Honey Bee Health: The Potential Role of Microbes

CHAPTER

1

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Introduction

Microorganisms, or microbes, are a diverse group of unicellular organisms, including bacteria, fungi, archaea, protists, and sometimes viruses. Microbial activity in honey bee colonies can be broadly divided into three areas: microbes that are pathogenic, causing disease within the colony; and those that are either benign to the honey bee (commensal) or beneficial. Bees carry a diverse assemblage of microbes (mostly bacteria and fungi), very few of which are pathogenic; most are likely commensal or even beneficial to the colony (Evans and Armstrong, 2006). Some beneficial microbes inhibit the growth of pathogenic bacteria and fungi; others aid in the digestion of food and the preservation of food stores (e.g., pollen). Microbes may even play a role in honey bee gene expression and affect colony-level traits such as social immunity.

While symbiotic microbes are found in many organisms, they may play a special role for honey bees. This is because bees obtain a majority of their nutrients from pollen, a food source resistant to enzymatic digestion. Furthermore, bees store pollen in the warm, moist environment of the hive that can provide ideal growth conditions for many pathogenic microorganisms. Honey bees rely on their symbiotic microbes to protect their food stores from spoilage. Indeed, it is in part through the action of microbes that the mixture of pollen and nectar is converted to a fermented food called bee bread that supplies the protein diet of honey bees. When honey bees feed on bee bread, some of the microbes are transferred to the bees where they might continue to aid in digestion and supply necessary components to the host metabolism.

Interactions between honey bees and microbes for the processing and preservation of food begin during foraging (Figure 1). Foragers inoculate the pollen they collect with nectar from their honey stomach and thus seed the pollen in their corbiculae with bacteria and fungi. Additional microbes are added to the pollen when it is packed into cells. These microbes cause the pH of the pollen mixture to be lowered and thus begin a fermentation process that converts the pollen into bee bread. Worker bees consume bee bread, metabolize the nutrients, and use them

Abstract Bees carry a diverse assemblage of microbes, mostly bacteria and fungi. Most microbes are commensals or even beneficial to the colony and few are pathogenic. Their role is to inhibit the growth of pathogenic bacteria and fungi, aid in food digestion and food store preservation, facilitate gene expression, and affect colony-level traits such as social immunity. This chapter summarizes the role of beneficial microbes, showing their importance for maintaining colony health, and discusses current molecular and metagenomic techniques (cf. sequence-based analysis of DNA and 16S rRNA) and findings that have expanded our knowledge about the complexity of the microbiome related to honey bees, their colonies, and community function. Of interest is that the current Human Microbiome Project (HMP) and questions related to host–microbe interactions could benefit microbiome analysis of honey bees. These studies reveal the significance of microbes in the health of all organisms and clearly indicate that optimum health depends on maintaining conditions that encourage microbial growth.

Figure 1. An overview of the role of symbiotic microbes in the conversion of pollen and nectar to bee bread and its use in the bee colony. (Top photo by P. Grebs; remaining photos by G. D. Hoffman.)

in anabolic and catabolic reactions and to generate immune responses. Young workers serving as nurse bees convert the bee bread to brood food. The brood food is used to rear larvae of all castes and also to feed adult nestmates via trophallactic interactions.

In this chapter, we will discuss beneficial microbes and their role in the nutrition and health of honey bee colonies. We will provide a historical summary of work on beneficial microbes and discuss new molecular and metagenomic tools and findings that can enable scientists to more deeply investigate the microbiome of honey bees and their colonies.

History of Research on Microbes in Honey Bees

Early studies on identification of microbes and descriptions of their function were done solely on species that could be cultured. Through the efforts of diligent and highly skilled microbiologists, hundreds of species of bacteria and fungi were isolated and identified from pollen, bee bread, and all stages of bees. The description of microbes in this section includes only those that could be cultured using the techniques available at the time. We now know that microbes that can be cultured make up only a small fraction of all those that actually reside in an organism. Therefore, when the assertion is made that certain life stages are devoid of microbes, it is based on only those that could be cultured.

