The Little Glass Chip
That Can Read Any Arthropod Bacterium, Fungus, Protozoan, Nematode, Mite, Virus or Microsporidian, Known or Unknown, and How We Are Going to Learn about Bees from It

M.E.A. McNeil

"Hi, I'm Joe." The affable man holding out his hand seemed improbably young to be one of the world's most innovative and celebrated researchers in molecular genomics. Even the building his lab inhabits, the freshly built QB3 Beyers Hall, at the new Mission Bay Campus of the University of California at San Francisco, is so new it is pictured on Google maps as a construction site.

The elegance of the expansive beige marble hallways there is a surprising contrast to typical architecture for a state university research facility (a style exemplified by its parent campus up the hill, a cobbled together bunch of functional hospitals, labs and converted houses). If entering QB3 brings a creepy appreciation for what must be vast corporate investment, that concern is dispelled by a visit to the lab on the fourth floor with its exhilarating dose of idealistically ethical bright minds and open hearts.

The surprises have only begun. Over the next year, honey bees will be examined at the DeRisi Lab at the molecular level in a unique and sustained study, and the results will be open to all of us to follow as it unfolds.

Joseph DeRisi, a professor of biochemistry at UCSF among a list of titles, has helped revolutionize the study of infectious disease with the use of molecular genomics. In graduate school at Stanford he contributed to the development of an altogether new way of measuring genes with a tool called a microarray, a DNA chip, making it possible to assay thousands of genes simultaneously and identify them by computer. This process has had an enormous impact on biology and medicine. What he does is now standard in the field for viral discovery.

At UCSF, DeRisi and his colleagues have applied microarray technology to human health, including malaria, a disease that devastates some of the most disenfranchised people in the world. The choice to do this research, which has resulted in a new understanding of how the malaria parasite grows, comes out of DeRisi's pervasive humanitarianism. The search for a cure has to be done by someone other than pharmaceutical researchers, he says. "People who have malaria don't carry credit cards." It is this altruistic motivation that will make his work on honey bees non-proprietary, freely available to everyone.

The ability of the microarray to find even unknown viral strains was demonstrated when, in a 24 hour period in 2003, DeRisi and his colleagues identified the novel coronavirus responsible for the outbreak of Severe Acute Respiratory Syndrome (SARS).

How could the chip, which carries only known pathogens, detect an undiscovered virus? "We catch everything," said DeRisi. The team has two approaches: First, since viruses evolve in families, the chip will show partial, evolutionary relationships that can be traced back through the nucleic acid and decoded.

Secondly, because the process won't find an unrelated, unique pathogen, the team will sequence all the nucleic acid in some samples. Stretches of DNA, which are known to assemble in genomes, can be seen. Additional clues, like start and stop signals, can lead them to previously unknown pathogens. Clearly they are confident that they can create a complete record.

DeRisi's accomplishments have attracted wide honors, including a MacArthur "genius" grant -- although one gets the impression that the laurels are stowed somewhere under a lab bench, not for resting on. Even so, the lab is named for him and he has attracted bright, passionate, even younger assistants. Among them are the principals in the honey bee chapter of this story, Charles Runckel and Michelle Flenniken. Runckel is a grad student in a program that spans traditional department boundaries (mathematics, physics, chemistry, and engineering). Michelle Flenniken, a PhD virologist, is the recipient of the Häagen Dazs grant for bee research through UC Davis. She summed it up: "Everyone finds Joe."

Among those who have found DeRisi is Charles Wick of the U.S. Army's Edgewood Chemical Biological Center in Maryland, where some diagnostic work on colony collapse disorder (CCD) in honey bees was being done. A couple of years ago,
Wick asked him to test some CCD bee samples.

DeRisi loves a mystery, an unknown cause. In the case of widespread and apparently unrelated die-offs of parrots, he was able to isolate the responsible virus. In the CCD bee samples he found a variety of viruses, but in one, 80% of what showed up was *Nosema ceranae*, not thought at that time to be widespread in this country. The speculative alarm resulting from these results surprised him, since the sample was so small.

