the herbivore is being investigated. The discussion is



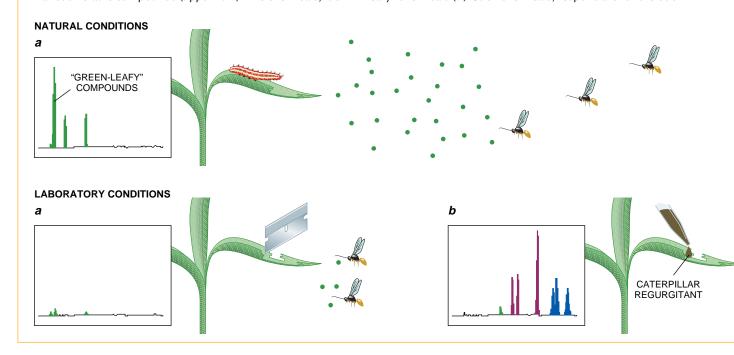
WIND TUNNEL is used to show that female wasps can be trained to respond to a particular odor and color. During the training period (*left*), two sets of plants, each marked with a different scent and color, are presented. Rewards of caterpil-

lars are given to the wasps only if they fly to the set labeled "odor A" and "color A." So trained (*right*), the wasps, when given the same choice, will fly immediately to that set, even if no caterpillars are present.

Distress Signals from Plants

Under natural conditions, corn seedlings attacked by beet armyworm caterpillars attract parasitic wasps by releasing various volatile compounds (*upper row*). The chemicals, iden-

tified through gas chromatography, are displayed as colored peaks. As a caterpillar feeds, the plant initially releases "greenleafy" chemicals (*a*). Such chemicals, responsible for the odor



akin to debating whether the chicken came before the egg. The current phenomena result from a prolonged evolutionary process of action and reaction. The larvae have evolved to become as inconspicuous as possible to avoid parasitization and predation. They must, however, feed to survive, and in feeding they damage the plant. The damage induces a reaction from the plant. The plant's reaction provides information that facilitates the wasp's attack on the caterpillar. In this process the carnivores exert an important influence on the defensive strategy of the plant.

This evolutionary game of hide-andseek leads to another question. Why do parasitic wasps learn? The answer lies in the incredibly complex collection of chemicals with which the wasps must contend. The signals will vary considerably if the hosts are feeding on different plant species. For parasitoids such as M. croceipes, whose victims feed on a wide variety of plant species, the different plants will send out completely different signals. For example, cotton, cowpea and soybean each produce a unique blend of volatile chemicals when eaten by corn earworm caterpillars. Moreover, the composition of the volatile compounds can differ when the hosts feed on different parts of the same plant. The picture is even more complicated if a parasitoid attacks caterpillars of several different species of moths, as does *C. marginiventris*. The same plant may release two different blends when fed on by two different species of caterpillar.

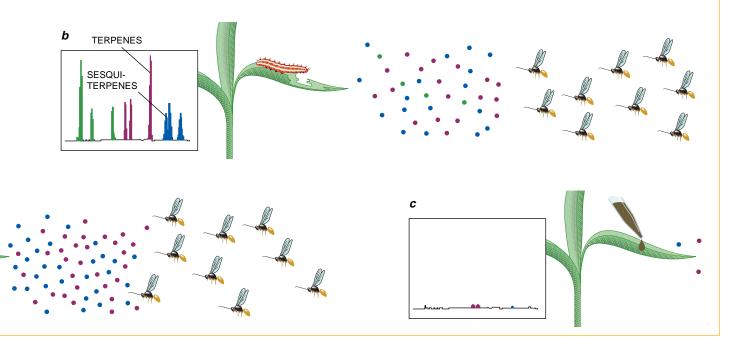
In such a complex, dynamic chemical environment, a simple, rigid search procedure would not permit the parasitoids to exploit the available resources efficiently. They must be able to detect a great variety of potentially important chemical cues and determine their significance in the context of the surrounding environment. They may encounter different host species or growth stages of plants of varying suitabilities in the same location, and they may have to "decide" whether to continue to search in a given area or to abandon it for another. The probability of survival of their progeny depends on making the right choices during foraging. When all the complexities of the environment in which the parasitoids must search are considered, it becomes clear why learning is an essential element of the foraging strategy of the wasp.