Studies on the establishment of the microflora in worker bees revealed that worker-destined eggs were free internally of microorganisms. After eclosion, most young larvae have microbially sterile digestive tracts, presumably because of the antibiotic properties of the worker jelly that is their sole food source during the first 36 hours of life (Burri, 1947; Kluge, 1963). However, as larvae age and begin consuming an admixture of worker jelly, pollen, and nectar, their intestine becomes inoculated with bacteria, molds, and yeasts (Gilliam and Prest, 1987).

Most (although not all) larvae continue to harbor a diverse microbial community within the alimentary canal until they approach pupation (Gilliam and Prest, 1987). After larvae eat their last meal, they discharge their gut contents into the comb cell (Snodgrass, 1925). Fecal analysis showed that the larval gut contains numerous *Bacillus* spp., Gram-variable pleomorphic bacteria, molds (primarily *Penicillia*), Actinomycetes, Gram-negative bacterial rods, and yeasts (Gilliam and Prest, 1987). Because gut contents are discharged prior to pupation, the digestive systems of pupae and newly emerged adults are free of microbes. Upon emergence as an adult, the gut is reinoculated via pollen consumption and trophallactic food exchange with older nestmates (Burri, 1947; Kluge, 1963; Gilliam et al., 1983). As the bee ages, the gut microflora will vary. Season and geographical location also affect the species composition of the microflora; however, certain microbes are prevalent (Cherepov, 1966; Gilliam and Valentine, 1974; Gilliam et al., 1988). These include Gram-variable pleomorphic bacteria of uncertain taxonomic status, *Bacillus* spp., Enterobacteriaceae, molds, and yeasts (Gilliam et al., 1988).

In addition to inoculating newly emerged adult bees with microbes, adult bees also inoculate the pollen they collect and thus begin the fermentation and conversion processes that lead to bee bread. Floral pollen begins to change microbiologically and biochemically as soon as a honey bee collects it (Gilliam, 1979a,b; Loper et al., 1980; Standifer et al., 1980; Gilliam et al., 1989). At first glance, it might seem that foragers are adding nectar to the pollen to pack it more efficiently in their corbiculae.

While this might be so, the bees are also inoculating the pollen with microbes that reside in their honey stomach. Many studies have demonstrated that pollen, corbicular loads, and bee bread differ in pH and biochemical composition, likely due to the fermentative action of lactic acid bacteria and yeasts acting on carbohydrates present in honey and nectar (Foote, 1957; Haydak, 1958; Gilliam et al., 1989; Human and Nicolson, 2006). For instance, bee bread contains more reducing sugars than pollen from the same plant species (Casteel, 1912). There is Vitamin K in bee bread but not in the pollen used to create it (Haydak and Vivino, 1950).

The fermentation and chemical changes of pollen stored by honey bees is similar to the microbial action occurring in green plant food materials that are ensiled to improve palatability, digestibility, and nutritional value (Gilliam, 1997). Indeed, there are analogies in the microbiology and biochemistry of silage and the production of bee bread. These include microbial succession; fermentation; increased availability of amino acids; enhanced stability of silage by *Bacillus* spp., yeasts, and molds; and the production of organic acids to act as preservatives (Woolford, 1978; Gilliam, 1979a,b; Standifer et al., 1980; Nouts and Rombouts, 1982; Gilliam et al., 1989; Gilliam, 1997).

The conversion of pollen to bee bread is a multi-phase process. The first phase in the microbial succession lasts about 12 hours and is characterized by the development of a heterogeneous group of microorganisms including yeasts. During the second phase, anaerobic lactic acid bacteria (*Streptococcus*) utilize growth factors produced by the yeasts and putrefactive bacteria and lower the pH of the pollen. The growth of *Streptococcus* ceases and the microbe eventually disappears during the third phase, but there is an increase in *Lactobacillus* (Chevtechik, 1950, cited in Gilliam, 1979). *Pseudomonas* also is present and possibly contributes to anaerobiosis required by *Lactobacillus* and to the degradation of the walls of pollen grains (Klungness and Peng, 1984). After pollen is packed in cells for 2 to 3 days, *Pseudomonas* can no longer be cultured from it. The first three phases of pollen conversion to bee bread last about 7 days. The lactic acid fermentation process in the fourth phase is complete in about 15 days, though the microbes remain for several months. The yeasts are present in small numbers initially, but then increase after fermentation and remain in the stored pollen longer than other microbes (Gilliam, 1979). The yeasts apparently play a significant role in the fermentation process. When pollen was sterilized using gamma radiation and then seeded with *Lactobacillus*, the lactic acid fermentation process yielded an unpalatable product of poor nutritive value for the bees (Pain and Maugenet, 1966).

