COOPERATIVE EXTENSION UNIVERSITY OF CALIFORNIA

from the U. C. APIARIES University of California

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Old Dog, New Knowledge

Please put up with me as I revise some of my thinking on honey bees, based on new research.

It isn't That Simple (I)

For decades I have told everyone that the difference between queen honey bee larval development and worker development is based on diet differences. That still appears to be true. However, I have indicated to many people that the fundamental mix of hypopharyngeal and mandibular secretions is "bee food." I related that bee food is the basic diet fed to adult queens so that they can lay so many eggs; the basic diet fed to all larvae at first; the food fed to drones as they mature; and the food shared by all the workers in the hive.

I also said that in order to get a queen, the diet had to be modified from worker diet to queen diet (royal jelly) by the addition of sugar and some undetermined proteins or peptides. I felt that my ideas were sound when Kamakura published a paper in 2011 ("Royalactin Induces Queen

Differentiation in Honeybees": http://www.nature.com/nature/journal/v473/n7348/full/nature10093.html) on the chemical compound they called "royalactin" that was found only in royal jelly being fed to queen larvae.

Avani Patel and six collaborators published a paper in 2007 (PLoS ONE 2(6): e509. doi:10.1371/journal.pone.0000509) demonstrating genetic control of queens *versus* workers by knocking out specific genes coding for "diphenic development."

But it isn't that simple. Just recently, another paper was published on this topic. This time a very thorough analysis was performed on worker jelly (my "brood food") and royal jelly being produced at the same time in the same colonies. Yes, there was a significant difference in the two types of gelatinous foods, but not what I had anticipated. The researchers determined that the basic diet, which I had been calling brood food was really royal jelly, not worker food.

With the exception of the additions mentioned previously, X. Guo and 11 collaborators determined that the big difference in worker jelly is the **removal of components that normally lead to queen production**. Thus, worker jelly is significantly less complex and less nutritious than royal jelly. Apparently, the missing ingredients fail to stimulate larval physiology to produce body parts and functions of a queen bee. So, it is not necessarily a more robust diet fed to queens versus larvae – due to additions to the royal jelly – but the subtraction of basic diet components that leads to the underdeveloped queens that we call workers.

I extend my apologies to Dr. Larry Connor and others with whom I have disagreed over the use of the word royal jelly when describing the basic mix of glandular secretions from the head glands of nurse bees. But even with this revised take on the differentiation process, how do the nurse bees regulate the jelly components to reach the desired results?

As a guess, the food produced by nurse bee glands changes with time, automatically. Apparently, the nurse bee physiological change induces a diminishing of queen bee nutrients from their brood food with time. If coordinated properly, the least robust food should be fed to worker larvae about to complete feeding and change into pupae (Guo, *et al*.).

The mechanism for that synchrony may be a chemical feedback from the larvae of various ages. Thus, "brood pheromone" may come in many different "flavors" as well, as the larvae develop.

Further studies by Guo Xiangqian *et al.* ("Recipe for a Busy Bee: MicroRNAs in Honey Bee Caste Determination": http://www.plosone.org/article/authors/info%3Adoi%2F10.1371%2Fjournal.pone.0081661) determined that those changes in jelly components result from the activities of microRNAs (miRNAs). The miRNAs modify the mRNAs (messenger RNAs) that carry the code for building peptides. The miRNAs in worker food were 7-215 times higher in worker jelly than in royal jelly. They must be turning off a lot of things. To me, this suggests that all nurse bees are not producing the same food at any given moment. The miRNA studies determined that the greatest changes in miRNAs occur on the 4^{th}-6^{th} days of larval development in both castes. So, the nurse bees must be changing with them.

Given all of this, why is it that when we purchase royal jelly, collected from late-term queen cells, and graft one-day-old female larvae onto the diet, they almost always develop into worker bees? We must be functioning as older nurse bees without knowing it! We have s-o-o-o-o much more to learn.

It Isn't That Simple (II)

For decades I have been explaining that when a honey bee stings someone, the antigens in the venom stimulate the immune system to form antibodies (the so-called "type 2" immune response) that perform various functions in the body. Classically, I thought of, and taught, that immunoglobulin E (gamma E) is the bad guy. It circulates around the body and attaches to mast cell receptors, particularly those mast cells associated with vital organs. Then, when the venom enters the body, again, if the antigens reached the gamma E, histamine would be released from the mast cells and the organs (and our health) would be negatively affected.

If, on the other hand, gamma G becomes predominant, it will remain mostly in the blood stream. With bee stings, the important role of gamma G is to tie up venom antigens before they get a chance to become hooked up with the gamma E on the vital organs. This prevents serious allergic reactions. But, of course, it is nowhere near that simple.

