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Current Opinion in Insect Science

Nutrition, immunity and viral infections in honey bees

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Viruses and other pathogens can spread rapidly in social insect colonies from close contacts among nestmates, food sharing and periods of confinement. Here we discuss how honey bees decrease the risk of disease outbreaks by a combination of behaviors (social immunity) and individual immune function. There is a relationship between the effectiveness of social and individual immunity and the nutritional state of the colony. Parasitic Varroa mites undermine the relationship because they reduce nutrient levels, suppress individual immune function and transmit viruses. Future research directions to better understand the dynamics of the nutrition–immunity relationship based on levels of stress, time of year and colony demographics are discussed.

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Current Opinion in Insect Science 2015, **10**:170–176

This review comes from a themed issue on **Social insects**

Edited by **Christina M Grozinger** and **Jay D Evans**

For a complete overview see the Issue and the Editorial

Available online 3rd June 2015

http://dx.doi.org/10.1016/j.cois.2015.05.007

Introduction

Honey bees and other eusocial insects comprise more than half of the insect biomass in the world making them one of the most ecologically successful insect groups [1]. Contributing to this success is the coordination of activities among members of a colony. Essential tasks such as thermoregulation, brood rearing and resource gathering are efficiently executed due to the architecture and organization of the nest and spatial proximity among individuals. However, crowded conditions, warm temperatures, high concentrations of resources and periods of confinement in the nest are ideal for pathogen invasion and transmission that can lead to epidemics [2,3•]. The risk of disease outbreaks is mitigated by specialized group behaviors (social immunity) and immune systems in individuals.

Honey bees are important pollinators in undisturbed ecosystems, but are essential for the production of numerous high-value crops [4]. Over the past decades, the health of honey bees has been in steady decline especially with arrival of parasitic Varroa mites (*Varroa destructor* Anderson and Trueman). There has been considerable effort to identify parasites and pathogens that threaten the health and survival of honey bee colonies. Viruses have received much attention due to the significant loss of colonies especially over winter from Varroa mite and virus associations [5,6••]. Greater attention also has been given to nutritional needs of colonies and how improvements in this area might reduce colony losses.

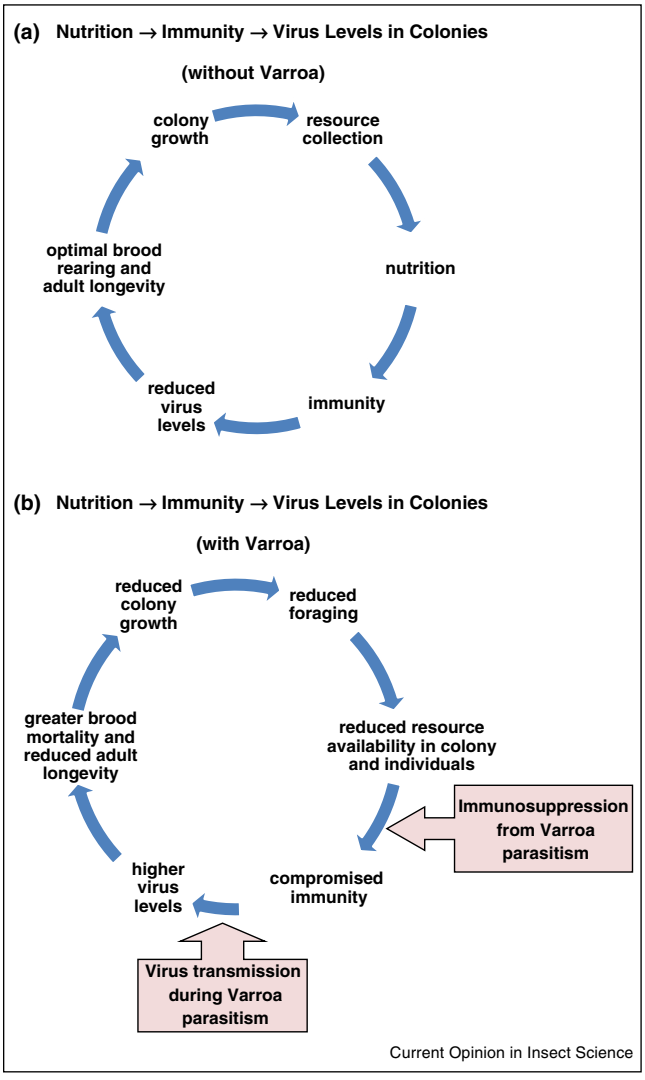
This review will focus on the role of nutrition in immune response to viral pathogens. We briefly describe the connections between nutrition and individual immunity, and speculate on the possible changing nutritional requirements of colonies throughout the year. These changes might revolve around trade-offs between colony growth and immune defense. Within this framework, we include the effects of parasitism by Varroa because when the mite is present, optimal nutrition alone might not be sufficient to keep virus levels low [7,8] (Figure 1).

