Bee researchers close in on Colony Collapse Disorder

Across the nation, beekeepers have seen hive after hive succumb to Colony Collapse Disorder (CCD); a team of entomologists and infectious disease researchers now report a strong correlation between the occupancy of CCD and a virus, Israeli Acute Paralysis Virus (IVAP).

"We have not proven a causal relationship between any infectious agent and CCD," the researchers report in today's (Sept. 6) issue of Science Express online. However, they note that the prevalence of IAPV genetic material in bees suffering from CCD, the timing of the outbreaks and the geographical circumstances "indicate that IAPV is a significant marker for CCD."

Many researchers are investigating CCD because domestic honeybees are vital to a variety of agricultural crops in the United States. Beekeepers truck their hives cross country to pollinate almond groves in California, field crops and forages in the Midwest, apples and blueberries in the Northeast and citrus in Florida.

Unlike other diseases that have plagued bees in the past, CCD leaves a hive with a few newly hatched adults, a queen and plenty of food. Researchers suspect a pathogen because while bees will not recolonize a CCD hive, once the hive is irradiated and therefore sterile, bees are happy to live there.

The disease was recognized in 2006, but beekeepers reported hive declines similar to CCD as early as 2004. An estimated 23 percent of all beekeeping operations in the U.S. suffered from CCD during the winter of 2006-2007.

After looking at other methods of identifying the cause of the disease, the researchers decided to sequence the genetic material in bees to try to find a potential pathogen.

"The genome of the honey bee had just been completed," said Diana Cox-Foster, professor of entomology, Penn State. "So it was possible to do the sequencing and then eliminate the genetic material of the bees."

W. Ian Lipkin, M.D., professor of epidemiology, neurology and pathology at Columbia University and director of the Center for

Infection and Immunity at Columbia University Mailman School of Public Health, and his team prepared samples for 454 Life Science -- the company that developed the array-based pyrosequencer -- to sequence cDNA from the RNA of the bees.

Researchers analyzed data using a unique set of algorithms generated at Columbia, did a large amount of viral sequence comparison, developed real time PCR assays and cloned the full length IAPV genome, among other things.

The samples sequenced included bees from four geographically separated CCD suffering operations, apparently healthy bees imported from Australia, non-diseased samples from Pennsylvania and Hawaii, and samples of royal jelly imported from China. Royal jelly is secreted by bees and used to feed all larvae, but those fed only with royal jelly become queens.

"We chose bees from Hawaii because at that time, those populations were free of varoa mites, a problem in all mainland hives," says Cox-Foster. "The royal jelly was not intended for bees, but for human consumption and cosmetics, but some beekeepers use it to create new queens."

The researchers grouped material for sequencing as presumed CCD positive, presumed CCD negative and royal jelly. The pooled RNA sequences were analyzed for bacteria, fungi, parasites and viruses matches.

Lipkin played a key role in the search for new or reemerging pathogens, contributing unique methods. The genetic sequences, minus that of the domestic honeybee, were eventually matched against GenBank, a database of genetic sequences maintained by the U.S. National Center for Biology Information, National Institutes of Health. Ninety-six percent of the genetic material matched that previously found in bees.

The bacterial sequences were those normally found in bees worldwide, analyzed by Nancy A. Moran, the Regents' professor of ecology and evolutionary biology, University of Arizona, and colleagues and Jay Evans, research entomologist, Bee Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service and colleagues.

"The bacteria found were the same as those found in two previous studies from two different parts of the world at two different times," says Cox-Foster. "They represent mutualistic or symbiotic relationships with the bees, similar to those of humans and the bacteria found in the human gut."

Protozoans and fungi analyzed by Liwang Cui, associate professor of entomology, and David M. Geiser, associate professor of plant pathology, Penn State respectively, were associated with both CCD and non CCD populations.

"We knew before we started that we would find a boatload of viruses in the bees given our preliminary research," says Cox-Foster. "Eighteen different types are known from serology and antibody work in England."

Cox-Foster's and Lipkin's groups analyzed the viruses. They found the expected viruses, and they found one that, while identified by researchers at Hebrew University in 2004, has just now appeared in scientific publication. This virus, IAPV, along with Kashmir bee virus (KBV), was found only in CCD populations. In the initial experiments, the researchers report that "IAPV was found in all four affected operations sampled, in two of four royal jelly samples and in the Australian sample. KBV was present in three of four CCD operations, but not in the royal jelly."