Floral pollen, corbicular pollen, and bee bread also differ in the predominant molds that can be cultured (*Mucor* sp. in floral pollen, *Penicillia* in corbicular pollen and in bee bread stored for 1 week, *Aspergilli* and *Penicillia* in bee bread stored for 3 weeks, and *Aspergilli* in bee bread stored for 6 weeks). The molds produce enzymes that are involved in lipid, protein, and carbohydrate metabolism (Gilliam et al., 1989).

The early work on microbes isolated from bee bread provided the first glimpse of how bees use a suite of microflora to preserve and possibly even pre-digest their food. The studies also defined the functions of the microbes with the tools available at the time. Based on these early studies, the importance of microbes for maintaining colony health became evident.

With current molecular tools we can expand on this early work, detailing the molecular mechanisms of known microbes and illuminating the functions of microbial communities that resist culturing.

New Tools for Studying Microbes: Metagenomics

Though numerous microbes were isolated, cultured, and identified from samples of honey bees, pollen, and bee bread, there are many more that could not be cultured. Indeed, most microorganisms (about 99%) cannot be grown in pure culture because microbes rarely live in isolation but rather in communities where they either enhance or constrain each other's growth. Recently, metagenomics has enabled the limitations of culturing to be circumvented by applying genomic analysis to entire microbial communities. Metagenomics bypasses the need to isolate and culture individual microbial species, thus enabling the study of microbes that cannot be isolated and cultured (Kowalchuk et al., 2007).

Metagenomic analysis begins with collecting a sample from a particular environment such as bee bread, and doing a mass extraction of the DNA from all the microbes in the sample (Figure 2). Protein or RNA also can be extracted from the microbes. Most metagenomics studies currently focus on microbes with relatively small genomes, such as bacteria or archaea (a group of microorganisms that differ from bacteria in cellular structure and function). Once the sample DNA is extracted, it can be analyzed directly or inserted into laboratory bacterial cultures for long-term storage as a metagenomic “library”—a living warehouse representing all of the DNA fragments from the sample's microbial community. Newer sequencing technologies generate gigantic volumes of high-quality sequence data for relatively little cost.

Information about the microbial community and its functions can be obtained in several ways. A sequence-based metagenomic analysis can be conducted to define the entire genetic sequence (i.e., the pattern of the four different nucleotide bases in the DNA strands). The sequence can then be analyzed to determine the complete metagenome of the community or the genome of an individual microbial species. Sequenced-based analysis can utilize evolutionarily conserved phylogenetic markers or “anchors” such as 16S rRNA, to indicate the taxonomic group that is the probable source of the DNA fragment. Such information can be used to determine the relative amounts and proportions of target microbes in a given sample.

The alternative to a phylogenetic marker-driven approach is to sequence random clones. Although relatively time-consuming, this method has produced dramatic insights, especially when conducted on a massive scale. Primary inference drawn from sequence-based analysis includes the uniqueness and redundancy of community function, and the way that microbial function may benefit or harm host function (see Handelsman, 2004). Metagenomic sequence data can be examined for genes that encode critical symbiont functions, genes involved in basic metabolism virulence, or antibiotic resistance genes. Another approach involves the direct extraction and identification of expressed proteins and metabolites (the products of cellular processes) from a microbial community. This approach has already been used to identify novel antibiotics, antibiotic resistance genes, membrane transporters, and secondary metabolites (see references in Handelsman, 2004). Nucleic acid probes labeled with fluorescent tags can also be used to quantify the relative abundance of

Figure 2. Flow diagram of a metagenomic analysis of a microbial community.(Source: http://books.nap.edu/openbook.php?record_id=11902&page=20. Used with permission.)

each organism within the community. This method can elucidate conditions that enhance or constrain the growth of particular microbes and can be particularly useful for understanding the effects of nutrition, antibiotics, or various agricultural chemicals.

