Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Insect Science

The power and promise of applying genomics to honey bee health

Christina M Grozinger[1] and Gene E Robinson[2]

New genomic tools and resources are now being used to both understand honey bee health and develop tools to better manage it. Here, we describe the use of genomic approaches to identify and characterize bee parasites and pathogens, examine interactions among these parasites and pathogens, between them and their bee hosts, and to identify genetic markers for improved breeding of more resilient bee stocks. We also discuss several new genomic techniques that can be used to more efficiently study, monitor and improve bee health. In the case of using RNAi-based technologies to mitigate diseases in bee populations, we highlight advantages, disadvantages and strategies to reduce risk. The increased use of genomic analytical tools and manipulative technologies has already led to significant advances, and holds great promise for improvements in the health of honey bees and other crucial pollinator species.

Addresses
[1] Department of Entomology, Center for Pollinator Research, Pennsylvania State University, University Park, PA 16803, United States
[2] Department of Entomology, Neuroscience Program, Institute for Genomic Biology, University of Illinois, Urbana-Champaign 61801, United States

Corresponding author: Grozinger, Christina M (cmgrozinger@psu.edu, christina.grozinger@gmail.com)

Current Opinion in Insect Science 2015, **10**:124–132

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

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

http://dx.doi.org/10.1016/j.cois.2015.03.007

Introduction

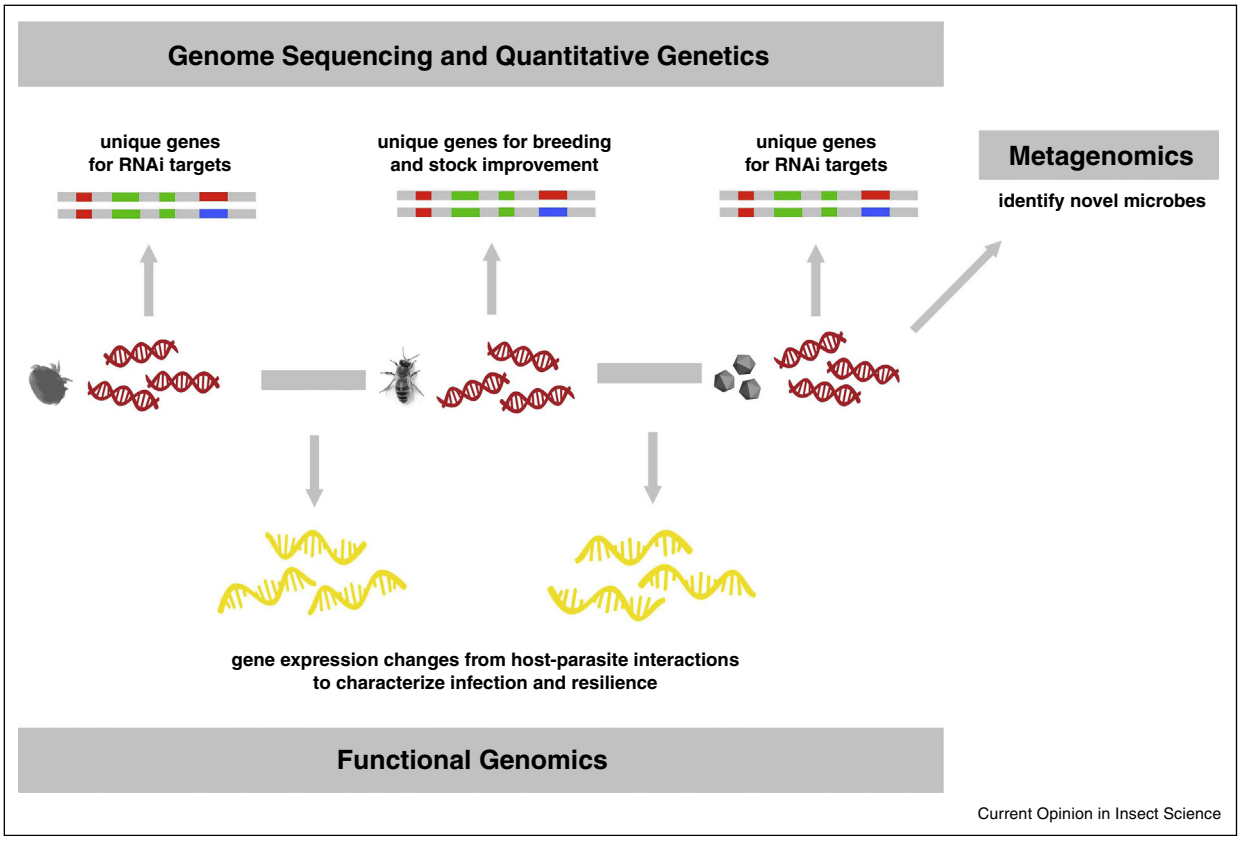
The winter of 2006–2007 ushered in a new era in bee biology, with the simultaneous discovery of the devastating effects of Colony Collapse Disorder on US honey bee populations [1] and the culmination of a multi-year, international effort to sequence and analyze the *Apis mellifera* honey bee genome with a large series of papers in *Nature*, *Science*, *PNAS* and elsewhere [2]. As for other topics such as social behavior [3], the knowledge and tools that derived from the honey bee genome sequencing project were quickly deployed to address CCD [4••]. In the following years these resources, for honey bees and soon for other bee species, have formed the basis for new approaches to the study of bee health. This review summarizes the progress and challenges associated with applying genomics to understand the mechanisms by which abiotic and biotic factors undermine bee health and to develop novel strategies to mitigate the effects of these stressors (see Figure 1).