Other viruses and Nosema parasites had been suggested as the cause of CCD, but the researchers found that those pathogens appear in both CCD and non-CCD samples. Only KBV and IAPV correlated with CCD in the genetic survey. In a recently published study, Jeffery S. Pettis, research leader, Bee Reseach Laboratory, and colleagues reported that Nosema ceranae had been in the U.S. for at least 10 years, along with Nosema apis.

Researchers then analyzed samples collected from 30 CCD colonies and 21 healthy colonies in the past three years for four pathogens: KBV, IAPV and Nosema apis and Nosema ceranae -- both fungi that infect bees. They found that all samples that had IAPV had KBV, but KBV also occurred in both sick and healthy samples.

"IAPV was found to increase the risk of CCD with a trend for increased CCD risk in samples positive for Nosema apis," the researchers said. "Neither KBV nor N. ceranae contributed significantly to the risk for CCD nor did they alter the influence of IAPV on CCD."

However, while IAPV may be a marker for CCD, proving that any organism is the cause of CCD is somewhat more difficult. The researchers will now try to infect bee colonies with CCD. Beside general health stress from the heavy load of pathogens normally carried by bees, other suggested contributors to CCD include pesticides, drought and nutritional stress.

Timing also may be the key to pinpointing the cause. The United States began allowing importation of bees from Australia in 2004, which coincides with early reports of CCD. The same year, IAPV, described by Israeli researchers with symptoms of shivering wings, progressed paralysis and bees dying outside the hive appeared. While CCD does not seem to have the same symptoms, this may reflect a different strain of the virus, co-infection with another pathogen or the presence of other stressors.

The researchers note that "the varroa mite, for example, absent in Australia, immunosuppresses bees, making them more susceptible to infection by other organisms." Beekeepers used mitocides, chemicals used to control varroa, on both CCD and healthy colonies.

Edward C. Holmes, professor of biology, Penn State, and Gustavo Palacios, Columbia University, were instrumental in determining the evolutionary relationships of the viruses found in CCD colonies compared to previously known viruses and isolates from Australia.

While unquestionably it is important to identify the cause of CCD, this total genetic study of bees and their fellow travelers also may lead to a better understanding of other disease causing agents in the population and to an understanding of the beneficial organisms that reside within the bee.

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Other researchers on the Penn State team include Dennis vanEngelsdorp, senior extension associate and State Apiarist for the Pennsylvania Department of Agriculture, and Abby Kalkstein, research technologist. Other researchers at Columbia University include Sean Conlan, Phenix-Lan Quan, Thomas Briese, Mady Hornig, Andrew Drysdale, Jeffrey Hui and Junhui Zhai. Vince Martinson, University of Arizona and Stephen K. Hutchison, Jan Fredrik Simons and Michael Eghom, at 454 Life Sciences, also contributed.

Progress made in research on mysteriously disappearing honeybees

Researchers identify virus possibly responsible for declining honeybee population using 454 Life Sciences' sequencing technology; findings published in Science implicate virus in deaths of tens of billions of bees

BRANFORD, Conn. – September 6, 2007 – 454 Life Sciences, a Roche company, today announced that researchers at Columbia University have identified a virus implicated in the deaths of 2.4 million honeybee colonies – tens of billions of bees – using the company's Genome Sequencer™ system.

The findings explain how foreign organisms living in and among the bees were identified by reading sequences of DNA isolated from the bee colonies. The study, entitled "A metagenomic survey of microbes in honey bee colony collapse disorder", appears online (ahead of print) today in the journal Science. Using 454 Sequencing technology, Dr. Ian Lipkin, Professor of Epidemiology at Columbia University's Mailman School of Public Health, and colleagues sequenced DNA and RNA samples that were extracted from collapsing and healthy bee colonies in search of any pathogen responsible for the collapse.

The research identified five major bacterial groups, four lineages of fungi and seven types of viruses. While most of the foreign organisms were found in both the collapsed and healthy bee colonies, one virus, Israeli Acute paralysis Paralysis Virus (IAPV), was found only in the collapsed colonies. As discussed today in Science: "Although we have not proven a causal relationship between infection and CCD, the prevalence of viral sequences in CCD operations … make IAPV a leading candidate."

