Colony Collapse Disorder: Many Suspects, No Smoking Gun

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Colony Collapse Disorder: Many Suspects, No Smoking Gun

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The cause of colony collapse disorder remains unknown, although some possible explanations for the loss of honey bee colonies can be ruled out.

David Hackenberg, a beekeeper who owns large apiaries in Pennsylvania and Florida and sends his hives all over the United States—to California to pollinate almonds, to Maine to pollinate blueberries—was the first to notice that some of his hives were empty. The adult honey bees were gone, but the healthy brood remained. He called Penn State entomologist Diana Cox-Foster, and the search to find the cause of the mysterious bee disappearances began. At the time, it was reported that 20 to 30 percent of beekeepers' colonies were affected, and among the hypothesized culprits were emerging pathogens, an environmental chemical or toxin, and stressful apicultural practices.

Although more has been learned since colony collapse disorder (CCD) was first identified in mid-November 2006, the mystery remains. Some possibilities—contamination with pollen from plants genetically modified to carry an insecticidal gene, radiation from cell phones, and perhaps even stress itself—can probably be ruled out as contributory causes of CCD, but the cause of the bee colony losses remains unknown. "We don't know any more today than we knew...when I stumbled onto these things in Florida," Hackenberg laments.

The evidence to date

Honey bees (*Apis mellifera*) can be loaded with parasites. Varroa mites (*Varroa destructor*) are relatively large ectoparasites that feed on bee hemolymph (insect "blood") and wreak havoc in hives. Tracheal mites (*Acarapis woodi* [Rennie]) attach to the bees' breathing apparatus and suck out hemolymph, injecting the bees with bacteria and weakening and killing adult bees. And two species of microsporidia, *Nosema apis* and *Nosema ceranae,* can infect a bee's gut, damaging its digestive tract, exposing it to numerous bacteria and viruses, and shortening its lifespan. Bees are also subject to all sorts of chemical insults, especially environmental and in-hive insecticides and in-hive antibiotics, as well as to stress. The most pressing question at present, however, is whether a virus is causing the die-off.

Cox-Foster led a study, published last fall (12 October 2007 *Science*), to identify microbial species associated with CCD-affected migratory bee operations. Sequences from at least eight species of bacteria (some uncultured), two species of fungi, the two *Nosema* microsporidians, one trypanosome, the varroa mite, and seven virus species were found in the affected bees. Cox-Foster and colleagues concluded that Israeli acute paralysis virus (IAPV), which was identified only recently, is a marker for CCD but not necessarily the cause. W. Ian Lipkin, from the Mailman School of Public Health at Columbia University in New York, who did much of the genetic work for the article, says that his group is now studying the distribution of IAPV.

First described in Israel in 2004, IAPV has been present in the United States since before 2006. It was identified in material found in the US Department

This is a honey bee trachea with tracheal mites, Acarapis woodi. *The Honeybee Act of 1922 prevented importation of European bees and kept the United States free of tracheal mites for decades. Photograph: USDA, Lilia De Guzman.*

of Agriculture's freezers dating from 2002. (Hackenberg remarks that there were similar-appearing die-offs in 2004 and 2005, though on a lesser scale than in 2006.) Cox-Foster explains that Lipkin's group has identified three complete viral genomes: one found in honey bees from Australia, another from Israel, and a third in affected bee operations in the eastern United States and from two sites in Canada (New Brunswick and British Columbia).

The Australian virus sequence matches sequences identified in bee operations in California and other states in the western United States. This makes sense, because beginning in 2005, under pressure from almond growers, the US Congress passed an exemption to the Honeybee Act of 1922, which forbade all importation of honey bees to prevent the spread of disease to US bee colonies. At the time the act was passed, Isle of Wight disease (caused by tracheal mites) was ravaging bees in Europe, and Congress wanted to make sure the disease did not enter the United States. But Congress' 2005 exemption allowed the importation of honey bees from Australia to pollinate the almond trees, easing almond growers' concerns about insufficient numbers of US honey bees. The apparently healthy Australian bees tested for Cox-Foster's CCD study were found to carry IAPV as well.

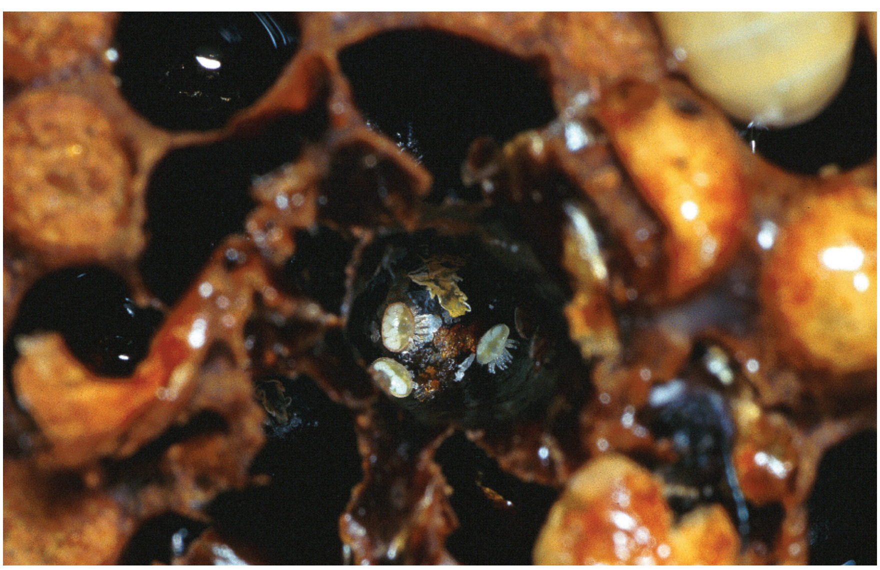
The IAPV virus found in beekeeping operations on the eastern US coast and

