A Beekeeper Reads The Paper

Be warned – this wasn't easy to write, so don't expect it to be easy to read. We are going to review a scientific paper entitled "***A Metagenomic Survey of Microbes in Honey Bee Colony Collapse Disorder***". Even the title is intimidating. Not to worry though, most of what we will consider will be simple things.

The paper has more authors than paragraphs, and was published in the prestigious journal "Science", so it should be definitive, authoritative, and crystal clear once we wrestle the jargon into submission. Mere beekeepers like ourselves should find the results compelling. We should be impressed by the clarity of the conclusions drawn from the data. Honest, the paper really does have more authors (23) than paragraphs (14).

How Science Really Works

Published peer-reviewed scientific papers are viewed by laymen as "proof" of something. Nothing could be further from the truth. Papers make claims, and good papers provide hard data to support the claims. But if you've only read the summary paragraph found at the beginning of papers, you've been reading the equivalent of a movie review written by the producer of the film. ***It is a sales pitch. They are "selling" ideas*.** I worked the day shift at the idea factory for a while, so I'll point out the items of interest, and try to help you to be a better-informed consumer of science.

What Does "Peer Reviewed" Imply?

For this paper, it meant that it was the subject of critique, correction, clarification, and arguments from the day it was submitted (June 14, 2007) until the day it was accepted for publication (August 30, 2007). Compromises are made, one by one until the reviewers, scientists not connected with the research presented, are satisfied that their concerns have been addressed. Significant changes can be required, and this one took all summer to turn into something acceptable for publication.

What's "Metagenomics"?

It is the examination of genomes recovered from "environmental samples". In this case, it means processing samples of bees and royal jelly.

What The Paper Claims, In 44 Words

Let's start with the essential core of the summary "abstract" paragraph: ***"The observation that irradiated combs from affected colonies can be repopulated with naive bees suggests that infection may contribute to CCD. ...pathogens were screened for significance of association with CCD. One organism, Israeli acute paralysis virus of bees (IAPV), was strongly correlated with CCD."***

Translating into plain English, they claim that hives that had died out from CCD were irradiated, and new colonies put into that equipment did better than new colonies put into equipment that was not irradiated. This led them to think that "infection" might "contribute" to CCD.

And, as a result of their work, they claim that IAPV "strongly correlated with CCD", meaning that when they looked at samples from colonies in yards suffering from CCD, the found IAPV so often that they two could be said to be "certain" to be related. They didn't claim that IAPV causes CCD, but the implication is that it is at least a contributing factor.

Of course, correlation works both ways. It is just as likely that CCD weakens a colony to the point that the bees get IAPV as a result of getting CCD. But let's stick to looking at the specific claims they make for now, and come back to correlation later.

Don't Be A Pest About Pesticides

What about pesticides? Let's get pesticides out of the way up front, just like the authors of the paper did. The word "pesticide" appears only once in the paper: ***"Crop pesticide use is similar in both the United States and Australia."*** This may be a surprise to many beekeepers. One would have hoped to hear something more detailed and convincing on this point, given the number of symptoms associated with CCD that can be matched up with the advertised effects of certain pesticides on targeted pest insects. One might have even hoped for some pesticide residue analysis from the exact same colonies analyzed for pathogens.

There's another research team working on pesticides and CCD. When asked for the status of that work in the press conference, Diana Cox-Foster of Penn State said in the press conference that they had found no correlation between CCD and any specific pesticide.

But Dr. Cox-Foster also said: ***"…in terms of chemical pesticides, the data there suggests that perhaps those could be helping to stress the bees, or acting as a potential trigger. We have some data from our lab suggesting that some classes of pesticides may cause amplification of specific viruses, not all. We need to do more work on that to look at that in particular."***

Her comment above is more than slightly at odds with the following statement in the paper:

"One hypothesis is that CCD is due to the introduction of a novel infectious agent. This is supported by preliminary evidence that CCD is transmissible through reuse of equipment from CCD colonies and that such transmission can be broken by irradiation of the equipment before [re]use."