Recent studies, conducted by a team of medical researchers from the Stanford School of Medicine, Boston Children's Hospital, and Allergie-Centrum-Charité in Germany, determined the gamma E associated with mast cells was essential for protection against exposure to lethal doses of honey bee venom in mice. The test mice that succumbed were those with genetic mutations that prevented the production of gamma E, prevented the gamma E from combining with mast cell receptors, or whose bodies had produced no mast cells. In each of these cases, the previously inoculated mice had produced gamma G at levels that would have been predicted as protective.

From their previous studies, the researchers had determined that gamma E is a very useful component of the immune system for battling worms and other parasites. However, when it gets mal-directed toward seemingly harmless antigens, it can become problematic. These studies (that included a little snake venom work) determined that gamma E can be protective against animal venoms.

There is a lot more detail in the paper: "Beneficial Role for Immunoglobulin E in Host Defense against Honeybee Venom" by Thomas Marichal *et al.* (http://dx.doi.org/10.1016/j.immuni.2013.10.005). They explain how they determined that gamma G is not a big performer in protection. I do not pretend to be familiar with all the jargon in the paper, but the main idea is pretty straightforward: gamma E can be essential to venom detoxification, or terribly disturbing to your vital organs, depending upon how your immune system is responding to honey bee venom.

It Isn't that Simple (III)

A broad overview of the information on honey bee-associated microbes, especially those involved with food digestion might sound pretty uncomplicated. The bees forage outside the hive and pick up the microbes they need from the blossoms they visit. But, of course, it isn't that simple.

The world of microbiology took a huge leap forward when researchers no longer had to try to grow microbes on various defined laboratory media, under specified temperature and oxygen regimes, to determine which species they had. Now, the microbes of interest are fractionated, their DNA and or RNA collected and cleaned up, and further processed. Most protocols result in DNA which is sequenced. That sequence is compared directly to other DNA or used to reverse engineer the RNA sequence that fostered the DNA sequence. Either way, an unbelievably large assemblage of DNA sequences now exists. By digging into them, or their reflections of RNA, all sorts of identities and relationships can be determined.

Not all researchers necessarily agree with each other on exactly which microbes are being found, but we are getting very deep into determining what is happening in the world of honey bee microbiology. At the recent AHPA convention, Dr. Kirk Anderson presented a synopsis of what is known and what he is studying at the USDA lab in Tucson. The presentation was crammed with information and went very quickly, so I hope I did not get too confused.

As I interpret what I heard, there are various groups of bacteria involved with honey bees, but they are separated spacially. The most common bacteria that arrive on freshly collected pollens fall into the Alpha 2.2 (one of the *Acetobacteraceae* "clades") and *Lactobacillus kunkeei* groups. Besides carrying enzymes that begin the breakdown of proteins and start the digestive process, they are very resistant to the extreme acid and osmotic stress that kill most other bacteria when they get into nectar, honey, and royal jelly. These bacteria are predominant in the adult worker bee crop, bee larvae, and throughout the queen bee intestinal tract. They also are two of the three most common bacterial types in bee bread. The additional species belong to the *Actinobacteria* group that are good producers of antibiotics against fungi and other food-spoilage microbes. *Actinobacteria* also are found in larvae, beeswax, larval cocoons, and the adult midgut, but they aren't predominant.

Moving beyond the crop, the microbes in the midgut tend to be transient visitors headed to the hindgut, where the digestive enzymes are produced, then moved forward into the midgut. Common midgut residents are *Bifidobacterium* spp., *Gilliamella apicola*, *Snodgrassella alvi*, and Alpha 2.1 spp. The hindgut contained the midgut bacteria but was very heavily populated with *Bifidobacterium* spp.

Outside the bees, the microbes most commonly found in bee bread were Alpha 2.2 spp. and *Lactobacillus kunkeei*. Mention was made of there being a number of "strains" of *L. kunkeei*. A quick Google search led to a paper that listed more than 50 different strains of that bacterial species.

Apparently, Dr. Anderson believes that there may be a honey bee probiotic commercially available, but he doubts that it would accomplish much. The microbes that are required at the front end of the process and in bee bread apparently come in with fresh pollens. The digestion-related microbes appear to be resident in the hive and can be picked up by feeding on previously stored pollens.

If you are a glutton for details on this topic, Kirk and eight collaborators published "Microbial Ecology of the Hive and Pollination Landscape: Bacterial Associates from Floral Nectar, the Alimentary Tract and Stored Food of Honey Bees (*Apis mellifera*), 2013, (PLOS ONE 8(12): e83125. doi:10.1371/journal.pone.0083125.)