Comparative analyses of immune genes

Over the past several years there has been a steady increase in the availability of genome sequence information for a variety of insect species, including several bee species [5]. Additional sequencing and analyses substantially improved the *A. mellifera* genome in 2014, resulting in the identification of more than 5000 additional protein coding genes [6]. Sequenced genomes were recently reported or are underway for a managed Asian honey bee species, *Apis cerana* [7], a halictid bee, *Lasioglossum albipes* [8], two bumble bee species, *Bombus terrestris* and *B. impatiens* [9], and several other bee species [10••]. In addition, transcriptomes for over ten bee species have been published (e.g., [11–13]).

Comparisons across a broad range of insect species have provided important insights into the molecular mechanisms regulating several traits of bees, including immunity. In the first such comparison, between *A. mellifera* and the only two other sequenced insect genomes at the time (*Drosophila melanogaster* and *Anopheles gambiae*) it was observed that though honey bees have a fully intact immune system with genes corresponding to all known branches of the immune response pathway, they appeared to have fewer of the canonical insect immune genes [14••]. However, as more genomes became available, this difference was not observed and honey bees are now thought to have a typical complement of canonical immune related genes [15,16••]. Comparisons across bee species suggest that these canonical immune genes are rapidly evolving, and thus may allow different species to adapt to species-specific immune challenges [13]. However, more recent studies suggest that this rapid evolution is not due to positive selection but rather relaxed selection [17••]. This might be the case because bees do not rely exclusively on canonical immune genes to fight infection, but can employ other genes and mechanisms to combat diseases and parasites, such as social immunity (behavioral mechanism to reduce disease load, [18]) or increased genetic diversity [19]. Consistent with this speculation, analyses of gene expression changes in response to immunostimulation revealed that honey bees

Figure 1



Genomic approaches to bee health. (1) Sequencing the genome of parasites and pathogens can provide information about unique gene sequences that can be targeted by RNAi approaches, allow for the development of efficient molecular diagnostic tools, and characterize mechanisms for host-parasite interactions and virulence. (2) Genome sequences and quantitative genetic studies of bees can identify gene variants associated with resilience to different stressors, which can be used in breeding and stock improvement programs. (3) Metagenomic approaches can identify and characterize pathogenic and beneficial microbes. (4) Functional genomic studies to identify host and parasite gene expression changes (changes in the levels of RNA produced by a given gene) associated with infection or other stressors can help characterize mechanisms for host–parasite interactions and resilience. Graphical design by Harland Patch and Nick Sloff (Penn State University).

(and other insects) may employ a much broader array of genes than those identified as part of the canonical immune pathways, though the functional significance of these gene expression changes remain to be determined [20,21••].

Using genomic tools to investigate the effects of biotic and abiotic stressors on bee health

