A more concise explanation of CCD - Iridescent Virus and Nosema ceranae

New technology finds pathogens that may reconcile contradictory claims on Colony Collapse Disorder

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A multi-institutional team of researchers sifted through the ever-growing zoo of new invasive, exotic pathogens of bees, and consistently found the same two disease organisms in beehives suffering from Colony Collapse Disorder (CCD) in samples collected from 2006 to 2009.

They discovered a new virus never seen before in North America, and found a well-known invasive variant of the intestinal bee disease Nosema. The overlooked virus may explain why prior studies presented mutually contradictory findings. This new evidence could create a basis for consensus among research teams who to date, lacked common ground in their conclusions.

Their paper appeared in the journal *PLoS ONE* http://dx.plos.org/10.1371/journal.pone.0013181
It reports on a multi-year study of Colony Collapse Disorder. Researchers used new technology and techniques to detect and unambiguously identify every pathogen in collapsing bee hives, rather than the smaller subset of possible pathogens detectable via other means.

An Invertebrate Iridescent Virus ("IIV") , newly-found in North America, in combination with Nosema ceranae, which arrived from overseas less recently, was found in "*Virtually all of the bees from CCD colonies*" sampled from widely dispersed USA hives from 2006 through 2009.

IIV was not found in bees from packages imported from Australia nor in bees from an isolated non-migratory commercial bee operation in Montana, both sites confirmed free of CCD-like symptoms.

Additionally, the researchers "*observed the progression of CCD in a collapsing colony… taking bee samples… over a three month period, ending when only a queen and four workers remained*."

Further still, some bees were inoculated with Nosema ceranae, while other bees were inoculated with the "IIV-6" strain of the IIV virus. Their mortality was then compared to bees inoculated with both pathogens, and a control group given a placebo. The results "*strongly suggest that the combination of N. ceranae and IIV is associated with increased bee mortality*."

Yet even further, the effort discovered two additional invasive exotic bee viruses never before detected in North America, but determined that they were not involved in CCD. The viruses found are "Varroa Destructor-1 Virus" and "Kakugo Virus", both native to Asia.

Dr. Jerry Bromenshenk of U Montana outlined the next steps "*We have a proposal pending to isolate, characterize, and then inoculate bees with the specific iridescent virus that occurs in USA bees. This is a critical step, since the virus does not appear to be any of the world's known iridescent viruses. Once we have the actual virus, we can complete the inoculation trials that are needed to test whether we've truly found the cause of CCD*."

Proteomics – A Brief Summary

The technology used in this study seems ideal for addressing the ever-growing list of pathogens carried across oceans by the globalization of trade. It can detect disease pathogens that need not be identical to any known pathogen. This describes the needs of beekeepers clearly, given the number of invasives that came to plague honey bees in the USA since the early 1980s.

"Mass Spectrometry-Based Proteomics" (MSP) starts with about 60 bees tossed in a blender, and mixed until homogenous, then filtered. Cells are chemically burst, and proteins are isolated from the mix and "digested", breaking them down to peptides. The resulting peptides are run through a device called a "Liquid Chromatograph" to separate them by density, which allows their structure and sequence to be determined by another set of devices, "Tandem Mass Spectrometers".

Each peptide sequence is then compared to the NIH National Center for Biotechnology (NCBI) database of peptide sequences. The database used is a collection of the peptides unique to specific organisms. This means that each match of a peptide sequence is a unique match to a single organism. Any peptide used in more than one organism would not be in the database.

Dr. Charles Wick of the US Army Edgewood Chemical Biological Center explained the level of certainty with which the virus was detected in colonies showing CCD symptoms: "*IIV has 18,900 unique peptides… When we detect a few of these, say 50-100, we have enough evidence for an unambiguous identification*."

But how did they make what Dr. Wick called an "*unambiguous identification*" of a virus that was said by Dr. Bromenshenk to not be "*any of the world's known iridescent viruses"?* How can anyone find what's never even been detected or identified before? The answer is that the unknown organism will match the closest organism in the database, which narrows things down to at least the "family" or "genus" level, if not "species". So, even without having sequenced the specific strain of IIV of interest, enough peptides matched the IIV strain in the database to confirm that what was found was a strain of IIV.

As an example of the wide net cast by this technique, Nosema was not well-represented in the NCBI database, so there was some ambiguity in the identification of the Nosema via proteomics alone, matching only the genus Nosema. The species and strain was confirmed as Nosema ceranae using Polymerase Chain Reaction (PCR) techniques.

