Bee Disease, Bee Sex and "Curiously Promiscuous" Queens

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Diversity, it seems, is all it's cracked up to be.

Most beekeepers become familiar with bee diseases – from chalkbrood, sacbrood, and the foulbroods, European and American – all too quickly. They learn there are many more, especially the viruses associated with parasitic mites. Viruses and mites (or is it mites and viruses) provide a "one-two" punch for a lot of beekeepers. I suspect a lot of beekeepers are like me when thinking about bee diseases – they think that the key to disease control it to keep a colony "healthy." But what do you have to do to achieve that healthy state? Do you avoid contact with all other colonies (good luck), keep your bees confined to a certain foraging area (please, please tell me just how to you plan to do that?) and well fed and growing? Perhaps you were taught that having disease in your hives meant that you were a bad beekeeper. Some of us act that way and try to hide the problem.

So, if you are like me, you might be surprised to learn that researchers have been looking at the relationship between disease (in all organisms as well as bees) and how they are responsible for two remarkable biological events: the development of sex (male-female exchange of genetic materials) and the evolutionary pressure for the development of queens that mate with more than one drone.

The Red Queen and the Evolution of Sex

Lets take these concepts – these evolutionary issues – one at a time. We will discuss the development of sex first. In evolutionary biology there is a concept that states that *sex evolved to overcome the constant pressure from disease*. Sex provides the mechanism for a constant genetic recombination of genes, and, the theory argues, is the best method to stay one step ahead of diseases, pests and parasites. This concept was described in a book by English science writer Matt Ridley, titled *The Red Queen: Sex and the Evolution of Human Nature*. The *Red Queen* in question here is not a bee or even an insect; but refers to the Red Queen chess piece in Lewis Carroll's *Through the Looking-Glass* that runs frantically yet remains stationary. Ridley uses the Red Queen as a metaphor to describe the nature of the genetic relationship between host and disease. It has been studied in humans and recently examined in honey bees. The Red Queen's speeding race that gets her nowhere puts reproductive sex in the center of the evolutionary spotlight. While not without controversy, the concept of the Red Queen's struggle on the evolutionary chess set has important lessons beekeepers must apply to our honey bee queens of many colors that we install into our white and multicolored beehives.

Social insects occupy nests that are crowded and humid, perfect conditions, and a highly favorable environment to promote the growth of pathogens and the rapid transmission of diseases from individual to individual. Social insects have evolved elaborate and complicated behaviors that help manage disease. For example, in cer-

Queen purchased in a queen cage from a Southern shipper shows a high degree of uniformity in the color and banding patterns of the workers. This strongly suggests that the drone supply was closely related.

Queen and daughter workers at the end of March (2006) after wintering in a five frame nucleus. The queen was reared in New England and mated to local drones (Summer 2005). No effort was made to control the composition of the drone supply. The appearance of several worker color patterns strongly suggests the presence of highly diverse drone genetics. The only sure way to determine differences in paternity is through DNA analysis. This colony had no apparent disease infestation upon inspection.

Appearance of bottom board of the colony headed by the queen in photo number 1. Research by Tarpy and Seeley suggest that colonies that lack genetic diversity may be more likely to have large disease outbreaks.

tain leaf-cutting ants, a caste of tiny farmer ants constantly monitor the fungi growing on the leaf pieces, and are small enough and quick enough to remove sections of any harmful fungus whenever it appears in the leaf farms of the colony. This allows the ants to grow without contamination only the species or strain of fungus that they use for food. Ironically, these tiny ants react much faster than human farmers who rarely note a disease or pest problem until it has become widespread and difficult to control – in this case there is a huge advantage to being tiny and able to work among food you are growing.

In honey bees, of course, we have discovered the process of hygienic behavior, where bees somehow detect defective brood cells – ones that have a bad odor or some other cue that stimulates the bees to remove the cell's contents. Remarkably, this same genetic mechanism has been shown to successfully work on a number of very different, highly unrelated diseases and even mites: American foulbrood, chalkbrood, virus-killed brood, chilled brood, and, of course, *Varroa* mites are all managed using the same basic cell cleaning mechanism. Some have predicted that the hygienic stocks from Minnesota, SMR/VSR, Russian and others will all be found to demonstrate some variation or even the identical genetic hygienic mechanism as outlined by Professor Walter Rothenbuhler in the mid 1950s.