Many studies have used analyses of gene expression as a way to probe effects of various stressors on bee health (see Box 1). The underlying idea is that changes in gene expression can provide a sensitive indication of effects that will eventually negatively impact a variety of physiological systems. This approach also has provided insights into the mechanisms underlying tolerance or resistance to these stressors (see also [22,23], this issue). For example, viral infections in developing honey bee pupae led to changes in expression of genes encoding ribosomal RNA and proteins, consistent with viral impacts on protein translation [24]. Changes in expression of these genes were also found in gut samples from bees collected from colonies exhibiting symptoms of CCD [25], in accordance with the possibility that CCD, at least in some cases, involves infections with multiple viruses [26]. Viral infection caused upregulation of genes in the RNAi pathway in honey bee fat body tissues [21••], supporting previous studies demonstrating that the RNAi pathway plays an important role in mediating antiviral responses in insects [27]. Introduction of non-viral double-stranded RNA also can reduce viral titers in honey bees (likely by non-specific activation of the RNAi pathway, [28]), and thus may serve as a therapeutic tool to reduce viral infections in bee colonies (see below for further discussion). Exposure of young bees to neonicotinoid pesticides altered expression of a gene that regulates NF-κB-mediated antiviral immune responses, resulting in increased

Box 1 From the bench to the beehive: using genomics to improve bee health

(1) Identification and characterization of bee parasites, pathogens and beneficial microbes. Metagenomic sequencing of honey bee populations has identified several new viruses and demonstrated that a largely ignored honey bee parasite, *Lotmaria passim* (previously identified as *Crithidia mellificae*), is prevalent in honey bee populations and associated with colony losses [45,46,55••,56]. Similarly, genomic approaches have demonstrated that honey bees host a multitude of species of microbes which may positively impact bee health (see [51], this issue). A comprehensive analysis of the parasites, pathogens and beneficial microbes circulating within bee populations is necessary for a complete understanding of bee health. Furthermore, since viruses and parasites apparently transmit between populations of bees and other insects readily, this screening should be performed on a broad array of species.

(2) Develop molecular diagnostics for rapid and inexpensive monitoring of bee diseases. It is impossible to fix what you do not see. Beekeepers need new, cost-effective tools to be able to rapidly diagnose their colonies for diseases, as well as information on treatment thresholds. This is the first step in an 'Integrated Pest Management' approach to bee diseases. At this point, beekeeper-accessible protocols for monitoring and treatment threshold have only been developed for *Varroa* mites. All other viruses and pathogens require specialized screening using relatively expensive molecular or microscopy instrumentation, and treatment thesholds have not been well-defined.

(3) Develop molecular tools to control bee parasites and pathogens.

Several studies have demonstrated that RNAi approaches can successful reduce parasite and pathogen loads in bees [87••,88••,90••]. These tools are very promising, since they can specifically target genetic sequences in pathogens and parasites, thereby reducing off-target effects and potentially reduce the likelihood for selecting for resistant strains. However, there has been indication that off-target effects can occur (see text), and thus additional testing should be performed to ensure that these treatments do not cause unintended effects on bees. Finally, as in all treatments, it is important to develop an Integrated Pest Management approach, to reduce off-target effects, reduce the likelihood of resistance development, and reduce costs and labor.

(4) Identify factors that improve resilience which can be incorporated into management practices. Genomic approaches have provided considerable information about the types of genes that mediate the effects of stressors on bees, and genes that can underlie sensitivity and resilience to these stressors. However, breeding and maintaining genetic stocks of bees is challenging. Thus, these studies should be examined to identify management practices that can be easily employed to improve bee health. For example, recent studies demonstrated that both pesticides and rich (honey/pollen) diets impacted the same suite of genes, and, based on those results, the authors developed and validated the hypothesis that complex diets (pollen) reduced mortality in pesticide-exposed bees [36••].

viral titers in pesticide-treated bees [29••]. Finally, viral infection also caused changes in DNA methylation patterns in fat body tissue for a set of genes previously associated with antiviral responses in vertebrates but not insects [21••], and thus this may represent a heretofore undescribed genomic response to viral infections. However, it is important to note that most studies only show correlations between stressors and changes in gene expression or methylation levels, and detailed functional analyses of these processes must be performed.

Characterizing transcriptional responses to *Nosema* microsporidia infections has also helped explain the bewildering diversity of effects that *Nosema* has on bees. *Nosema* are gut parasites, and infections lead to increased hunger, accelerated behavioral maturation from brood care to foraging, reduced flight capabilities, and premature death [30]. Examination of genome-wide expression patterns demonstrated that the primary impact of *Nosema* in honey bee fat body tissue is on expression of genes in metabolic and nutritional pathways, which appears to subsequently lead to the transcriptional and physiological changes associated with accelerated behavioral maturation, altered immune function, and reduced longevity [31].