Application of Metagenomics: Insights from the Human Microbiome Project

Research in the study of the microbiome in honey bees will be greatly advanced because of the work being done in the Human Microbiome Project (HMP). Many of the questions posed in studies of the human microbiome are similar to those of honey bee colonies. For instance, how stable and resilient is an individual's microbiota throughout the day and during his or her lifespan? How similar are the microbiomes between members of a family or members of a community or across communities in different environments? Do all humans have an identifiable "core" microbiome; and if so, how is it acquired and transmitted? What affects the genetic diversity of the microbiome, and how does this diversity affect adaptation by the microorganisms and the host to markedly different lifestyles and to various physiological or pathophysiological states? Such questions are directly transferable to honey bees, their colonies, and even beekeeping practices. The many similarities in conceptual framework and molecular techniques between human and honey bee systems will enable us to leverage findings in the HMP to advance our knowledge of the role of microbes in honey bee colony health.

The HMP is an extension of an earlier project to map the entire human genome (i.e., the Human Genome Project). At the onset of the Human Genome Project, it was thought that about 100,000 human genes would be found. Many were surprised by the finding that the human genome contains only about 20,000 protein-coding genes, not much different from the fruit fly genome. However, microorganisms that live on and within humans—the human microbiota—are estimated to outnumber human somatic and germ cells by a factor of ten. Together, the genomes of some of these microbial symbionts encode traits important to human health that humans either did not derive on their own, or have subsequently lost. Thus, the human genetic landscape is an aggregate of the genes in the human genome and in the microbiome, such that the metabolic phenotype that arises is a blend of human and microbial traits. The same is potentially true for honey bee colonies.

To understand the range of human genetic and physiological diversity, the microbiome and the factors that influence the distribution and evolution of the constituent microorganisms must be characterized. This is one of the main goals of the HMP (Turnbaugh et al., 2007). The characterization of the microbiome will provide perspective on contemporary human evolution, and on whether and how human lifestyles and environmental factors influence health and predisposition to various diseases. A similar paradigm can be applied to the honey bee microbiome and the beekeeping practices that shape it. Additionally, studies from the HMP that define the parameters needed to design, implement, and monitor strategies for intentionally manipulating an individual's microbiota to optimize physiological performance can provide a framework for manipulating the honey bee microbiota to improve colony health.

Research for the HMP has focused on bacterial communities that reside on or in the human body, including the skin and in the mouth, esophagus, stomach, colon, and urogenital region. The largest reported data sets are for the gut, although sample sizes using these culture-independent surveys are limited. Based on the studies though, it appears that more than 90% of all phylogenetic types (phylotypes) of colonic bacteria belong to just two of the 70 known divisions (phyla) in the domain Bacteria: the Firmicutes and the Bacteroidetes.

Though the phylotypes of bacteria in the human colon are limited and stable over time, there appear to be large differences in microbial-community structure among individuals (Zoetendal et al., 2001). Such high interindividual diversity in the gut microbiota might be explained by the neutral theory of community assembly, which states that most species share the same general niche or the largest niche, and therefore are likely to be functionally redundant (Hubbell, 2006). This theory predicts that highly variable communities (as defined by 16S rRNA gene lineages) will have elevated levels of functional redundancy between community members. The same might be true for honey bee colonies in that the microbiota might differ among colonies, depending upon their geographic location and diet. There also appear to be significant effects of genotype on the microbiota of humans, and the same may apply to bees and their colonies. In humans, the overall species composition of gut microbiota is significantly different between individuals of different ethnic groups (Li et al., 2008). Epidemiological observations of metabolic phenotypes also indicate differences based on ethnicity (Dumas et al., 2006); this supports the concept that host metabolic phenotype is strongly influenced by the gut microbiome (Nicholson et al., 2005). This is expected because gene sets annotated in the intestinal microbiomes identified significant numbers of bacterial genes that are not encoded in the human genome. Similarly in honey bees, the colony phenotype relative to population growth or overwintering success might also be at least partially influenced by the microbiome residing in the bees and food stores.