Advantages of multiple mating

If we accept the idea that the best way to distribute adaptive genetic mutations and changes protective to the genome of a species is through sex, we are still faced with an explanation of why bees of the genus *Apis* have evolved the behavior of mating multiple times, using Drone Congregation Areas (DCA's) where queens mate with a dozen or more drones during a short time interval. What is the advantage of one queen mating so many times?

Most insects mate only once, and within the social insects the examples of multiple mating have been reported in *Vespula* (yellow jacket wasps), *Atta* (leaf-cutter ants), *Pogonomyrmex* (harvester ants), and *Dorylus* and *Eciton* (army ants). But the social insects with the highest level of multiple mating are all in the genus *Apis*, with queens of *Apis mellifera* mating with 12 drones on average as based on molecular genotyping.

North Carolina State University researcher David Tarpy and Cornell scientist Tom Seeley have worked with queens mated to different numbers of drones and examined their ability to counteract disease. We will review some of their work to examine the role of multiple mating in the overall success of the honey bee.

Tarpy and Seeley studied the "curious promiscuity" of queen bees and how the queen's multiple mating behavior impacts the hive's disease levels. There must be a huge evolutionary payback for a species to invest in the complicated mating system found in the honey bee species. Rather than mating in or around the nest, virgin honey bee queens and drones fly to drone congregation areas (DCAs) to mate. On their flight they are exposed to predators, high winds, sudden changes in the weather, and may make orientation mistakes returning to the proper hive. Why does a queen mate multiple times and then stop for the rest of her life?

Sex and the Single Queen

To answer this question, I reviewed two related papers. The first is by David Tarpy and compares queens that have been instrumentally inseminated with either one drone (SDI) or with many drones (MDI).[1] In the second paper, Tarpy and Cornell's Tom Seeley (where Tarpy had worked as a post-doc before being hired by NCSU) set up a similar study to evaluate different colonies[2]. In each paper they produced two groups of colonies. One group consisted of highly related virgin queens that were instrumentally mated with the semen of just one drone, each drone taken from an unrelated colony. The second group of queens was instrumentally mated with an identical volume of semen collected from unrelated drones – one each from the colonies used in the first group. After the semen was collected and mixed, one micro-liter of semen was given to each queen. This is the same amount of semen given to each queen mated to a single drone. This second group of queens were mated in such a way as to duplicate the number of drones the average queen mates with in the wild, but under highly controlled conditions. Once established in production hives, the colonies were examined for disease and general aspects of beekeeping. The two groups of colonies allowed the researchers to compare colonies mated to one drone, called monandrous colonies, where the worker bees were genetically uniform; with colonies mated with multiple drones, called polyandrous colonies, and where the resulting colonies had genetically diverse worker bees. The experiment allowed them to examine any possible advantage of polyandry.

Colonies were set up and managed for six weeks so they had worker

[1] Tarpy, D.R. 2002. Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. Proc. R. Soc. London B 270: 99-103

[2] Tarpy, D.R and T.D. Seeley. 2006. Lower disease infections in honeybee (*Apis mellifera*) colonies headed by polyandrous vs monandrous queens. *Naturwissenschften*, 93: 195-199.

bees from the queen. At that time, each colony was inoculated with spores of chalkbrood fed in pollen patties. Infected brood was counted and compared between the two groups.

The results show that the colonies with a diverse number of drones had a lower *variation* in disease development when compared to the colonies with a queen mated to a single drone. Tarpy concluded that genetically diverse colonies (polyandrous) may be better able to resist severe infections of chalkbrood because they reduce the variability found in the occurrence of disease. These colonies did express some chalkbrood, but not at the extremely high or the extremely low levels found in the monandrous colonies. Genetic variation has been shown to exist in bee populations for most bee diseases and mites, including chalkbrood, *Varroa destructor, Acarapis woodi, Nosema apis*, and American foulbrood. Tarpy's study indicated that polyandrous colonies were less likely to experience a severe chalkbrood infestation. He wrote "that increased genetic diversity within colonies provides them with several benefits, and thus should be viewed as a trait with pluralistic consequences."