Apitherapy Studies

For centuries, if not millennia, people in various parts of the world have used products from honey bee hives for medicinal purposes. Belief in, and use of, these hive products continues, especially in countries where "modern medicines" are either not available or too expensive for the local economy.

Western medicine has tended to view the medicinal use of hive products with a jaundiced eye. Despite voluminous testimonials and rather lightly designed scientific studies, doubt still persists. Few western researchers are willing to invest time and money in such studies.

Finally, studies are beginning to be conducted in more sophisticated manners with more sophisticated protocols, using current instrumentation. I am sure that the results of current studies still will be debatable, but at least they are more "scientific."

Professor Peter Molan has long been an advocate of using honey as an antibiotic and wound healing accelerator for mammals, including humans. He lauds manuka honey, in particular, for its anti-biotic properties. Eventually it was determined that manuka honey contains particularly high levels of a natural antibiotic, methylglyoxal.

Professor Molan recently published a review on "The Immuno-Stimulatory Activity of Honey." The review can be found at http://waikato.academia.edu/PeterMolan. The review contains a list of 19 references to published articles dealing with studies, mostly conducted on rats, that demonstrate positive immune responses to various treatments with honey.

A few additional articles on such topics have been brought to my attention recently, and I would like to share them with you.

The first involves the question of, "Can consumption of local honey really help with hay fever (allergic rhinitis) problems?" I have seen the results of some studies that were not very positive, but the practice has a huge following. Researcher Zamzil Amin Asha'ari and his colleagues from the International Islamic University Malaysia published a paper titled "Ingestion of Honey Improves the Symptoms of Allergic Rhinitis: Evidence from a Randomized Placebo-controlled Trial in the East Coast of Peninsular Malaysia. 2013. Ann Saudi Med 33(5): 469-475.

A group of 40 patients was divided in half and matched for severity of their symptoms. One group was fed corn syrup with a honey taste and the other group a honey with a high antioxidant level. The participants consumed the equivalent of 1 g of honey or syrup per kg of body weight, daily, for four weeks, divided into four portions each day. That dose was chosen because previous studies suggested that consumption of 50-80 g per day was the minimum for positive results. At the experimental dose, an 180-pound adult would be consuming just less than 3 ounces (81 g) of honey per day.

Detailed symptoms were documentted at the start of the experiment and at four and eight weeks later. Symptoms were similar at the start of the experiment. Symptoms for both groups improved progressively up to the fourth week. However, improvement tapered off on the syrup at week four but continued to show significant improvement up to the eighth week with the honey eaters. Although it was not part of the study, symptoms remained reduced in the honey consumers for about a month after treatment terminated.

The authors postulated three possible mechanisms for these results: 1) reduction of immuno-response by inhibiting Ig-E-mediated mast cell activation, 2) honey may have carried a low dose of the allergens and induced "tolerance" in the immune system, or 3) honey provided an anti-inflammatory property into the patients.

In the next article, by a Korean researcher at the College of Korean Medicine, Seung Min Lee and associates studied "Bee Venom Treatment for Refractory Postherpetic Neuralgia: A Case Report." Once again, this is not a controlled experiment.

The 72-year old patient developed severe pain following an outbreak of herpes zoster ("shingles"). The pain continued through medicating with antivirals, painkillers, steroids, and analgesic patches. After being tested for possible bee venom allergy, the patient was treated with subcutaneous injections of 1:30,000 dilution bee venom along the margins of the rash, once a week for four weeks.

After a month of treatments, the patient described his pain as subsiding from 8 to 2 on a 10-point scale. At three, six, and 12 months post-treatment, he described his improvement as permanent.

The problem in this case is to determine if this change for the better was natural remission or cause-and-effect due to the venom treatment. The authors suggest that a large group of patients should be tested, collecting specific neurological details to try to determine whether the pain is due to nociceptors or nerve degeneration.

Finally, researchers Mohamed Amin and Ihab Abdel-Raheem in Egypt worked on developing a wound dressing that would encourage faster and better wound-healing, while avoiding problems with microbial growth in the dressing. They mixed various concentrations of polyvinyl alcohol (PVA), chitosan hydrogel (Chit), and venom (BV). They froze and then thawed the mixtures three times before use. They then applied the mixture to wounds on diabetic rats. Diabetes often precludes normal wound-healing.

"The pharmaceutical activities including wound-healing and anti-inflammatory effects … were determined." The most effective (most swellable, flexible and elastic) mixture was 10 percent PVA, 0.6 percent Chit, and 4 percent BV. The addition of BV speeded up healing, reduced inflammation, and prevented bacteria from growing in the gels. The authors suggested use of this gel as a promising wound dressing for diabetic wounds.

