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Mysterious Honeybee Disappearance Linked to Rare Virus **Researchers isolate possible cause of "colony collapse disorder" but stress that other explanations are still in play**

By JR Minkel

The mystery illness that has bedeviled U.S. beekeepers since 2006 may stem from a bee virus that apparently spread to the U.S. from Australia three years ago, according to a new study that marks the first big break in the puzzling case of the disappearing bees.

Researchers performed a sophisticated genetic comparison of healthy and diseased U.S. colonies that revealed the presence of Israeli acute paralysis virus (IAPV), an obscure but lethal bee bug, in almost all beekeeping operations affected by "colony collapse disorder" (CCD), but in only a single healthy one they examined.

"We haven't proven this is the cause. It is a candidate for being a trigger for CCD," says W. Ian Lipkin, director of the center for infection and immunology at Columbia University's Mailman School of Public Health, one of the study's lead members.



The disorder may also result from a combination of poor nutrition, pesticides and other factors, including infection, Lipkin and his colleagues say. They add that time-consuming tests are needed to determine whether IAPV can trigger CCD alone or in concert with other stressors, or whether certain combinations of stressors instead make hives vulnerable to the virus.

Israeli virologists discovered IAPV three years ago after investigating unexplained cases of dead bees piled in front of hives. The new study found the virus in samples of Australian bees, which were first imported to the U.S. three years ago.

If IAPV is the main trigger, researchers say, honeybees worldwide could be bred with strains of bees resistant to the virus, perhaps rescuing our nation's most economically valuable pollinator.

Bees are estimated to provide pollination valued at \$15 billion every year and are already worked to the limit. Half of the nearly 2.5 million hives in the U.S. alone are needed to pollinate almond crops.

Late last year, reports surfaced that adult honeybees were mysteriously abandoning commercial colonies, leaving ghost hives full of honey, larvae and unattended queens. The disorder wiped out an average of 45 percent of bees among the 23 percent of commercial U.S. beekeepers affected last winter.

Researchers and conspiracy theorists have offered a number of potential explanations, from parasitic varroa mites to chemical pesticides to cell phone radiation that leads bees astray.

Entomologists Diana Cox-Foster of Pennsylvania State University and Jeffery Pettis of the U.S. Department of Agriculture's Agricultural Research Service Bee Research Laboratory formed the Colony Collapse Disorder

Working Group to try to solve the mystery.

A key line of evidence suggested that infection plays a major role: The group found they could restore empty CCD hives to health by restocking them with fresh bees—but only if they first irradiated the hives with gamma rays, which destroy DNA.

Based on that evidence, Cox-Foster and Pettis convinced Lipkin, who led the discovery of West Nile virus, to take up the case, using specialized technology manufactured by genome sequencing firm 454 Life Sciences in Branford, Conn.

The trio and their colleagues lumped together RNA—the chemical that encodes active genes—from four geographically separated commercial operations stricken with CCD and compared it with the combined RNA of apparently healthy bees from Hawaii and Pennsylvania. They also scanned seemingly healthy bees from Australia and imported royal jelly from China, which queen bees use to nourish young workers.

Overall, CCD bees carried more types of harmful microorganisms than healthy bees, the researchers report in the online edition of *Science*. To identify potential culprits, they analyzed individual hives.

IAPV showed up in 25 of 30 diseased operations but in only one healthy colony from the U.S., they report. In contrast, all CCD hives contained a related virus called KBV along with a single-celled parasite *Nosema ceranae*, which a prior study had linked with CCD—but both organisms were present in about 80 percent of the healthy hives, too.

"The paper is a model of careful investigation," says entomologist Gene Robinson, director of the University of Illinois at Urbana-Champaign's bee research facility, who was not involved in the study. IAPV seems to be either a cause of or a marker for the disease, he says. "Either way, it's the first big breakthrough in the CCD story, so it's very exciting and very encouraging."

The smoking gun, Lipkin says, would be to infect healthy or stressed bees with IAPV and see if they catch CCD. The researchers plan to carry out such tests, but isolating the virus is challenging, he adds.

A broader sampling of diseased and healthy colonies from around the world would also help narrow down what causes the disease, Robinson says.

Lipkin and co-workers found that seemingly healthy Australian bees were infected with the virus and point out that all of the CCD hives they examined included or spent time near imported Australian bees. Beekeepers from Down Under have reported a "disappearing disease" but not on the scale of CCD, Pettis said during a press conference Wednesday.

One difference, he said, could be parasitic varroa mites, which suppress bees' immune systems and have driven down the U.S. bee population by 30 percent in the last 25 years, but are not found in Australia. "We know it's a primary stressor," he added. "I still believe that multiple factors are involved in CCD and we must test [them] in a more rigorous fashion."

The researchers only found varroa mites in half of the diseased U.S. hives. They note, however, that it is possible a mite-killing chemical applied by beekeepers may have killed the parasites off after they did their damage, or the chemical itself could have harmed the bees.

IAPV seems to have first killed bees in Israel in 2002, and since then has caused a varying number of deaths each year, says plant virologist Ilan Sela of The Hebrew University of Jerusalem, whose group identified the virus, which causes dying bees' wings to shiver.

"I was told by the Israeli [beekeeping] extension people that there are some recent indications for a small-scale CCD-like phenomenon in Israel," he says, adding that he has yet to test the afflicted bees for the virus.

If IAPV is causing CCD, there is hope of stopping its spread. About 30 percent of the bees Sela examined have incorporated pieces of the IAPV genome into their chromosomes and are resistant to the virus. Other bees

could be bred to carry those fragments and presumably survive infection, too, he says.

But he cautions that IAPV could in theory be causing CCD by inserting its genetic material into bee genes for pheromones or other molecules that coordinate hive behavior, thereby disrupting those genes, a possibility that he and the CCD working group plan to test.

Until researchers have cracked the CCD mystery, Cox-Foster advised beekeepers Wednesday to keep their bees well fed and free of mites.

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