Global gene expression studies have also suggested that nutrition and diet can mitigate the effects of pesticides. Exposure to pesticides caused upregulation of detoxification genes in abdominal tissues, which should reduce the impacts of the pesticides, and altered expression of immune genes, which is consistent with studies demonstrating that pesticide-exposed bees are immunocompromised [32–35,36••]. Interestingly, the effects of pesticide exposure on gene expression in fat body tissue are similar to those caused by consuming a rich diet of honey and pollen (vs sucrose) [36••]. Honey and pollen contain a variety of chemically complex secondary plant compounds and thus may trigger similar 'detoxification' responses as pesticides. Indeed, feeding honey bees p-coumaric acid, a constituent of honey, or quercetin, found in both honey and pollen, caused upregulation of detoxification genes and improved detoxification abilities [37,38••,39]. However, while short-term feeding with pollen before pesticide exposure does confer some benefit in terms of longevity (consistent with a priming effect), long-term feeding is significantly more beneficial, suggesting that natural pollen/honey based diets result in improved overall health, which in turn improves responses to pesticides and other stressors [36••].

Do different stressors elicit common or distinct transcriptional responses in bees? Parasitization with *Nosema* and *Varroa* cause similar changes in brain gene expression, despite the fact that these parasites infect their hosts at different life stages (adult for *Nosema*, pupae for *Varroa*) [40]. *Nosema* parasitization, injection with *E. coli* bacteria, and exposure to pesticides all triggered similar changes in gene expression in fat body tissues [20,31,36••]. In contrast, gene expression changes induced by viral infection appear to be fairly distinct, and the effects of infection vary with the type of virus, developmental stage of infection, duration of infection, and tissue [21••]. Similarly, while pesticide exposure generally causes changes in expression of detoxification genes, the identities of these

genes can vary greatly across pesticides and studies [32,33,35,36••]. Comparing and contrasting the effects of biotic and abiotic stressors on gene expression in a variety of tissues is a promising avenue to understand how these stressors affect bee health.

Genome sequencing of bee parasites and microbial communities

Genomic resources for honey bees have been used to generate great insight into the possible mechanisms underlying molecular and physiological responses to stressors, but it is also necessary to generate genomic resources for the parasites and pathogens infecting bees to fully understand and potentially mitigate the effects of the diseases they cause (see also [41], this issue). Recently, genomic information for several key parasites of honey bees has been produced, including a partial genome sequence for the parasitic mite *Varroa destructor*, a major cause for bee decline across the world [42]; whole genome sequences for the gut microsporidian parasites *Nosema apis* and *Nosema ceranae*, which have been associated with increased mortality and colony loss [43,44]; and a draft genome sequence for the gut trypanosomatid parasite *Lotmaria passim* (previously identified as *Crithidia mellificae*), which has been linked to colony losses in Europe [45,46]. Additionally, genome sequences have been generated for two key honey bee brood parasites: *Paenibacillus larvae*, the bacterial species that causes American foulbrood, and *Ascosphaera apis*, the fungal species that causes chalkbrood [47–49]. Genome sequencing has been used to define and characterize variants of two major viral pathogens of honey bees, Deformed Wing Virus (DWV) and Israeli Acute Paralysis Virus (IAPV) [50].

Genomic information obtained from these sequences can be used to identify pathogen/parasite-specific gene sequences for RNAi-based control measures (see below), develop molecular diagnostic markers to efficiently monitor parasite/pathogen load and spread, or to generate insights into the mechanisms mediating host–parasite interactions and potentially host resilience to these disease agents. Furthermore, metagenomic approaches have allowed for characterization of the beneficial microbes found in honey bees, and the factors that may perturb these communities (see [51], this issue).

Using genomics to reveal hidden diseases

Genomic approaches have greatly facilitated the identification of previously unknown or uncharacterized pathogens and parasites in honey bee populations. However, it must be noted that if parasites and pathogens are very different from previously sequenced species, identification based simply on genomic sequence alone can be challenging. Furthermore, while genomics approaches can reveal an association of a parasite or pathogen with particular symptoms, additional testing is necessary to provide causation. For example, historically, 18 viruses were known to infect honey bees [52]. Metagenomic sequencing of control and collapsing honey bee colonies indicated that prevalence of a relatively understudied virus, IAPV, was higher in collapsing colonies [4]. It was subsequently shown that IAPV was present in the US before the occurrence of CCD [53], but more comprehensive longitudinal studies indeed demonstrated that colonies with high levels of IAPV are less likely to survive the winter [54].