Honey bee viruses

More than 20 viruses have been identified to infect honey bees worldwide [9]. The most common are: *Deformed wing virus* (DWV), *Black queen cell virus* (BQCV), and *Israeli acute paralysis virus* (IAPV) [10••]. IAPV, *Acute bee paralysis virus* (ABPV) and *Kashmir bee virus* (KBV) often are referred to as the Acute–Kashmir–Israeli complex or AKI, and share similar characteristics [11] (Table 1). Viruses infect all developmental stages and castes [9,12]. Though always present in colonies, viruses often persist as covert asymptomatic infections. However, if colonies are under stress, virus levels can increase causing reduced worker longevity and brood survival and colony loss in winter or early spring [13–15]. Viruses such BQCV also can cause colony death by preventing the development and emergence of a new queen following queen loss.

A factor that has increased virus levels in managed colonies of European honey bees in the U.S. and Europe is Varroa. The mite weakens bees by feeding on hemolymph of larvae, pupae and adults. Varroa also can transmit viruses among nestmates and suppress host immunity thus leading to elevated virus replication [9,16–20,21•]. In colonies with large Varroa populations, brood cells are invaded by multiple foundress mites causing higher DWV levels than in singly infested cells even in Varroa-resistant stocks [22,23]. Multiple infestations are

Figure 1



Schematic of the relationships among nutrition, immunity and virus levels and the effects on colony growth **(a)** and changes in the relationships when bees are parasitized by Varroa mites **(b)**.

common in the fall because mite populations are peaking and there are fewer cells to invade. The combination of multiply infested cells and greater virus levels in autumn ultimately causes colonies to die over winter [5,6••,15].

In addition to the threat viruses pose to honey bee colonies, recent studies indicate that the viruses can cross the species barrier and infect non-*Apis* species (e.g., bumble bees) [24,25]. Bumble bees have experienced dramatic population declines, and might acquire viruses while foraging on flowers previously visited by infected honey bees. Therefore controlling viral diseases in honey bee colonies is vital for stopping the spread of viruses among wild pollinators [26•].

Honey bee immune system

The risk of disease outbreaks is reduced in colonies of honey bees and other social insects by group-level behaviors ('social immunity') and individual immunity. Together these provide multiple levels of disease prevention and responses to challenges from pathogens and parasites.

Social immunity

The collective defense against parasites and pathogens that emerges from the behavioral cooperation among individuals in colonies is 'social immunity' [2,3•]. With social immunity, many individuals do small tasks that collectively have a colony-wide impact on reducing the spread of parasites and pathogens. For example, workers remove adults that die in the colony (undertaking or necrophoric behavior) and brood that are diseased or parasitized (hygienic behavior). Adults that die outside the nest also contribute to social immunity if they have high pathogen loads [3•]. Thermoregulatory behaviors also are a type of social immunity particularly when worker bees generate a behavioral 'social fever' against heat-sensitive pathogens such as chalkbrood fungus (*Ascosphaera apis*) [27].

In addition to group behavior inside the nest, bees collect plant resins (propolis) and use them to create a water and airtight antimicrobial and antiviral envelope around their nest [28–32]. Some compounds in propolis such as p-coumaric acid up-regulate immunity genes [33]. Other compounds might limit the growth of Varroa populations because they have miticidal properties [32,34].

Individual immunity

At the individual level, honey bees have several lines of innate immune defense against foreign pathogens. Physical and chemical barriers including the exoskeleton cuticle and the peritrophic membranes lining the digestive tract are a first line of defense that prevent pathogens from adhering to or entering the body [3•]. If a pathogen breaches the physical and chemical barriers, honey bees can protect themselves from infection with cellular and humoral immune responses which represent a second line of defense [35,36]. The activation of the innate immune responses involves recognition of the highly conserved structural motifs on the surface of pathogens, termed Pathogen-Associated Molecular Patterns (PAMPs), by Pattern Recognition Receptors (PRRs) that are germline-encoded proteins [8]. The binding of PAMP by PRRs triggers signaling cascades that lead to the activation of hemocyte-mediated cellular immune response including phagocytosis, nodule formation and encapsulation of the invading pathogens, the initiation of phenoloxidase cascade that regulates coagulation or melanization of hemolymph, or the synthesis of antimicrobial peptides (AMP). Several AMPs such as abaecin, apidaecin, hymenoptaecin, and defensin have been identified in the hemolymph of honey bees upon induction of microbial infections [37–40].