The Claims In Spain Can Mainly Be Explained

Research led by Mariano Higes of the Bee Pathology Laboratory, Centro Apícola Regional in Marchamalo, Spain has repeatedly pointed to Nosema ceranae as the sole proximate cause of rapid colony collapse. This seemed unlikely to researchers in the USA and elsewhere, as Nosema has not appeared to be as virulent outside of Spain. But this new work provides an explanation that could support the Higes work with nothing more than the addition of the newly-detected IIV.

As in previous US studies, no one in Spain would have had reason to suspect that a DNA virus like IIV would be involved, as the bulk of bee viruses are RNA viruses. So they've yet to look for IIV in Spain, and they have not had the wider net of MSP to find what was not being sought. The good news is that Dr. Higes has historical samples frozen. Dr. Jerry Bromenshenk reports that the Higes team is willing to engage in a joint effort to screen the Spanish samples using MSP.

Does This Explain CCD In The USA?

The samples analyzed in this study showed a wide range of pathogens, including Nosema, Invertebrate Iridescent Virus ("IIV"), Black Queen Cell Virus, Acute Bee Paralysis Virus, Israeli Acute Paralysis Virus, Deformed Wing Virus, Sac Brood Virus, Kashmir Bee Virus, Varroa Destructor-1 Virus, and Kakugo Virus. None of the suspect pathogens named by other research efforts were missed, two new and novel pathogens were found, and the use of MSP implies that no pathogens were overlooked. Even a new, unknown, and unnamed pathogen would have resulted in a partial peptide match to some other living thing.

So, while the counts or mix of pathogens might have been skewed by an insufficient number of samples, or collecting samples from an insufficient number of operations, it is difficult to imagine that there are additional pathogens yet to be found that could be implicated in CCD.

Insecurity About Biosecurity

Since the 1980s, "Globalization" has increasingly consisted of shipments of goods from Asian ports to Western shores. This research connects the dots by consistently finding specific bee pathogens native to Asia, unknown to USA beekeepers in the early 1980s, but that have since become far too familiar to everyone in beekeeping, including the authors of the paper: *"We know that in the Asian honey bee, Apis ceranae, a combination of parasites and pathogens co-exist, including: (1) Nosema ceranae, (2) an iridescent virus, (3) parasitic and predacious mites, and (4) two other RNA-type viruses, Kashmir bee virus*

and a Sacbrood virus. We have had both Kashmir bee virus and Nosema ceranae in North America going back a decade or more. We need to see how similar the CCD strain of iridescent virus is to the IIV-24 strain from Apis ceranae. It is possible that US bees acquired IIV from the Apis ceranae along with Nosema ceranae and Kashmir bee virus."

While unsubstantiated "fringe" explanations for CCD abound, ranging from cell phones to pesticides to GMO crops, the common factor is that pathogens previously found only in Asia have spread to countries lacking effective biosecurity, such as the USA, but not to countries with more robust approaches to biosecurity, such as New Zealand. The research team suggests "*Standard quarantine practices such as testing of imported bees before they are added to colonies, and disinfection of equipment would likely help*."

Practical Implications For Beekeepers

The team has two suggestions of interest to beekeepers:

1) "*Most IIVs replicate at about 21 C (70 F) and do not replicate above 30-32 C (86 – 89 F). Higher temperatures may suppress the virus by halting replication, whereas cool weather and damp conditions may speed up replication of both IIV and Nosema. Many instances of CCD have occurred following extended periods of cool, damp weather. Several beekeepers have reported to us that they have more problems with bees in areas with frequent fog or in hill areas where the weather is cooler. Placing bees in warm, sunny locations appears to help*."

2) "*Varroa may act as a vector for the dispersal of IIV among bee colonies. Varroa is known to increase damage caused by other viruses, and beekeepers who fail to control varroa levels are likely to sustain high colony losses*."

This may not sound like much, but it is a vast improvement over the usual vague platitudes we've been handed over and over about "maintaining strong colonies" and "minimizing stress". It also ups the ante in the age-old debate among beekeepers over placing hives in sun versus placing hives in shade.

"**Iridovirus and Microsporidian Linked to Honey Bee Colony Decline**"
Jerry J. Bromenshenk, Colin B. Henderson, Charles H. Wick, Michael F. Stanford, Alan W. Zulich, Rabih E. Jabbour, Samir V. Deshpande, Patrick E. McCubbin, Robert A. Seccomb, Phillip M. Welch, Trevor Williams, David R. Firth, Evan Skowronski, Margaret M. Lehmann, Shan L. Bilimoria, Joanna Gress, Kevin W. Wanner, Robert A. Cramer Jr. (2010) PLoS ONE 5(10): e13181. doi:10.1371/journal.pone.0013181

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