Another area where the HMP parallels the interests of honey bee health is in the role of nutrition in immune response. Microflora play important roles in the defense of the host by limiting the colonization of potential pathogens (Ashara et al., 2001, 2004; Kelly et al., 2007). Additionally, the interaction between a human host and microflora increases the concentrations of immunoglobulins, and the production of specific antibodies modifies the subpopulations of mucosal lymphocytes and boosts overall immunological function (Corthsy et al., 2007). In insects, humoral immunity involves the synthesis of antimicrobial peptides in response to infection by bacteria, fungi, or parasites (Hetru et al., 1998; Lamberty et al., 1999; Yamauchi, 2001; Klaudiny et al., 2005). Useable energy and the structural components required to synthesize the antimicrobial elements of the immune system are obtained from food. Without adequate nutrition and the microflora required for its thorough digestion, the immune system is clearly deprived of the components needed to generate an effective immune response (Gershwin et al., 2000).

In addition to their role in supplying the necessary nutrients for mounting an immune response, there are direct interactions between microflora and host defense. These interactions are modulated by a class of proteins in the immune system called Toll-like Receptors (TLRs) that recognize molecules produced by pathogens. Studies in humans and animal model systems indicate that macrophages play a central role in the

regulation of inflammatory and immune responses. The functions associated with macrophages are triggered by the stimulation of TLR expressed on the surface of macrophages (Rakoff-Nahoum et al., 2004; Akira et al., 2006; Mitchell et al., 2007). The eradication of the intestinal microflora by antimicrobials plays a role in the downregulation of the surface expression of TLRs on the peritoneal macrophages and impaired immunological responses (Umenai et al., 2010). Whether microbes in the gut of the honey bee have similar effects on immune response requires further investigation, especially because colonies are commonly exposed to antibiotics and fungicides that might eliminate or severely curtail the growth of intestinal symbionts.

Applying the techniques and information generated by the HMP to studies on the honey bee microbiome can provide a framework for generating hypotheses and interpreting results from metagenomic and physiological studies. In turn, the HMP could benefit from studies on the honey bee microbiome because this insect can serve as an ideal model system that can be easily manipulated to answer basic questions on host-microbe interactions. Worker bees emerge as adults microbe-free and as such can be used for studies detailing the mechanistic basis for the molecular interactions that occur at the interface between microbes and their host. Studies on the role of genotype, diet, and environment on the composition of host microbiota also can be conducted using honey bees because it is possible to make specific genetic crosses using instrumental insemination and controlling diet through feeding specific protein and carbohydrates either in colony or small cage studies (DeGrandi-Hoffman et al., 2007; DeGrandi-Hoffman et al., 2010).

Recent Studies on the Honey Bee Microbiome

Recent application of metagenomic techniques indicates that the great number of microorganisms uncovered using classic culturing techniques represents only a small portion of the actual microbiota in bees and their food. Current investigations of the microflora are conducted by sequencing the 16S rRNA genes (16S rDNA). These genes encode RNA of the small subunit of the bacterial ribosomes (Egert et al., 2003; Hongoh et al., 2003; Jeyaprakash et al., 2003; Reeson et al., 2003). Genetic profiling techniques such as denaturing gradient gel electrophoresis (DGGE) (Reeson et al., 2003; Schabereiter-Gurtner et al., 2003), terminal restriction fragment length polymorphism (TRFLP) (Broderick et al., 2004), or single-strand conformation polymorphism (SSCP) (Czarnetzki and Tebbe, 2004) based on the 16S rDNA allow a straightforward comparison of the bacterial communities from a relatively large number of samples.

Using molecular techniques, qualitative and quantitative differences were found in the bacterial community structure of larvae and adult worker bees (Mohr and Tebbe, 2006). A metagenomic survey of adult worker bees from four widely separated beekeeping operations across the United States, including some affected by CCD, revealed a bacterial community that included an abundance of Gammaproteobacteria and several less frequent but widespread organisms from the Betaproteobacteria, Alphaproteobacteria, Firmicutes, and Actinobacteria groups (Cox-Foster et al., 2007). The bacterial analysis also indicated community composition similar to that in samples collected in Africa, Switzerland, and Germany (Babendreier et al., 2007; Jeyaprakash et al., 2003; Mohr and Tebbe, 2006), suggesting that *Apis mellifera* have similar bacterial flora worldwide.