High-throughput sequencing of RNA extracted from honey bees from colonies of US migratory beekeeping operations led to the identification of four additional viruses (Aphid Lethal Paralysis virus, Big Sioux River virus, and Lake Sinai viruses 1 and 2), some which reached high levels of prevalence [55••]. Similar molecular screens later identified Lake Sinai viruses 3 and 4 in US and European honey bee populations [26,56]. Genomic and molecular diagnostic approaches also demonstrated that US and European colonies are frequently infected with the trypanosome *Lotmaria passim* (previously identified as *Crithidia mellificae*) [46,55••,56]. *L. passim* was not previously considered to be a significant threat to honey bee health, but more recent studies in Europe demonstrated that levels of *L. passim* are strongly associated with winter colony losses [56]. Recent studies also have suggested that a plant virus (tomato ringspot virus) can also infect honey bees [57], though further analyses are necessary to confirm replication with bee hosts and negative health effects.

Genomic approaches have also helped disentangle the complex pathogen-parasite-host interactions that have been observed between DWV, *Varroa* mites, and honey bees. DWV is found in nearly all honey bee populations, with *Varroa* mites both transmitting the virus and triggering elevated viral titers [58]. Interestingly, DWV titers also reach very high levels in bees when the cuticle is pierced by a needle [59,60••], and thus it may be the mechanical trauma from *Varroa* feeding that immunocompromises the bee, rather than factors introduced by *Varroa* during feeding. Recent studies have demonstrated that when DWV is introduced to a host bee by *Varroa* or injection, there is selective amplification of genotypically distinct, highly virulent strains of DWV, which in some cases correspond to a recombinant DWV-Varroa destructor virus strain [60••,61••]. Further studies are needed to determine the mechanisms by which these strains specifically amplify under these conditions, though it has been hypothesized that this represents a trade-off between anti-Varroa/melanization and anti-viral immune responses [62].

New diagnostic tools derived from genome sequences have facilitated our ability to detect pathogens and track their spread across bee populations. For example, *Varroa* mites were recently introduced into honey bee

populations in Hawaii, New Zealand, and Kenya, and molecular techniques allowed for the rapid analyses of these populations to determine the extent of the distribution of *Varroa*-associated viruses, and their impacts on bee health [61••,63,64]. Furthermore, molecular approaches have demonstrated that pathogens from commercial bumble bee colonies can spill over into wild bumble bee colonies [65] and may cause declines in wild species [66]. Similarly, pathogens and parasites of honey bees can infect populations of other bees and insects, likely via horizontal transmission by feeding on common flowering plants [67,68••,69–71].

Using genomic approaches to combat stressors

Ever since the discovery of honey bees resistant to the bacterial disease American Foulbrood [72], it has been known that there can be considerable heritable variation in the sensitivity of different bee genotypes to parasites and pathogens. These discoveries have fueled an interest in identifying the underlying genetic factors that drive this variation to breed more resilient stocks of bees. Breeding programs have generated stocks of bees that are more resistant to *Nosema* in Denmark, and this difference is associated with increased expression of immune genes [73] and inferred sequence variation in four locations in the genome [74,75]. Similarly, there is variation in resistance to *Varroa* mites in both natural and selected populations of honey bees. Resistance to *Varroa* is driven by multiple physiological and behavior traits, and different quantitative trait loci (QTLs) have been found that are related to these different traits, including grooming (where mites are removed from a nestmate's body, [76]), hygienic behavior (removal of parasitized brood, [77]) and suppressed reproduction of female mites feeding on developing pupae [78]. Genetic differences in resistance to viruses have also been observed, though these have not been mapped to specific genomic regions [79]. Thus far, genetic differences in response to other major stressors of bees (pesticides and poor nutrition) have not been investigated.

Although it has been possible to identify several genomic regions associated with variation in resilience to different stressors, identifying the specific genes and using this information to breed and maintain improved stocks of bees can been challenging (for review, see [80], this issue). Variation in many of these traits is influenced by variation in many genes, thus setting up the possibility of many complex interactions among genes in determining phenotypic differences. In other words, a particular genetic variant that is associated with variation in grooming behavior or pathogen resistance in one population may not be causally relevant in a different population. Furthermore, honey bee queens typically mate with an average of 12 drones, always outside the hive [81]. Thus, beekeepers must use instrumental insemination or tightly controlled breeding yards to limit uncontrolled gene flow into selected stocks. In addition, there can be negative effects of inbreeding or low genetic diversity in a colony [19,82,83], requiring that stocks include considerable genetic diversity at non-selected loci.