That female wasps need experience to learn the odor of their hosts raises a question. How do females newly emerged from their cocoons, which are formed on the outside of or near the caterpillar, find their first hosts? The answer is somewhat speculative, but enough experiments have been performed to enable us to piece together a reasonable conjecture. There appear to be two possibilities.

First, wasps seem to have some innate preference for certain odors. We know from the work with *Drosophila* larval parasitoids that odors from some sources are more attractive to inexperienced female wasps than those from others. In our laboratories, Eller observed that *M. croceipes* females that had had no contact with hosts were nonetheless slightly attracted to volatile chemicals from some plants. Wasps thus seem to have a genetic inclination for odors from substances that have served as food sources for hosts.

The second possibility is that compounds in their cocoons can to some extent train the wasps. Franck Hérard, working in our Tifton laboratory on Microplitis demolitor, a species from Australia that attacks corn earworm larvae, found that wasps reared on plant-fed hosts responded well to the odors of host-infested plants in a wind tunnel. The response, however, occurred only if the wasps had been allowed to emerge naturally from their cocoons. In contrast, wasps cut out of their cocoons responded poorly to odors from plantfeeding hosts. Their reaction improved only when they could walk on and touch their antennae to their cocoons. The cocoons undoubtedly contain odors and of freshly cut grass, attract wasps only slightly. But later, the seedling also produces terpenes and sesquiterpenes, which are highly attractive to wasps (*b*). The plant continues to produce such compounds even after the caterpillar has stopped feeding. In the laboratory (*lower row*), simulated caterpillar

damage using a razor blade (a) produces few compounds. But when oral secretions from the caterpillar are placed on artificially damaged leaves, a plant produces terpenes and sesquiterpenes, though no green-leafy compounds (b). Requiring on an intact leaf elicits virtually no response (c).



other substances from the hosts and their food plants. This initial conditioning provides the "experience" that aids the wasps in locating their first host.

A level of innate ability and contact with the cocoon seem sufficient to prime the wasps to respond to the odors of host-infested plants. In studies, wasps usually had trouble finding their first hosts, often requiring 20 to 30 minutes. Efficiency improved significantly with each success for the first few encounters. The wasps began to uncover subsequent hosts sometimes in less than five minutes.

Because parasitic wasps learn readily and respond efficiently to chemical cues, researchers have begun to explore ways to exploit the wasps' abilities. Specifically, they are trying to determine whether the natural proclivity of parasitic wasps to control caterpillar pests can be significantly enhanced. Appropriately trained, the wasps could "police" plants and protect them from crop-feeding caterpillars.

Using beneficial insects to control pests is not a new idea. The first truly notable example is the biological control of the infamous cottony-cushion scale, which was on the verge of destroying the citrus industry in California in the late 1800s. Importation of the vedalia beetle (ladybug), a predator