(Table 1). In addition to bacteria, 81 distinct fungal 18S rRNA sequences were recovered from the pooled samples. The fungal sequences are primarily from four distinct lineages: Saccharomycotina, which includes a variety of presumed commensal yeasts; Microsporidia, including the important bee pathogens *Nosema apis* and *Nosema ceranae* (Higes et al., 2006); Entomophthorales/Entomophthoromycotina, a diverse group of insect pathogens; and Mucorales. Mucoromycotina includes *Mucor hiemalis*, a species known to kill honey bees under certain conditions (Burnside, 1935).

Bacteria from larvae were isolated, cultured, and described using 16S rRNA and protein-coding gene sequences. Sixty-one bacterial isolates reflecting a total of 43 distinct bacterial taxa were identified (Evans and Armstrong, 2006). There was a high frequency of bees harboring bacteria from the *Bacillus cereus* group suggesting a stable symbiosis between bees and this taxon. Twenty-three bacterial isolates consistently inhibited *Paenibacillus larvae larvae*, the causative agent of American foulbrood. Isolates that inhibited *P. larvae* were evenly distributed across the sampled taxa. The survey indicated that older larvae (7 days old) have more bacteria and bacterial species per individual than younger larvae (1 day old). Diversity of bacteria species did not differ between younger and older larvae. The frequency of bacteria species varied among colonies and sites where they were sampled. This suggests that genotypic and environmental factors, especially as they affect the types of nectar and

Table 1. Microbes in worker honey bees as determined by a metagenomic survey of samples from four widely separated beekeeping operations across the United States. (From Cox-Foster et al., 2007)

Kingdom	Taxon (rank)	Organism
Bacteria	Firmicutes (phylum)	<i>Lactobacillus</i> sp.* Uncultured Firmicutes
Bacteria	Actinobacteria (class)	<i>Bifidobacterium</i> sp.*
Bacteria	Alphaproteobacteria (class)	<i>Bartonella</i> sp.* <i>Gluconacetobacter</i> sp.*
Bacteria	Betaproteobacteria (class)	<i>Simonsiella</i> sp.*
Bacteria	Gammaproteobacteria (class)	Two uncultured species*
Fungus	Entomophthorales (order)	<i>Pandora delphacis</i>
Fungus	Mucorales (order)	<i>Mucor</i> spp.
Fungus/microsporidian	Nosematidae (family)	<i>Nosema ceranae</i>
Fungus/microsporidian	Nosematidae (family)	<i>Nosema apis</i>
Eukaryota	Trypanosomatidae (family)	<i>Leishmania/Leptomonas</i> sp.
Metazoan	Varroidae (family)	<i>Varroa destructor</i>
Virus	(Unclassified)	Chronic Bee Paralysis Virus
Virus	Iflavirus (genus)	Sac Brood Virus
Virus	Iflavirus (genus)	Deformed Wing Virus
Virus	Dicistroviridae (family)	Black Queen Cell Virus
Virus	Dicistroviridae (family)	Kashmir Bee Virus
Virus	Dicistroviridae (family)	Acute Bee Paralysis Virus
Virus	Dicistroviridae (family)	Israeli Acute Paralysis Virus

pollen bees collect, might influence the diversity of microbes individuals harbor and in their ability to inhibit the growth of pathogenic bacteria.

Recent investigations of the microflora in the honey stomach of adult workers have revealed the presence of novel lactic acid bacteria (LAB) of the genera *Lactobacillus* and *Bifidobacterium* (Olofsson and Vásquez, 2008). The species vary with the sources of nectar and the presence of other bacterial genera within the honey bee. Similar species to those found in the honey stomach were not found in nectar from flowers but were isolated from nectar stores, indicating that the LAB originated from the honey stomach. The nectar sugars probably act as inducers for the resident honey stomach flora and, depending on the types of flowers that the honey bees visited, enhance their numbers. When the collected nectar is regurgitated from the honey stomach into comb cells, the bacteria are transferred into the stored nectar and might play a role in its conversion to honey and conferring its antibiotic properties. The majority of the honey stomach LAB flora also is present in a viable state from both the pollen and 2-week-old bee bread. Older bee bread (i.e., > 2 months) does not contain LAB. Bee bread is fermented at least in part by the honey stomach LAB that are added to the pollen via regurgitated nectar. The presence of the honey stomach LAB and its antimicrobial substances in bee bread indicate that these bacteria might play a role in the defense against honey bee diseases for all colony members as the bee bread is consumed by both the larvae and the adult bees.