Healthy honey bees on a honeycomb show what a normal colony looks like. With CCD, hives contain few or no adult bees. Photograph: Stephen Ausmus, USDA.

Commercial beekeeping operations may have tens of thousands of hives. Here, hives wait in a "holding yard" for transport to the almond orchards for pollination. Photograph: USDA-ARS, Bart Smith.

Canada cluster together genetically, which, according to Cox-Foster, means that the virus came into North America from another, yet unknown, source. A tantalizing piece of evidence is that the small hive beetle (*Aethina tumida*)—first found in the southern United States in 1998 and native to tropical and subtropical Africa, where "it doesn't cause much problem," Cox-Foster says—carries IAPV, though no one knows how it got the virus. Cox-Foster says the beetles are genetically identical throughout their range worldwide, but they devastate beehives only in North America. "One of the questions," Cox-Foster posits, is "Is it possible that the beetle was carrying the virus into the United States?"

A third possible source of introduction for the virus is royal jelly imported from Manchuria. Although imported royal jelly is for homeopathic and cosmetic use, not for use in bee colonies, Cox-Foster notes that some beekeepers use it to try to rear queens, as royal jelly increases queen production. She adds that New Zealand banned the importation of royal jelly from China in 2002 because its use led to severe cases of foulbrood, a bacterial infection that destroys bee larvae.



*Varroa mites (*Varroa destructor*) are visible at the bottom of a honey bee brood cell. The mites or mitocides may be contributory to CCD. Photograph: Scott Bauer, USDA.*

Cox-Foster and colleagues also found IAPV on cockroaches that had infested equipment used in bee colonies that died from CCD. Her group decided to use the German cockroach as a model of bee disease: they injected German cockroaches with a mixture of infectious materials from bees. The roaches died four days after injection, and the researchers were able to recover IAPV but not other viruses from the dead roaches. "This clearly proves that the virus is capable of causing disease in insects," Lipkin remarks. But, to date, "we don't know if it's [IAPV] necessary or if it's sufficient" to cause CCD.

Jeffery Pettis, research leader at the US Department of Agriculture's Bee Research Laboratory in Beltsville, Maryland, reports that IAPV has been found in healthy colonies as well as in unhealthy ones, so there may be differences between the virulence of the IAPV strains.

Bumblebees are in trouble, too.

While colony collapse disorder (CCD) has focused researchers and the public on the threat to domestic honey bees, other pollinators are in trouble, too. Last year, the National Research Council issued the report *Status of Pollinators in North America* (Washington, DC: National Academies Press), which included a section on wild pollinators and noted a spillover of pathogens from greenhouse-raised bumblebees to wild populations. Habitat loss, fragmentation, more grazing land, loss of hedgerows and grasslands, planting of monocultures, and urbanization are taking their toll on pollinator populations.

Bombus ternarius is one of the less common bumblebees in Ontario, Canada, but in surveys in southern Ontario conducted between 2004 and 2006, this species was found to be significantly more abundant than it was in surveys conducted in the same area between 1971 and 1973. One of the most common bumblebees found in the early 1970s, Bombus affinis, *was just about gone, represented by one specimen. Photograph: Sheila R. Colla, York University, Toronto, Canada.*

In North America, a unique situation may have led to the loss or the near loss of a number of species of bumblebees. Unlike honey bees, which are heavily managed introduced bees, bumblebees are native pollinators and the only social wild bees, though some commercial operations rear bumblebees for greenhouse pollination. A problem arose when North American queens were exported to Europe, where they were infected by European bumblebee parasites, and were then reimported into North America for greenhouse pollination of tomatoes and other plants. Greenhouses are not airtight, and bees can easily escape, come into contact with wild bumblebee populations, and transmit infection to wild bees. In 2006, Sheila R. Colla, a doctoral student at York University in Toronto, and colleagues documented that bumblebees captured a short distance outside commercial greenhouses in which bumblebees are reared are more likely than bumblebees captured far from greenhouses to be infected with *Nosema bombi* or *Crithidia bombi,* two microsporidian parasites.