The details were published in: Archives of Pharmaceutical Research. DOI 10.1007/s12272-013-0308-y. "Accelerated Wound Healing and Anti-inflammatory Effects of Physically Cross Linked Polyvinyl Alcohol-chitosan Hydrogel Containing Honey Bee Venom in Diabetic Rats."

Cover Crops in Orchards

The value of cover crops in certain cropping systems have been discussed for decades. The current need for abundant sources of forage for honey bee colonies has reignited the discussion. Should beekeepers ask their growers, and other growers, to plant crops specifically for bees to use as forage? The advantage to the beekeepers is pretty obvious. Some advantages to the growers will be described below, since our UC Farm Advisors have worked around this question for years. But some of the growers would just have to be altruistic about providing food for bees that they may never need for pollination services. It will be a hard sell, especially when there is little water left in California to irrigate anything.

A 2001 “The Pomology Post” from the Madera County Cooperative Extension office contained the following information on “Cover Crops in Orchards” by Brent Holtz. Brent wrote that cover crop choices are numerous but they have to fit the soil type, management practices, and irrigation systems. Soils can be improved and nitrogen added by growing and incorporating winter and summer legumes. Cowpeas can add between 100 to 130 pounds of nitrogen per acre (*California Agriculture* 48(5): 43-48). Left on the soil surface, the same plants tend not to release as much nitrogen into the ground. It is released into the air. Grasses take up a lot of nitrogen which is returned to the soil over time, but slowly.

Cover crops can reduce water and wind erosion; reduce dust; suppress weed growth; attract and sustain beneficial insects; and improve soil structure and water penetration. About 20 percent of California’s irrigated crop land has water penetration problems, and crops like alfalfa, cotton, fruit and nut trees are adversely affected by that problem.

Cover crops are not without their problems. They can suppress root growth near the soil surface in vineyards and orchards, especially in young vineyards. They consume quite a bit of water and can increase the possibility of frost damage. But if the cover crop is mowed periodically and the top 12 inches of soil kept moist, freezing should not be a problem. In the summer, a cover crop keeps the soil surface cooler, and also increases relative humidity, keeping dust down and reducing potential spider mite populations.

Often growers will plant self-reseeding cover crops that will produce a new stand of plants in the fall. Be sure to provide enough water to get the seed mature in the spring. Early-maturing varieties of crimson clover, subterranean clover and burr medic are commercially available. That may work for a while, but the weeds are sure to resurge eventually. Buying and planting new seed on a three-to-five-year basis is typical. Also consider the future. If you do not wish to have the cover crop around perpetually, try to select “less invasive” species. White and strawberry clovers can get out of hand under some circumstances.

Another word to the wise: “Cover cropping, like any new practice, should be approached slowly and methodically. It may be valuable to test several species or mixtures to find the most appropriate cover crop. It usually is a good idea to begin on a small scale in order to learn from mistakes without incurring unnecessary expenses. With persistence and creativity, cover cropping can provide many benefits with little extra cost.”

Farm Advisor Chuck Ingels from Sacramento County offered further advice. Problems can occur when cover crops are grown perennially as monocrops. For example, when Cahaba white vetch is grown for a while, it can be affected by diseases in the soil, and alfalfa weevils can become quite plentiful in continuous stands of burr medic. Portions of fields with sand streaks or different soil nutrients may not be compatible with certain species.

High biomass cover crops, like bell beans, vetch, and field peas are normally easy to grow. Lana wollypod is one of the most vigorous vetches that blooms and it matures earlier than purple vetch. Common vetch has extrafloral nectaries on the stipules that bees like to visit. Cahaba vetch is interesting in that it has been shown to suppress root knot nematodes. These plants are disced in the spring to serve as "green manure." The periodic addition of organic matter to the soil enhances microbial populations, and improves soil structure and nutrient cycling. Often a grass, such as oats or barley, is included in the mix to give the vetch something to climb on.

More information on cover crops can be found at the website of the UC Sustainable Agriculture Research and Education Program: http://www.sarep.ucdavis.edu. There is a very long list of articles on an extremely varied choice of cover crop-related topics.

A final contribution from Wilbur Reil, a Yolo County Farm Advisor (retired), explains how to know if the cover crop is helping with soil nutrition. "I strongly recommend a leaf analysis from each orchard to "fine-tune" a nitrogen fertilizer program. Leaves should be collected in late June or July annually. While a single year will show if the nitrogen level is deficient or adequate, it does not indicate if the current program is increasing or decreasing. Collecting leaf samples every year will provide information on whether the level is adequate and also whether the current fertility program needs range adjustment."

Happy New Year,

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