"From our perspective, what was most obvious was that we did not know what the normal prevalence, the natural background, of pathogens was, so we could not really know what we were looking at. Most organisms have viruses. You can take spit from a human being and sequence it, which we've done, and most don't cause disease. So what is the natural load in honey bees? We need to know in order to discuss disease."

With his curiosity sparked, the timing was right for Chris Heintz of Project *Apis m* (P*Am*) to find DeRisi. P*Am* was formed two years ago as a non-profit to support practical bee research, and Heintz, who has managed pollination research for over a decade, came with an offer. "I went over to see what it would take to get him committed to bees. I was thrilled with the potential. My job was to snag him." In her pocket was a grant put together from beekeeper and almond grower donations along with a Specialty Crop Grant from the California Department of Food and Agriculture.

And so the bee chip was born. The DeRisi Lab used the model created for human pathogens to create a microarray of all pathogens found in arthropods – insects (e.g. bees), arachnids (e.g. mites), crustaceans (e.g. lice). The number of arthropods is vast: although honey bees aren't known to host, say, lobster infections, the idea is to create a global microbial survey that will find any pathogen, even those that cross species – which was the case for the parrot virus.

"In the old days," which for DeRisi is 15 years ago, "Science was done by hypothesis, because the technology was such that you only had resources to look at a tiny number of things. That's completely changed. In this current age you can look for everything simultaneously with a similar number of resources." The bee chip can look for bacteria, fungi, protozoa, nematodes, parasites, viruses or microsporidia – anything living, in fact, or on the cusp of life, which is the case with viruses.

"It's amazing to have this," said Heintz. "Never before have we had this level of science and technology working for bees."

Starting in February, the DeRisi Lab will test weekly samples taken from the entrance and interior of each of 20 colonies in a large commercially managed apiary. All the samples will be collected and stored the same way. "Often, samples from all over the country have been kept under varying conditions. We will have a more carefully controlled systematic study," said Flenniken.

Samples at the lab are stored in a freezer at -80° C. Runckel donned thick gloves to access it because contact with the surfaces would cause instant frostbite. He put his mitts on a tray of Randy Oliver's cryogenic bees, explaining that the stored material deteriorates far less at that temperature than at freezing. He put a bee into a vial with a bit of liquid and a ball bearing and turned on the tissuelizer, a machine that vibrates 30 times a second to dissolve the bee particles.

Runckel placed the remains of the sacrificial bee in a robot that takes out her DNA and RNA (nucleic acid). Flenniken explained that this step can be done by hand, with solvents separating out the nucleic acid for analysis.

"You can multiply nucleic acid, replicate it, make a billion copies like a Xerox machine," said Runckel. "You can start from a single virus and create enough material to work with. The sensitivity of this technique is many orders of magnitude better than protein detection. People in the biomedical field ultimately rely on nucleic acid. If you have HIV, you determine the viral load by nucleic acid."

Runckel and Flenniken take the extracted nucleic acid, which contains not only the genetic material from the bee but that of any other organism in or on that bee. The famous chip looks like nothing more than a simple glass slide. On it, in specific sequences, is stuck the huge master library of nucleotides. A machine called an arrayer meticulously spots the nucleic acid of the test sample onto the chip.

Labeled with a fluorescent dye molecule for tracing, the sample DNA latches onto its complementary sequence. That is to say, where the sample and the master chip match, the DNA will form a dimer, that is, join in a molecule of two identical, simpler molecules. As Flenniken put it: They stick like velcro. "Where it matches we will be able to detect the signal and locate the signal to identify it with a laser and computer." Most viruses will show 5-20 spots on the printout showing up as green against a red background.

As Runckel put the sample through the process he remarked that DeRisi designed and built much of the technology they were using. Flenniken added, "He is a really hands-on scientist – kind of like beekeepers, it's a good fit. He can build stuff and do the science, too."

As data is collected for the P*Am* research, failing hives can be compared to strong hives. It will be possible to look at the viral load and see how it relates to the information tracked by the beekeeper: temperature, location, travel and treatment. Synergistic patterns can emerge. "We don't necessarily need to have a cure to make an impact," said DeRisi, who expects that the data will create a valuable baseline.