Lower Disease Infections With Multiple Mating

In the second study Tarpy conducted in cooperation with Tom Seeley, various techniques were refined, so while the methods used in the two experiments were not identical they were very similar. Again single-drone inseminated queens were produced and compared to multiple-drone inseminated queens and their resulting colonies. In the second study, the colonies were exposed to natural conditions and evaluated for the presence and frequency of bee diseases. Of note is that of the 20 final colonies, 80% had one or more of four diseases: sacbrood, chalkbrood, European foulbrood or American foulbrood. Of these, the MDI colonies had less variability in both chalkbrood and in all disease combined. Colonies headed by MDI queen had significantly more comb and more frames of brood.

These results show that with increased genetic diversity multipley inseminated queen colonies may produce subgroups (sometimes called *super sisters*) of worker bees with unique behavioral skills. It is thought these subgroups may be specialized in doing certain chores, like finding food resources or maintaining a more uniform brood nest environment. Second, the multiple drone mating reduces the chance of having the same allele for the sex gene, and reduces the amount of missed brood due to diploid drone removal. Third, the increased diversity of the colony may mean that colonies are more likely to moderate the levels of diseases and parasites in the colonies.

Of these three ideas, this research supports the third concept, of reduced disease. But the study does not contradict the first two concepts, for "the genetically more diverse colonies may have benefited in multiple ways."

The ecology of honey bee colonies is significantly affected by disease; even in colonies started as swarms on new equipment showed four brood pathogens in less than two months. It reflects the overwhelming role of disease in our colonies' life cycle. The test colonies were kept in pairs and were widely separated and not manipulated during the experiment.

Lessons for Beekeepers

Since I grew up with the old Starline/Midnite technology of creating inbred lines to cross via instrumental insemination to create hybrid bees, I was trained to develop large numbers colonies of genetically uniform hives. Queens were very closely related and so were the drones they mated with. The key was to develop highly uniform bees, hives with hybrid vigor that made them more productive. The goal was to produce hybrid colonies that all required the same management at the same time – every hive gets two supers today – that sort of thinking. Our diseases were the two foulbroods, a little sacbrood, the newly introduced chalkbrood, and silent battles with *Nosema apis*. Many beekeepers used drugs (we were likely to call them medications), and we kept treating on a regular basis.

I suspect this genetic uniformity had no tolerance against either the tracheal mite or the *Varroa* mite. We lost a lot of colonies, and while I have argued that we lost a great deal of genetic diversity, we certainly lost a huge number of very uniform, highly susceptible stocks.

We did not talk about diversity except to test new queens for possible integration into the hybrid program. Many queen lines were evaluated but precious few were integrated; the queens that failed to make the cut were pinched off and the stock rejected from the program.

Now beekeepers are looking at ways to increase diversity in the bees. The work of Tom Seeley and David Tarpy shows the advantage in having a large number of unrelated drones mate with queens and thus provide a mechanism to reduce the extreme cases of disease. Over the next decade I hope that individual beekeepers, or some of the new bee breeding groups that are developing around the country, will be able to identify certain genetic strengths in their stocks, and that all beekeepers may include such stocks in a drone saturation plan to increase the genetic diversity of the drones their virgin queens will mate to.

At the end of Tarpy and Seeley's second paper, the authors write: "The curiously high promiscuity of honey bee queens is an adaptation to minimize the impact of the litany of parasites that threaten their colonies. More generally, the findings of this study, together with the many studies supporting the Red Queen hypothesis for the evolution of sexual reproduction, suggest that disease is a fundamentally important agent in natural selection and that genetic diversity plays a key role in minimizing fitness losses imposed by disease." BC

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