Areas for Future Research

We are just beginning to learn about the role of microbes in the health of all organisms, including honey bees. We know that many metabolic processes are not directed by host genes but rather those of symbiotic microbes. This implies that optimum health depends on maintaining conditions that encourage the growth of those microbes needed to process food, and either convert it to energy or use it to synthesize more complex molecules via anabolic pathways. Though most of what we know about microbes in honey bees is their role in food processing and preservation, there are many other areas yet to be explored. For example, the role of microbes in the conversion of bee bread to brood food is unknown. The microbial communities of queens and drones that might have roles in fertilization and reproduction also have not been explored. Whether microbial communities in queens and drones are different from those in workers or are similar but differ in structure needs further investigation. The role of microbes in the synthesis of pheromones also should be studied. There are greater supersedure rates now than in the past, and whether nutritional factors, environmental contaminants, or antibiotics cause the microbial community in the queen to be compromised—thus affecting the synthesis of pheromone signals that communicate her presence—is worth further study.

Queen fertility is another area where microbes might play a role, but a link between microbial communities and fertility has not been investigated in honey bees. However, this connection might exist based on studies of human fertility. These studies have uncovered a putative link between microbial communication systems and host infertility (Rennemeier et al., 2009). The presence of the opportunistic pathogenic yeast *Candida albicans* and the Gram-negative bacterium *Pseudomonas aeruginosa* caused multiple damage to spermatozoa, including reduced motility,

premature loss of the acrosome (a cap-like structure of the sperm head that is essential for fertilization), apoptosis, and necrosis. The damage was caused by the quorum-sensing molecules that the microbes produced. This previously unknown interaction between microorganisms and human gametes might also occur in queens or drones, especially if the growth of beneficial microbes in the reproductive organs is impaired due to antibiotics or fungicide exposure.

Another substantially unexplored area involves possible changes in microbial communities particularly in workers throughout the year. In the spring, colonies are converting food to brood, but in the fall, brood rearing declines and food is stored so the colony can survive the winter. It seems plausible that the changes in the metabolic processing of food might have underlying microbial drivers.

Differences in food processing also seem to be based on genotype. For instance, the conversion of pollen to hemolymph protein and to brood differs among lines of bees, particularly between African and European genotypes (see references in Schneider et al., 2004; Cappelari et al., 2009). An in-depth comparative study of microflora of European and African bees could determine if differences in the composition of microbial communities are a contributing factor in the success of the Africanized honey bee throughout the tropics and subtropics of the Americas.

Determining the role of microbes in the health of honey bee colonies is essential for evaluating the impact that exposure to sublethal doses of fungicides and other antibiotics might have on colonies. Currently, fungicides are considered safe for bees so they are applied while crops are in bloom. Fungicides negatively affect the growth of culturable molds commonly found in bee bread (Yoder et al., 2011—this volume), and might disrupt the structure of other microbial communities that cannot be cultured but are necessary for colony health. Without knowing the roles that specific microbial communities play in maintaining the health of a colony and the effects that fungicides have on them, it is not possible to evaluate the safety of these compounds to pollinators.

A deeper understanding of the microbiota of honey bee colonies cannot help but cause a rethinking of beekeeping practices. For example, the full effect of using antibiotics—especially prophylactically—becomes clear when the effects on symbionts bees need for food preservation and colony-level metabolic functions are considered. The broader effects of carbohydrate and protein supplements on colony health will be revealed if they are studied within the context of whether the supplements enhance the growth of honey stomach and gut microbes or have deleterious effects on them. The need for clean pollen sources that can be converted to bee bread becomes especially urgent as it may represent the best solution for reestablishing the microflora in colonies and preventing the growth of pathogenic microbes. The development of probiotics to stimulate the growth of beneficial microbes especially in colonies under stress might become a common beekeeping practice.

The microbiota in honey bee colonies is critical in sustaining their health and vigor. Currently, most beekeeping practices are therapeutic rather than preventative. However, honey bee colonies have evolved strategies to process food and combat pathogens. Beekeeping practices that augment these strategies might help prevent the occurrence of numerous diseases and mitigate the impact of others. Preventing problems is always easier than solving them. In the case of developing beekeeping practices to prevent colony losses, perhaps the best solutions reside in the bees themselves.