There are exciting new technical developments that will greatly improve our ability to functionally characterize pathways involved in mediating bee health (rather than relying simply on correlations) and potentially breed more resilient bees. Recently, the *piggyBac*-derived transposon was used to transform honey bees and drive expression of an exogenous *green fluorescent protein* gene [84••], which, together with the development of general genome editing tools such as CRISPRs and TALENs [85], lays the groundwork for the development of transgenic bees with enhanced genetic resistance to different stressors. It should also be possible to generate transgenic strains of beneficial bee gut microbes [86], which could produce key nutrients, pesticide detoxification enzymes, or biotic factors targeting parasites or pathogens.

The use of double-stranded RNA (dsRNA, which activates the RNAi pathway and reduces RNA levels of target genes) has greatly enhanced our ability to study the function of genes involved in bee health [54,62], and is a promising new tool for mitigating the impacts of parasites and pathogens. Feeding parasitized honey bees with dsRNA corresponding to *Nosema* or *Varroa* genes reduces expression levels of these genes in populations of *Nosema* and *Varroa* collected from these parasitized bees, and reduces levels of both parasites in bees [87••,88••]. Importantly, in the case of the experiments using *Varroa*, there was bidirectional transfer of the dsRNA: dsRNA was fed to the bee, passed from the bee gut to the hemolymph, from the hemolymph to the *Varroa*, and then back to the bee. Similarly, feeding honey bees viral dsRNA can reduce viral titers, reduce mortality of individual bees, and have positive effects on colony health parameters [89,90••].

Using RNAi to reduce levels of pathogens and parasites has both advantages and disadvantages. dsRNA should in theory be highly specific for its target gene sequence, which should limit off-target effects [91]. However, if relatively long sequences are used to develop dsRNA constructs, they are likely to contain fragments that match the host bee's genome sequence. Indeed, feeding bees with dsRNA corresponding to *green fluorescent protein* (a gene sequence not found in bees) resulted in altered developmental timing and significant gene expression changes, specifically in bee genes that had small regions matching sequences found in the introduced dsRNA [92••]. Furthermore, a previous study indicated that non-sequence specific dsRNA can trigger a general antiviral immune response [28], which can be both beneficial, since it can impact a broad range of viruses and viral

strains, and potentially problematic, if external, non-target dsRNA is introduced in large quantities for other applications, such as to control crop pests or weeds [93]. Though a second study found no effect of non-specific dsRNA on viral titers [90••], further testing is needed to determine whether chronic exposure to dsRNA impacts bee immune function in a positive or negative way. Additionally, because most pathogens and parasites are broadly circulating in the environment, even if the dsRNA treatments are very effective, they will have to be frequently applied, or detailed studies will be needed to determine the most effective time period for treatment (e.g., treatments for *Varroa* are most effective in the fall, before the production of winter bees; for review see [94] this issue). As with all efforts to manage pests, parasites, and pathogens, an Integrated Pest Management approach should be employed, to reduce off-target effects, reduce the likelihood of resistance development, and reduce costs (see [95], this issue, for a discussion of IPM approaches to pollinator health).

Conclusions

The development of genomic resources and tools in honey bees has tremendously facilitated our ability to dissect the intricate mechanisms that regulate bee health. Furthermore, genomics has allowed us to make discoveries that have launched new fields of inquiry, including the identification of new parasites, pathogens, and genetic mechanisms that combat these. Finally, genomics is providing desperately needed tools to better diagnose and manage bee diseases. These tremendous advances have all been made possible with completion of the sequencing of the honey bee genome in 2006. With the development of the next generation of genomic tools and resources for a broader array of bee species, the next decade will bring even greater advances in our understanding and management of bee health and biology.

Acknowledgements

We would like to thank Harland Patch and Nick Sloff for graphical design of Figure 1, and members of the Grozinger lab for helpful discussion and critical reading of the manuscript. The development of this review was supported by funding from an NIH Pioneer Award to GER, USDA-APHIS to CMG, and NSF and USDA-AFRI.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Cox-Foster D, Vanengelsdorp D: **Saving the honeybee**. *Scientific Am* 2009, **300**:40-47.

2. Weinstock GM, Robinson GE, Gibbs RA, Worley KC, Evans JD, Maleszka R, Robertson HM, Weaver DB, Beye M, Bork P *et al.*: **Insights into social insects from the genome of the honeybee *Apis mellifera***. *Nature* 2006, **443**:931-949.

3. Zayed A, Robinson GE: **Understanding the relationship between brain gene expression and social behavior: lessons from the honey bee**. *Annu Rev Genetics* 2012, **46**:591-615.