Robbin Thorp, bee expert and professor emeritus of entomology at University of California–Davis, suspects that the introduction of European bee diseases into the North American bumblebee population was responsible for the loss of short-tongued species of bumblebees. He admits that the evidence is circumstantial, but he points out that his data on bumblebee species prevalence in northern California and southern Oregon over the past 10 years demonstrate a rapid decline of some species of bumblebees, particularly the western bumblebee, *Bombus occidentalis.* Thorp has data from 40 years ago showing that *B. occidentalis* was common in an area just south of western San Francisco. By the early 2000s, the species had disappeared.

Colla and her doctoral adviser, Laurence Packer, have just shown that *Bombus affinis,* which used to be one of the most common bumblebees of the eastern coast of Canada and the United States, has been supplanted by other species of *Bombus,* especially *Bombus impatiens.* In fact, only one specimen of *B. affinis* was found throughout its historical range in Canada, and not one was found on US collecting trips. Colla and Packer note that although the effects of parasites on bumblebee colonies may be sublethal in most cases, they affect behavior and foraging. Furthermore, some pesticides—particularly the neonicotinoids—may be a threat to bumblebees, as they are to honey bees. Colla is now pursuing laboratory studies on the effects of sublethal concentrations of neonicotinoids on bumblebee ovary development.

Scott Hoffman Black, executive director of the Xerces Society for Invertebrate Conservation, explains that the society helped US Senator Barbara Boxer organize a congressional hearing in April on the plight of pollinators. "The silver lining of CCD," says Black, "is [that it] got people to look at pollinators and pollination."

Cox-Foster is now giving bees that came from Hawaii and are free of IAPV preparations from colonies that had CCD. The Hawaiian bees are also free of varroa mites, the number one cause of honey bee loss, and they have not been exposed to pesticides. "We are beginning to see some mortality in the colonies," says Cox-Foster. They are sequencing genetic material from the Hawaiian bees to determine whether IAPV is present.

Environmental insults and weakened defenses

Christopher Mullin, professor of insect toxicology at Penn State, is leading a team that is looking at pesticides and their metabolites in wax, pollen, nectar, and bees. They are studying what began as a shopping list of 171 pesticides and metabolites and has now been narrowed down through liquid chromatography-tandem mass spectrometry. These chemicals are both in-hive chemicals (mitocides) and agrochemicals from outside the hive. "We're seeing a lot of chemicals, a lot of residues," he says. "We're still trying to understand it." In addition, Mullin says, bees in the summer are physiologically different than they are in the winter, and environmental factors vary with the season as well, making it even more difficult to tease out what may be leading to CCD.

Entomologist Kimberly Stoner, whose work is supported with federal Hatch funds from her employer, the Connecticut Agricultural Experiment Station in New Haven, looks at pesticides in pollen collected by bees. Hatch funds are allocated to state agricultural experiment stations to allow those centers to react quickly to changes that affect agriculture. Because the experiment station has not been able to confirm any cases of CCD in Connecticut, Stoner is looking for a baseline for contamination in healthy hives. In fact, she notes, so far this year, Connecticut beekeepers have had very healthy hives.

When Dewey Caron, professor of entomology at the University of Delaware in Newark, stressed bees, he found that stress alone did not duplicate the symptoms of CCD. Caron explains that it may not be one pesticide or herbicide causing CCD, but rather a synergistic action among several chemicals or, perhaps, their metabolites. He wonders if the problem is related to some loss of the beehive's natural defense mechanism. Honey bees deposit a resinous material collected from trees and plants, called propolis, or "bee glue," in their hives. Propolis has antibiotic properties and serves as a hive's initial defense against infection. But US beekeepers have selected against strains of bees that produce a lot of propolis, thus removing one of the bee's initial defenses. Although bees do not have much of an immune system, Caron adds, animals that live in massive colonies, as bees do (with between 40,000 and 60,000 individuals), can afford to lose a percentage of individuals to disease. Without propolis, a pathogen that would never make its way into the hive can move right in.

The United States is not the only place with sick bees. Europe and the Middle East are also experiencing something similar to CCD in their honey bee colonies. Cox-Foster suggests that IAPV has caused hive deaths in Israel. Caron says that in Spain, where losses are similar to those from CCD, the culprit appears to be *Nosema.* Some of the European nations have blamed the deaths on pesticide contamination. "Is it exactly the same as what we see here?" Pettis asks. "We don't know."

There is more US funding available to study bee pathology than there was before the first report of CCD, although many note that it still is not enough money for the sophisticated chemical assays that need to be completed. Häagen-Dazs, the ice-cream maker, is beginning a national campaign to help bee pollinators through the reintroduction of their vanilla-honey-flavored ice cream—a portion of the proceeds from ice cream sales will go to pollinator research. Whatever is causing CCD is likely to be multifactorial, several researchers note. And the American public will be watching closely to see what the scientists have found. "The general public can identify with bees," says Pettis. "If bees are in trouble, they want to know why."

For more information, visit these sites:

http://maarec.cas.psu.edu/ColonyCollapseDisorder.html

http://riley.nal.usda.gov/nal_display/index.php?info_center=8&tax_level=2&tax_subject=10&want_id=1322&topic_id=1006&placement_default=0

www.xerces.org

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