"Unbiased 454 Sequencing technology enabled us to rapidly assemble a comprehensive inventory of microflora in Colony Collapse Disorder (CCD) and non-CCD populations and provided the sequence information needed to identify candidate pathogens," stated Dr. Lipkin. "CCD is a model for investigating epidemics of unexplained infectious disease"

Bees play an integral role in the world food supply and are essential for the pollination of more than 90 fruit and vegetable crops worldwide. The economic value of these agricultural products is placed at more than $14.6 billion in the United States alone. In CCD, honeybee colonies inexplicably lose all of their worker bees. CCD has resulted in a loss of 50% to 90% of colonies in beekeeping operations

across the United States. The observation that irradiated honeycombs from affected colonies could be repopulated with healthy bees, while non-sterilized combs could not, suggested an infectious basis for CCD. Suspected pathogens were screened for association with CCD by examination of samples collected from several sites over a period of three years.

"We are very pleased to see our technology applied to solve real-world problems. There were a lot of examples during the last months, from cancer research, infectious diseases research, drug discovery, marine biology, anthropology, paleontology, and many more. We are hopeful this latest research will help eliminate the threat of CCD to global agriculture" said Christopher McLeod, president of 454 Life Sciences. "The chief advantage of 454 Sequencing technology is how it enables researchers to identify the organisms present in complex environments without any advance knowledge of the sample."

CCD was first reported in the fall of 2006 in the Unites States. Since then, CCD has been reported in Germany, Switzerland, Spain, Portugal, Italy and Greece. A recent survey of 13 states by the Apiary Inspectors of America showed that over a quarter of U.S. beekeepers have lost, on average, half of their bee colonies between September 2006 and March 2007.

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Virus named as possible factor in honey bee disorder

A comparison of healthy and unhealthy bee colonies points to a virus contributing to Colony Collapse Disorder (CCD), according to a report being published by the journal *Science*, at the *Science* Express web site, on 06 September. *Science* is published by AAAS, the nonprofit science society.

"Our extensive study suggests that the Israeli Acute Paralysis Virus (IAPV) may be a potential cause of Colony Collapse Disorder," said W. Ian Lipkin, director of the Center for Infection and Immunology at the Mailman School of Public Health, Columbia University. "Our next step is to ascertain whether this virus, alone or in concert with other factors such as microbes, toxins and stressors, can induce CCD in healthy bees," he added.

CCD is a puzzling phenomenon occurring in the United States – and possibly other countries where it is not yet confirmed – in which all adult bees disappear from the hive, leaving the honey and pollen behind. Few, if any, dead bees are found around the hive.

Between 50 and 90 percent of the commercial honey bee (*Apis mellifera*) colonies in the United States have been afflicted by CCD, and the disorder is making it difficult for U.S. commercial beekeepers to pollinate crops.

Researchers including Lipkin and Diana Cox-Foster, entomology professor at Pennsylvania State University, and colleagues have taken a new approach to investigating infectious disease outbreaks. To find the cause of CCD they used a rapid genome sequencing technique called pyrosequencing to catalogue the entire variety of microorganisms that honey bees harbor. After comparing their sequences with known sequences held in public databases, they identified symbiotic and pathogenic bacteria, fungi and viruses found in both healthy and CCD-afflicted colonies.

They tested samples collected over three years across the United States from normal and CCD-affected hives. They also tested royal jelly imported from China, which is fed to bee larvae to start up a new colony, as well as apparently healthy bees imported from Australia, in an attempt to locate a source for an infectious agent. After detailed statistical comparison of all the samples, the molecular signs of Israeli Acute Paralysis Virus appeared to be associated with CCD.

"This research gives us a very good lead to follow, but we do not believe IAPV is acting alone," said coauthor Jeffery S. Pettis, research leader of the Bee Research Laboratory, United States Department of Agriculture. "Other stressors to the colony are likely involved," he said. Those stressors could be poor nutrition, pesticide exposure and parasitic mites.

The next research steps include inducing CCD in healthy bees, determining the global distribution of IAPV and CCD and studying bees that appear to be resistant to CCD.

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"A Metagenomic Survey of Microbes in Honey Bee Colony Collapse Disorder," by D.L. Cox-Foster, E.C. Holmes, D.M. Geiser, D. vanEngelsdorp, A.L. Kalkstein and L. Cui at Pennsylvania State

University in University Park, PA; S. Conlan, G. Palacios, P-L. Quan, T. Briese, M. Hornig, A. Drysdale, J. Hui, J. Zhai and W.I. Lipkin at Columbia University in New York, NY; E.C. Holmes at Fogarty International Center, National Institutes of Health in Bethesda, MD; J.D. Evans and J.S. Pettis at U.S. Department of Agriculture (USDA) Agricultural Research Service in Beltsville, MD; N.A. Moran and V. Martinson at University of Arizona in Tucson, AZ; D. vanEngelsdorp at Pennsylvania Department of Agriculture in Harrisburg, PA; S.K. Hutchison, J.F. Simons and M. Egholm at 454 Life Sciences in Branford, CT.