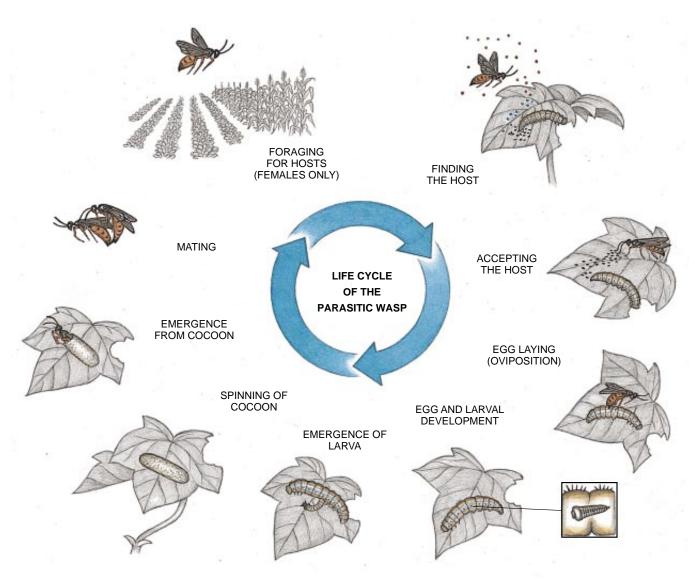
from the pest's native home of Australia, saved the crops. More recently, successful pest control combines beneficial insects with such sound management practices as crop rotation. Within the past 20 years, this latter approach has become the primary means in Europe for averting the ravages of whiteflies and other pests in greenhouses. In the Netherlands the greenhouse area protected in this way increased from 400 hectares in 1970 to 14,000 hectares in 1991. In this case, biological control has clearly proved to be cheaper and more reliable than chemical control.

Yet the vast potential of these natural weapons remains for the most part untapped—in part because researchers have failed to unlock the secrets of how beneficial insects find their prey. The success of biological control has often been hit or miss. Consequently, control for most of our major crop pests remains centered on synthetic pesticides, despite the urgent problems associated with the extensive use of toxic chemicals: environmental pollution, possible food contamination and development of resistance by the pests.

Recent experiments have shown that properly trained parasitic wasps are vital components in ecologically based pest control. Two of our colleagues, Randy Martin and Daniel R. Papaj, each conducted small-scale field studies showing that training in the laboratory improved the initial foraging success of wasps released in the field. Furthermore, the wasps tended to stay in the field for which they were trained. Keeping the beneficial insects on a plot had been a difficulty in some previous attempts at biological control.

Still, it is not yet possible to provide a specific prescription for developing reliable control with parasitic wasps. We think two criteria must be met before wasps become suitable pest-control weapons. First, female parasitic wasps must be lured into and retained in a target area. Second, the wasps must sustain a high level of efficiency in finding and parasitizing hosts.

Several possible ways exist to achieve these goals. Wasps learn easily, so great care must be exercised to make sure the stimuli that appear during laboratory rearing accurately reflect those encountered in the field. Laboratory artifacts could lead to incorrect "programming" of parasites, a problem that might have contributed to failure of such biological control in the past. For example, Vinson and his co-workers found that laboratory-reared Bracon *mellitor*, a parasitoid of the boll weevil, would look for material containing methyl parahydroxybenzoate. This substance, an antimicrobial agent, had been added to the diet used to rear the hosts



LIFE CYCLE of a parasitic wasp, from egg to adult death, typically takes about five weeks. After locating a host, a pregnant female wasp will confirm her find by touching her antennae to the caterpillar's feces. She then injects one of her eggs into

the caterpillar. In about a week, the egg develops into a larva, which feeds on the caterpillar and thereby kills it. After emerging from its host, the larva spins a cocoon around itself. It pupates and emerges as an adult in about 10 days.

and is not present under natural conditions. Aileen R. Wardle and John H. Borden of Simon Fraser University in British Columbia encountered a similar laboratory problem: wasps trained on caterpillars reared artificially (in plastic egg cups) had difficulty finding hosts.

To maintain an efficient response to volatile stimuli, wasps need to receive steady rewards of caterpillars while they are learning to forage. Studies with *M. croceipes* and *Drosophila* parasites suggest that actually laying eggs is the most important form of reinforcement. Wasps seem to "remember" the smells better after laying eggs than if they had only come into contact with host products. Preliminary field results suggest that such a reward system can be an effective tool.

Plant breeding or genetic engineering

may also play a role in biological control. The techniques could produce strains of plants that generate greater amounts of the attractive signals. Although more information is needed to put these ideas into routine practice,

the underlying principles are sound enough to develop useful methods. For useful applications, we will need to learn as much about the wasp and its world as the wasp needs to learn to find its host.

FURTHER READING

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