4. •• Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, Moran NA, Quan PL, Briese T, Hornig M, Geiser DM *et al.*: **A metagenomic survey of microbes in honey bee colony collapse disorder**. *Science* 2007, **318**:283-287.
Demonstrated the power of using a metagenomics approach to identify pathogens involved in declines of honey bees or other species.

5. Poelchau M, Childers C, Moore G, Tsavatapalli V, Evans J, Lee CY, Lin H, Lin JW, Hackett K: **The i5k Workspace@NAL-enabling genomic data access, visualization and curation of arthropod genomes**. *Nucleic Acids Res* 2015, **43**:D714-D719.

6. Elsik CG, Worley KC, Bennett AK, Beye M, Camara F, Childers CP, de Graaf DC, Debyser G, Deng JX, Devreese B *et al.*: **Finding the missing honey bee genes: lessons learned from a genome upgrade**. *BMC Genomics* 2014:15.

7. Park D, Jung JW, Choi BS, Jayakodi M, Lee J, Lim J, Yu Y, Choi YS, Lee ML, Park Y *et al.*: **Uncovering the novel characteristics of Asian honey bee, *Apis cerana*, by whole genome sequencing**. *BMC Genomics* 2015, **16**:1.

8. Kocher SD, Li C, Yang W, Tan H, Yi SV, Yang XY, Hoekstra HE, Zhang GJ, Pierce NE, Yu DW: **The draft genome of a socially polymorphic halictid bee, *Lasioglossum albipes***. *Genome Biol* 2013:14.

9. Sadd BM, Barribeau SM, Bloch G, de Graaf DC, Dearden P, Elsik CG, Gadau J, Grimmelikhuijzen CJ, Hasselmann M, Lozier JD *et al.*: **The genomes of two key bumblebee species with primitive eusocial organization**. *Genome Biol* 2015, **16**:76.

10. •• Kapheim KM, Pan H, Li C, Salzberg SL, Puiu D, Magoc T, Robertson HM, Hudson ME, Venkat A, Fischman BJ *et al.*: **Genomic signatures of evolutionary transitions from solitary to group living**. *Science* 2015 http://dx.doi.org/10.1126/science.aaa4788.
Compative genomic study of ten bee species.

11. Woodard SH, Fischman BJ, Venkat A, Hudson ME, Varala K, Cameron SA, Clark AG, Robinson GE: **Genes involved in convergent evolution of eusociality in bees**. *Proc Natl Acad Sci U S A* 2011, **108**:7472-7477.

12. de Sousa CS, Serrao JE, Bonetti AM, Amaral IMR, Kerr WE, Maranhao AQ, Ueira-Vieira C: **Insights into the *Melipona scutellaris* (Hymenoptera, Apidae, Meliponini) fat body transcriptome**. *Genet Mol Biol* 2013, **36**:292-297.

13. Viljakainen L, Evans JD, Hasselmann M, Rueppell O, Tingek S, Pamilo P: **Rapid evolution of immune proteins in social insects**. *Mol Biol Evol* 2009, **26**:1791-1801.

14. •• Evans JD, Aronstein K, Chen YP, Hetru C, Imler JL, Jiang H, Kanost M, Thompson GJ, Zou Z, Hultmark D: **Immune pathways and defence mechanisms in honey bees *Apis mellifera***. *Insect Mol Biol* 2006, **15**:645-656.
Examined the repetoire of immune genes across multiple species.

15. Fischman BJ, Woodard SH, Robinson GE: **Molecular evolutionary analyses of insect societies**. *Proc Natl Acad Sci U S A* 2011, **108**:10847-10854.

16. •• Barribeau SM, Sadd BM, du Plessis L, Brown MJ, Buechel SD, Cappelle K, Carolan JC, Christiaens O, Colgan TJ, Erler S *et al.*: **A depauperate immune repertoire precedes evolution of sociality in bees**. *Genome Biol* 2015, **16**:83.
Examined the repetoire of immune genes across also multiple social and solitary bee species.

17. •• Harpur BA, Zayed A: **Accelerated evolution of innate immunity proteins in social insects: adaptive evolution or relaxed constraint?** *Mol Biol Evol* 2013, **30**:1665-1674.
Demonstrated that the rapid evolution observed in immune gene sequences of in social insects is likely due to relaxed constraint rather than positive selection, providing evidence that other immune mechanisms are playing a large role in social insect immunity.

18. Wilson-Rich N, Spivak M, Fefferman NH, Starks PT: **Genetic, individual, and group facilitation of disease resistance in insect societies**. *Annu Rev Entomol* 2009, **54**:405-423.