Table 1

Viruses commonly detected in honey bee colonies.

Virus	Transmission	Lifestage infected	Symptoms	Reference
Acute bee paralysis virus (ABPV)	Horizontal primarily through feeding, Varroa parasitism	Brood and adults	Paralysis, trembling, inability to fly, darkening and loss of hair on thorax and abdomen	[9,11]
Black queen cell virus (BQCV)	Horizontal primarily through feeding, Varroa parasitism, possible vertical transmission through eggs	Brood and adults	Dead queen larvae or prepupae sealed in queen cells with dark brown to black walls	[12]
Chronic bee paralysis virus	Horizontal primarily through feeding and contact, possible transovarial	Adults	Trembling inability to fly, bloated abdomens, black hairless bees	[12]
Deformed wing virus	Horizontal primarily through feeding, venereal, transovarial, transspermal, Varroa parasitism	Brood and adults	Deformed wings in emergent bees, premature aging of adults	[12,17,21•]
Israeli acute paralysis virus (IAPV)	Horizontal primarily through feeding, transovarial, venereal, transpermal, Varroa parasitism	Brood and adults	Similar to ABPV. Also, reduced mitochondrial function, and possible disturbance in energy-related host processes.	[10,18]
Kashmir bee virus (KBV)	Horizontal primarily through feeding, transovarial, Varroa parasitism	Brood and adults	Weakening of colonies but no clear field symptoms	[9,16]

There are several signaling pathways including Toll, Imd, Jak-STAT as well as JNK, that have been experimentally demonstrated to control the expression of many AMP genes in Drosophila in response to virus infection [41–43]. While a study reported that honey bees infected with ABPV did not trigger either cellular immune or humoral responses [44], a more recent study showed that a diverse range of signaling pathways implicated in the cellular innate immune responses are regulated in IAPV infected honey bees [10••].

Recent studies indicate that RNA interference (RNAi) is the major antiviral innate immune response in insects [45–49]. This innate antiviral pathway is triggered by the detection of exogenous double-stranded RNA (dsRNA), an intermediate generated during RNA virus replication. The response includes an RNase III-like enzyme called Dicer 2 (Dcr2) that recognizes virus dsRNA as a PAMP and cleaves long stretches of it into short interfering RNAs (siRNAs) that are 21–23 nucleotide-long duplexs. The resultant siRNA duplex, in association with Dcr-2 and the dsRNA-binding protein, is loaded onto RNA Induced Silencing Complex (RISC) which comprises multi-subunit effectors with Argonaute 2 (Ago2) as the catalytic core of this complex and degrades the passenger strand of siRNA. The guide strand of the siRNA remains bound to RISC and guides the RISC to cognate viral RNAs that are sliced by the endonuclease activity of Ago2 at the point of complementarity, thereby restricting viral replication (reviewed in Brutscher *et al.*, 2015). The honey bee genome encodes the core components of the RNAi pathway including Dicer enzymes, Argonaute endonucleases, a Drosha homologue, dsRNA-binding proteins Loquacious, R2D2, Pasha [50] and homologue of systemic RNA interference defective protein (SID-1), a gene essential for transporting of dsRNA between cells and the systemic spread of RNAi signals [51].

The role of RNAi in mediating dsRNA-induced antiviral response in honey bees was confirmed in several studies. IAPV is a widespread RNA virus of honey bees that was initially linked with colony collapse disorder (CCD) [52]. Deep-sequencing analysis of honey bee workers from CCD-colonies revealed abundant siRNA matching the nucleotides of IAPV and other viruses associated with colony losses, indicating the activation of RNAi pathway in CCD-colonies for combating viral infections [53]. Injection and feeding of dsRNA corresponding to a segment of the intergenic region (IGR) and a segment of gene encoding the capsid structural protein can reduce the intensity of IAPV infection in honey bees [54,55]. Feeding siRNA targeting an Internal Ribosomal Entry Site (IRES) of IAPV required for protein translation can confer antiviral activity in bees [56]. Additionally, feeding dsRNA that is specific to DWV can lead to reduction in DWV infection in DWV-inoculated bees [57]. dsRNA-mediated non-specific antiviral response was demonstrated by a study showing that the administration of dsRNA, regardless of sequence could trigger an antiviral response that controls virus infection in honey bees [58]. More recently, a study of the global gene expression in both IAPV infected and uninfected bees indicated that RNAi pathway had increased activity in the virus infected bees, further confirming the role of RNAi in antiviral immunity. The study also showed alterations in DNA methylation patterns in response to viral infection, suggesting that honey bees may possess parallel epigenomic and transcriptomic mechanisms to respond to viral infection [59].