Note the use of the passive voice "one hypothesis is…" Who's hypothesis? The authors of the paper, of course. They don't have any final pesticide data yet. They admit that pesticides could be "***acting as a potential trigger"***, but the paper still focuses on the **"*novel infectious agent",*** giving the impression that pesticides have nothing to do with CCD.

So it seems that we will just have to wait for more detail on pesticides. But the comments made in the press conference indicate that CCD is highly likely to be caused by more than just IAPV alone.

Irradiation Kills Pathogens

They explain why they eliminated other possible factors from consideration before even starting their work: "***transmission can be broken by irradiation of the equipment before [re]use****."*

One might think it strange that an up-front assumption so broad would be based upon admittedly "preliminary evidence", and one would be right. We fact-checked this claim with Dave Hackenberg, the beekeeper who allowed some of his hives to be irradiated, and he is very enthusiastic about the difference between packages installed in irradiated dead outs versus packages installed in non-irradiated dead-outs. But not for the reason the researchers assume.

Irradiation May Also Break Down Some Pesticides

Dave does not agree that the positive results from irradiation imply that any "infectious agent" is behind CCD. Dave points out what should have been obvious to the researchers, that irradiation also can break down or volatize off pesticide residues on comb, in pollen, and in honey. I wasn't too sure about this myself, so I checked with Bayer Cropscience. Their chemists stated that ***"[Sprayed] Imidacloprid breaks down very rapidly in the presence of sunlight, with a half-life (time for half the applied dose to degrade) ranging from a few hours in water to approximately 39 days on soil surface."***

Sunlight is pretty weak as compared to the levels of gamma radiation used to kill American Foulbrood spores, so Dave's point is insightful, at least for sprayed Imidacloprid. But Bayer pointed out that this would not be the case for all neonicitinoid insecticide products. Maryann Frazier of Penn State plans to test this theory, even though the pollen samples she has been shepherding through

the process of analysis have yet to reveal any residue levels that appear toxic to bees or brood.

Pesticides? No Resolution

So, the paper fails to eliminate pesticides from the list of suspected contributing causes of CCD. There's nothing to refute a claim that CCD might be solely due to pesticides, and all the viruses nothing but opportunistic infections.

How Many Samples?

The paper uses inconsistent terms to refer to samples, so it is not clear to the reader how many samples were taken from how many yards, nor is it clear if some samples were taken from the same hives at different times

Jeff Pettis, who runs the Beltsville Bee Lab for USDA-ARS was able to put things in clearer terms in an e-mail: ***"The 30 CCD samples represent bees from 5 operations. The 21 non-CCD samples represent bees from 6 operations".***

So, we can look at the data as representing a total of 51 yards with one sample representing each yard, or look at the data as representing 5 beekeeping operations that had CCD and 6 operations that did not.

Were Diseased Samples Consistently Classified?

One basic step that can skew results is how one classifies the samples as being from "diseased" or "disease-free" colonies. If samples are labeled "diseased" when the specific colony is not diseased, the result is a misleading sample, and the results are much less accurate. Given that a total of only 30 "CCD Samples" were analyzed, a single misclassified sample could make a difference of 3% in the "CCD Samples" results.

The paper says:
"Diseased apiaries were selected based on evidence of recent collapse of some colonies within the apiary and a lack of dead bees in collapsed colonies. Up to three dead, collapsing or stronger colonies were selected for sample collection in each diseased apiary."

So, if only some of the colonies in an apiary had collapsed, samples were collected from any colony in that apiary, as if all colonies in the apiary were certain to be "diseased". This implies that some of the samples called "CCD Samples" could actually be "Non-CCD Samples". There's no way to tell, as there is no fool-proof test that confirms the presence of CCD. The best that could have been done would have been to only use samples collected from hives that showed obvious symptoms of CCD. That wasn't done, so the results attributed to the "CCD Samples" are questionable as to their accuracy.

With so few samples, there isn't a lot of room for any of them to be misclassified. The assumption that all colonies in a yard will have CCD when only some of the

colonies are showing symptoms is clearly a guess. The resulting data is dependent upon those guesses, and cannot be said to be any better than those guesses.