At this initial stage of the project, the variables will be limited to those of typical commercial management. As Runckel put it, "If you turn all the knobs scientifically you won't know what you get." Later, more elements can be varied. In addition, the lab will also fully sequence the *Nosema apis* and the *N. ceranae* genomes.

All of the information will be openly available on a Website from a new server that will be updated monthly and linked to the P*Am* site. The study can be followed month by month at http://www.projectapism.org/.

DeRisi is committed to open-access scientific publishing, making the body of his work freely accessible. When he first developed the microarray, he posted the design on the internet, making it possible to build a working replica from Web instructions, something that was successfully done. He has not pursued a patent for the ViroChip, allowing researchers around the world to use the invention for free. He believes that all research funded by public sources should be openly available.

"If the USDA or universities want to use this new chip, we will give the whole design. If they want to have a collaborative research project we are happy to do it." He is also eager to compare samples with labs testing with other technologies such as proteomics and the Integrated Virus Detection System (IVDS).

Such are the gifts of the DeRisi Lab and the P*Am* study – not the least of which is passion. And hope. Stay tuned for much more to come.

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SIDE BAR:
BEE DIAGNOSIS

Although DeRisi's lab is limited to research, the bee chip will be available to be used elsewhere in a testing service for beekeepers. DeRisi estimates that such a diagnostic facility could possibly run a microarray sample for bees for $20 to $40, testing for 2,000 things with the same scientific rigor used for human diagnosis. Until that time, the following testing options are available to beekeepers now:

Mites and Nosema
Washington State University
At present accepting samples from all interested beekeepers from California, Oregon, Idaho, Washington and Montana. So far, 100 of the *Nosema* positive samples examined with PCR (polymerase chain reaction) have found only *Nosema ceranae* in all samples.
Presently funded by the State of Washington (for WA beekeepers) and beekeeper groups. For the future, a possibility is to put this diagnostic laboratory on more secure funding by making it a pay-for-service diagnostic center. Steve Sheppard, **shepp@wsu.edu**

Randy Oliver has demonstrated the possibility for a beekeeper to examine and differentiate between *Nosema apis* and *Nosema cerana* with a $500 microscope. *The American Bee Journal, March 2008.*

Diana Summataro of the USDA Tucson lab has a video of an easy dissection method to check for tracheal mites. http://www.ars.usda.gov/pandp/docs.htm?docid=14370

Pesticide screening
Pennsylvania State University
Half of the cost of screening is subsidized by grants, including one from P*Am.* Pesticide screening for beekeepers is now $125 for 171 chemicals. Requires a fresh bee sample.
Contact SaraAshcraft at SAA15@psu.edu

Viruses, bacteria and fungi
Bee Alert
The University of Montana-Missoula
Viruses, bacteria and fungi can be named with proteomics. Deliver sample frozen in dry ice or in ice packs not alcohol for results in about two weeks. $250 per sample of 100 bees. A new project in cooperation with the Calif State Beekeepers Association will follow ten beekeepers from each of three areas in California -- the north where there is more queen rearing, the central area where there is more pollination, and the south where there is more honey production. At $125 per test, half the cost to the beekeeper (the other half borne by CSA), each will be tested for three seasons. A fourth test would be unsubsidized and paid by the beekeepers. This will create a profile -- pre-almond, post-almond, and end of season. In addition to being informative to the beekeepers, this benchmark will be useful for creating a center for disease control for bees, which Jerry Bromenshank at Bee Alert hopes to create.
Contact Bromenshank at BeeResearch@hotmail.com

Viruses
Integrated Virus Detection System (IVDS)
Readout of bee viruses based on size and titer will show presences of viruses but not identify them. Further analysis can be done in the adjoining lab at Bee Alert by proteomics. $40-45 per test.
Contact Dave Wick at BeeResearch@hotmail.com

High Fructose Corn Syrup
Diana Sammataro (USDA-ARS, Tucson)
If HMF breaks down to formic acid, it perforates the intestinal tract of adult bees.
The Tucson lab is accepting samples, which are analyzed at other labs.
Contact Diana.Sammataro@ARS.USDA.GOV

REFERENCES
An animated description of how microarrays (DNA chips) work:
http://www.bio.davidson.edu/Courses/genomics/chip/chip.html