These findings and those reported by others are very encouraging for RNAi development as a tool for managing virus diseases in honey bees.

Nutrition and immunity

Honey bees meet all their nutritional needs with nectar and pollen. These resources are collected in quantities that exceed colony demands and are stored for periods of dearth as honey and beebread. Nectar and honey contain carbohydrates and are the energetic fuel for all stages and castes. Pollen and beebread provide protein and nutrients required for physiological processes such as brood rearing, growth and immunity [60•,61].

The connection between nutrition and immunity has been demonstrated in numerous organisms where immune function is affected by caloric restriction [62••,63]. Dietary protein (pollen) provides essential amino acids needed for the synthesis of peptides in immune pathways [64,65] including components of AMP [66••]. Carbohydrates (nectar and honey) provide energy for metabolic processes associated with innate humoral and cellular immune reactions, and can provide secondary plant metabolites that have antimicrobial properties [67].

The relationship between nutrition and immunity is compromised when bees are parasitized by Varroa. Workers that are parasitized during development emerge with lower protein levels that cannot be raised even if sufficient pollen is available [68]. Varroa-infested pupae also can have significantly lower protein content, elevated free amino acid levels, and lower emergence weights than uninfested pupae suggesting that protein synthesis, and ultimately growth, are inhibited by Varroa [69].

Recently, nutrigenomic studies have revealed the effects of both carbohydrate and protein sources on transcriptional profiles of adult bees. Constituents in honey upregulate detoxification pathways in the gut [33] and genes associated with protein metabolism and oxidative reduction [70]. These effects were not found in other carbohydrate sources commonly fed to bees in managed colonies (e.g., sucrose solution or high fructose corn syrup). Pollen activates nutrient-sensing and metabolic pathways, and influences the expression of genes affecting longevity, immune function, the production of certain AMP [7] and pesticide detoxification [71]. However, if bees are parasitized by Varroa, there is a decrease in protein metabolism, inhibition of certain immunity genes and increased virus levels that cannot be reversed by pollen feeding. Thus, there are limitations to the benefits of diet on immune function in Varroa parasitized bees [7,8,69].

Conclusions and future directions

Honey bee nutrition is one of the most rapidly expanding research areas in bee biology largely due to colony losses from malnutrition and the accompanying pathologies. Though honey bee nutrition has been investigated for many years, molecular tools and the availability of the honey bee genome are enabling more comprehensive studies on the role of nutrition in honey bee health. To this end, we suggest several areas for future investigations. The first is a comprehensive evaluation of the nutritional value of pollen and nectar within the context of the nutritional needs of colonies throughout the year. An underlying assumption in comparing the nutritional value of pollens and in the development of protein supplements is that the nutritional needs of colonies are constant, and the relationship between diet and immunity is simply driven by energy consumption rather than specific nutrient blends that are key in determining an individual's immune response. Honey bee colonies go through yearly cycles. Brood production and colony demographics change throughout the year, so it is reasonable to assume that so do nutritional needs. As demonstrated in other insects, diets that are optimal for growth are not necessarily optimal for immunity [62••]. Thus, colonies that are building in the spring may require nutrients geared toward growth while in the fall when brood rearing is reduced and colonies are preparing for overwinter confinement, nutrients needs may be directed at supporting immune function. Nutritional analyses of pollen collected at different times of year in combination with nutrigenomic studies examining the effects on metabolic and immune gene expression could broaden our perspective on the nutritional needs of colonies and how they are met by the seasonal pollens bees collect.

The second area needing greater study is the role of the microbiome in nutrient processing and immunity. The composition of nutrients obtained from food influences microbial communities in the gut [72,73,74••]. The communities could affect immune function by providing essential nutrients, inducing host immune responses or reducing the growth of pathogens [75–79]. While there is evidence for these benefits in other organisms, the role of microbial communities as extensions of social and individual immune systems has only begun to be explored in honey bees.

Though improved nutrition can optimize colony growth and immune responses to virus, Varroa parasitism might undermine any benefits that nutrition might offer. Abundant resources stimulate brood rearing and population growth throughout the spring and summer. However, as the colony grows, so does the Varroa population. In the fall, when less brood is available the large Varroa population generates high parasitism rates [13,14,80]. Going into winter, the colony will be comprised of a majority of adults that were parasitized during development and harbor virus [5]. Colonies such as these have high overwintering mortality rates. A final research area that needs further study is the role of

nutrition on Varroa reproductive success and virus transmission. If improvements in bee nutrition affect either of these factors, then the relationship between nutrition and immunity could be re-established even when Varroa are present.

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