Were Healthy Samples Consistently Classified?

While it may seem simple to tell a healthy colony from a colony suffering from CCD, it would be premature to call the samples taken from such colonies "disease free" unless the colonies were inspected again at a later time to verify that they had remained disease-free.

No one knows what a colony about to collapse from CCD looks like, but it is reasonable to conclude that there aren't any obvious symptoms, or the many highly trained and experienced people investigating would have noticed something unusual. The paper did not mention this aspect of the sample collection process, so we asked Dr. Jeff Pettis of the USDA Beltsville Bee Lab about it during the press conference where the paper was announced to the press. He answered ***"No, we didn't have the luxury of going back to those same colonies."***

At risk of sounding flippant, couldn't someone at least have called the beekeepers who owned the hives, and asked "how are the hives that were sampled doing right now"?

They didn't even do that, so it is impossible to know if the samples classified as "non-CCD" were taken from hives that collapsed from CCD soon after, and thus should have been classified as samples from "CCD" colonies. Each misclassified non-CCD hive would result in a 4.7% error in the "Non-CCD Samples" results.

Chance, Luck, Statistics

If you think that I'm being unreasonable here, understand that being pedantic and picky about sample collection is central to being certain about the results. Given the lack of certainty about the samples, nothing can be said about the results with any certainty. The results could only be accurate by chance! All sorts of statistical analysis was done, but we can ignore it all, as the samples can only be properly classified if everyone was very lucky.

I'm surprised that something this basic slipped by the reviewers. The problems with sample classification invalidate the entire paper in my view. But we won't abandon the slog here, as there are other problems to consider.

Were Viruses Found, Or Mere Evidence Of Prior Exposure?

A great deal of emphasis was placed on the finding of genetic evidence of "Israeli Acute Paralysis Virus", but another paper in the June 2007 issue of the journal "Virology" presents findings that force the reasonable reader to question the claim that the virus was "found".

The "Virology" paper found that the bee genome itself is modified by exposure to IAPV. Bees that survive IAPV infection end up with part of the IAPV genome in their genome, which may give those bees immunity to IAPV. It is possible that this immunity can be inherited, so what was found could also be the result of exposure to IAPV several bee generations ago. There's simply no way to tell.

The "Virology" paper refers to a 400 base-pair long sequence that was found in the bees, while the CCD paper found much shorter sequences, "***averaging 150 base-pairs long***".

Could the 150 base-pair sequences viewed as evidence of the IAPV virus actually be nothing but part of the 400 base-pair sequences said to be evidence in the bee genomes of prior exposure and survival of IAPV? We aren't virologists, nor are we geneticists, so we handed both papers to several qualified people who all agreed that there was simply no way to compare the information presented in the two papers to determine this.

Sequencing the complete genome of the virus in question would answer the question, as would using alternatives to polymerase chain reaction (PCR) and sequencing, but when you toss bees into a blender with liquid nitrogen, and then extract DNA from the resulting margarita (perhaps we should call it a "marga-bee-ta"?), there's no way to be certain if you are looking at DNA from multiple organisms, or just one organism.

So, was a virus actually found, or wasn't it? There's no way to be certain from the evidence presented in the paper.

Is IAPV Really KBV?

Let's assume that the IAPV virus actually was found in many of the samples sequenced. It should be acknowledged that what was found may have been called "IAPV" in the paper, but the researchers are less than certain if they "found" IAPV, or if they found the more familiar Kashmir Bee Virus (KVB). Dr. Edward Holmes said in the press conference:

"As for IAPV itself, again, the big unknown that comes out is 'what is IAPV?'… Is this a distinct virus in itself, is it a distinct lineage of an another virus called KBV? We really don't know that yet."

Well, if they aren't sure they found IAPV or KBV, why did they make such a big deal about saying that IAPV was "strongly correlated with CCD", where KBV was not? The key word is "distinct". They are convinced that IAPV is different from KBV, even if it is nothing but a variation of KBV. But Dr. Holmes went on to say:

"We know from other viruses, like West Nile… that very small genetic changes I mean, one amino acid change, can turn a benign virus into a very

virulent one… it is quite possible that very small genetic changes… may make this virus behave differently in Israel, Australia, and the USA."