Connection between virus and Colony Collapse Disorder in bees

A team led by scientists from the Columbia University Mailman School of Public Health, Pennsylvania State University, the USDA Agricultural Research Service, University of Arizona, and 454 Life Sciences has found a significant connection between the Israeli Acute Paralysis Virus (IAPV) and colony collapse disorder (CCD) in honey bees. The findings, an important step in addressing the disorder that is decimating bee colonies across the country, are published in the journal Science this week.

In colony collapse disorder, honey bee colonies inexplicably lose all of their worker bees. CCD has resulted in a loss of 50-90% of colonies in beekeeping operations across the U.S. The consortium of scientists who have been studying the role of infection in this phenomenon includes Diana Cox-Foster, professor in the Department of Entomology at Pennsylvania State University, Ian Lipkin, director of the Center for Infection and Immunity at Columbia University Mailman School of Public Health, Jeffery Pettis, research leader of the ARS Bee Research Laboratory, and Nancy Moran, Professor at the University of Arizona, Tucson.

Ian Lipkin, MD, professor of Epidemiology, Neurology, and Pathology at Columbia, and his team at the Mailman School's Center for Infection and Immunity, together with a team at 454 Life Sciences, used revolutionary genetic technologies, to survey microflora of CCD hives, normal hives, and imported royal jelly. Candidate pathogens were screened for significance of association with CCD by examining samples collected by the USDA and Penn State from several sites over a period of three years.

Using the 454 Life Sciences high-throughput DNA sequencing platform, and analytical methods developed at Columbia, Dr. Lipkin's team searched for footprints of viruses, bacteria, fungi, and parasites in thousands of sequences. Candidates were further characterized by more detailed sequence analysis to ascertain their specificity for CCD and relationship to known and unknown pathogens.

IAPV, an unclassified dicistrovirus not previously reported in the U.S. that is transmitted by the varroa mite, and Kasmir bee virus were only found in CCD hives. The researchers report that IAPV was found in all four affected operations sampled, in two of four royal jelly samples, and in the Australian sample. KBV was present in three of four CCD operations, but not in the royal jelly. One organism was significantly correlated with CCD: finding IAPV in a bee sample correctly distinguished CCD from non-CCD status 96.1 percent of the time.

"This is a powerful new strategy for looking at outbreaks of infectious disease and finding cause. Dr. Cox-Foster recruited us into this project, making a persuasive case for applying our state-of-the-art methods for differential diagnosis of infectious disease in humans, to this challenge in agricultural epidemiology," said Dr. Lipkin. "The profound synergy within the group—bringing entomology, microbiology, and bioinformatics together—enabled us to work toward a solution to this extraordinarily complex problem."

This is the first report of IAPV in the United States. IAPV was first described in 2004 in Israel where infected bees presented with shivering wings, progressed to paralysis and then died just outside the hive. Importation to the U.S. of bees from Australia began in 2004, coinciding with early reports of unusual colony declines.

IAPV was found in non-CCD hives in some cases, which could reflect strain variation, co-infection, or the presence of other stressors, such as pesticides or poor nutrition. The varroa mite, for example, absent in Australia, immunosuppresses bees making them more susceptible to

infection by other organisms, including viruses. Other stressors may include chemical pesticides used on plants pollinated by bees and in hives to control pests.

"Our results indicate that IAPV is a significant marker for CCD. This discovery may be helpful in identifying hives at risk for disease. The next step is to ascertain whether IAPV, alone or in concert with other factors, can induce CCD in healthy bees," added Dr. Lipkin.

Bees play an integral role in the world food supply, and are essential for the pollination of over 90 fruit and vegetable crops worldwide, with the economic value of these agricultural products placed at more than $14.6 billion in the U.S. In addition to agricultural crops, honey bees also pollinate many native plants within the ecosystem. Recently, the increased deaths in bee colonies due to CCD seriously threaten the ability of the bee industry to meet the pollination needs of fruit and vegetable producers in the U.S.

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