So, it really doesn't matter which virus is which, or which one is found where. If very small changes can turn a benign virus into a virulent one, and tiny changes could appear in local populations of viruses in different countries, the names of viruses don't really have any meaning at all. We had better start numbering them, so that next year's IAPV is not confused with this year's version. One could be deadly, and the other harmless. Same thing with location, so we had better start adding zip codes onto the end of the "names", to track where they were found. This could get very messy.

Correlation Is Not Causation

While the authors of the paper cannot be blamed for the actions of the press, the wording of the paper hinted strongly that a specific pathogen, IAPV, was both the likely causative disease agent, and came here from Australia.

Even "Science Magazine", published by the same organization that publishes the journal "Science", ran an article with the headline "***Puzzling Decline of U.S. Bees Linked to Virus From Australia"***. Now, if the AAAS, who publishes the journal "Science" and "Science Magazine" can't read the paper they themselves published, and take the time to understand what is proven versus what is not, or bother to listen and comprehend what is said in the press conference they themselves held, can we expect any other media outlet to "get the story right"? I don't think so.

So let's look at causation. To start, understand that viruses are found often, even in healthy colonies. Work done in 2004 in France is a good example. ("Prevalence and Seasonal Variations of Six Bee Viruses in Apis mellifera L. and Varroa destructor Mite Populations in France" in "Applied and Environmental Microbiology", Dec. 2004)

A total of 36 apiaries were sampled across France.

For Adult Bees:

Virus	% Of Apiaries
DWV	97%
Sacbrood virus	86%
Chronic bee paralysis virus	28%
Acute bee paralysis virus	58%
Black queen cell virus	86%
Kashmir bee virus	17%

For Pupae:

Virus	% Of Apiaries
=====	==========
DWV	94%
Sacbrood virus	80%
Chronic bee paralysis virus	0%
Acute bee paralysis virus	23%
Black queen cell virus	23%
Kashmir bee virus	6%

For Varroa:

Virus	% Of Apiaries
=====	==========
DWV	100%
Sacbrood virus	45%
Chronic bee paralysis virus	0%
Acute bee paralysis virus	36%
Black queen cell virus	0%
Kashmir bee virus	5%

So which came first, the viruses or the CCD? It is perfectly possible that the claimed "strong correlation" between IAPV and colonies with CCD was actually a result of IAPV being nothing more than a very good opportunist.

Let's assume that a non-virus event weakened the colony, like, oh, I dunno, let's blame "infections of BOTH Nosema apis and Nosema ceranae at the same time" just for example.

Once the colony is weakened, it is doomed. The viruses are merely opportunistic infections, and IAPV is merely the most opportunistic of all of them, showing up most often in the weakened, dying colonies. The viruses might even contribute to the death of the colony, without being a proximate cause of death.

MY THEORY AND MY THEORY ALONE

As an aside, the detailed data provided in the supplemental materials for the paper does show that "CCD colonies" always had ***both*** Nosema apis and Nosema ceranae, as did the Australian bees. Non-CCD colonies had one type of Nosema or the other, but never both, and had lower levels of Nosema than the CCD-colonies.

So, I could explain the “strong correlation” between IAPV and colonies with CCD as nothing more than IAPV’s superior ability to be an opportunistic infection as compared to other viruses, and point to two kinds of Nosema at the same time as the actual cause of CCD. (I don’t have the full data set to work with, so I may be laughably wrong in my theory.)

My point here is that the authors might have considered examining the data using a “multivariate approach”, which means considering combinations of pathogens rather than merely comparing individual pathogens against each other. They didn’t.

But how do I explain the strange behavior associated with CCD if I blame the combination of two kinds of Nosema for the problem? I do exactly what the authors of the paper did, and don’t even try to explain the “behavior” issue.

See, any number can play this “science” game.