19. Seeley TD, Tarpy DR: **Queen promiscuity lowers disease within honeybee colonies**. *Proc R Soc B-Biol Sci* 2007, **274**:67-72.

20. Richard FJ, Holt HL, Grozinger CM: **Effects of immunostimulation on social behavior, chemical communication and genome-wide gene expression in honey bee workers (*Apis mellifera*)**. *BMC Genomics* 2012:13.

21. •• Galbraith GA, Yang X, Nino EL, Yi SV, Grozinger CM: **Parallel epigenetic and transcriptomic responses to systemic viral infection in honey bees (*Apis mellifera*)**. *PLoS Pathog* 2015, **11** e1004713.
Demonstrates that viral infection causes changes in expression levels of one set of genes, and DNA methylation levels of a second set of genes. Interesting, several of the differentially methylated genes correspond to genes involved in anti-viral immune responses in vertebrates.

22. Brutscher LM, Daughenbaugh KF, Flenniken M: **Antiviral defense mechanisms in honey bees**. *Curr Opin Insect Sci* 2015 http://dx.doi.org/10.1016/j.cois.2015.04.016.

23. Poppinga L, Genersch E: **Molecular pathogenesis of American foulbrood: how Paenibacillus larvae kills honey bee larvae**. *Curr Opin Insect Sci* 2015 http://dx.doi.org/10.1016/j.cois.2015.04.013.

24. Boncristiani HF, Evans JD, Chen Y, Pettis J, Murphy C, Lopez DL, Simone-Finstrom M, Strand M, Tarpy DR, Rueppell O: **In vitro infection of pupae with Israeli acute paralysis virus suggests disturbance of transcriptional homeostasis in honey bees (*Apis mellifera*)**. *PLoS One* 2013, **8**:e73429.

25. Johnson RM, Evans JD, Robinson GE, Berenbaum MR: **Changes in transcript abundance relating to colony collapse disorder in honey bees (*Apis mellifera*)**. *Proc Natl Acad Sci U S A* 2009, **106**:14790-14795.

26. Cornman RS, Tarpy DR, Chen YP, Jeffreys L, Lopez D, Pettis JS, vanEngelsdorp D, Evans D: **Pathogen webs in collapsing honey bee colonies**. *PLoS One* 2012:7.

27. Wang XH, Aliyari R, Li WX, Li HW, Kim K, Carthew R, Atkinson P, Ding SW: **RNA interference directs innate immunity against viruses in adult Drosophila**. *Science* 2006, **312**:452-454.

28. Flenniken ML, Andino R: **Non-specific dsRNA-mediated antiviral response in the honey bee**. *PLoS One* 2013, **8**.

29. •• Di Prisco G, Cavaliere V, Annoscia D, Varricchio P, Caprio E, Nazzi F, Gargiulo G, Pennacchio F: ***Neonicotinoid clothianidin* adversely affects insect immunity and promotes replication of a viral pathogen in honey bees**. *Proc Natl Acad Sci U S A* 2013, **110**:18466-18471.
Provided the molecular mechanisms linking pesticide application to increased viral titers in bees

30. Holt HL, Chen J, Grozinger CM: **Towards an integrated pest management approach for Nosema parasites in honey bee colonies**. *J Econ Entomol*. submitted.

31. Holt HL, Aronstein KA, Grozinger CM: **Chronic parasitization by *Nosema microsporidia* causes global expression changes in core nutritional, metabolic and behavioral pathways in honey bee workers (*Apis mellifera*)**. *BMC Genomics* 2013, **14**:799.

32. Boncristiani H, Underwood R, Schwarz R, Evans JD, Pettis J, vanEngelsdorp D: **Direct effect of acaricides on pathogen loads and gene expression levels in honey bees Apis mellifera**. *J Insect Physiol* 2012, **58**:613-620.

33. Derecka K, Blythe MJ, Malla S, Genereux DP, Guffanti A, Pavan P, Moles A, Snart C, Ryder T, Ortori CA *et al.*: **Transient exposure to low levels of insecticide affects metabolic networks of honeybee larvae**. *PLoS One* 2013, **8**.

34. Garrido PM, Antunez K, Martin M, Porrini MP, Zunino P, Eguaras MJ: **Immune-related gene expression in nurse honey bees (*Apis mellifera*) exposed to synthetic acaricides**. *J Insect Physiol* 2013, **59**:113-119.

35. Gregorc A, Evans JD, Scharf M, Ellis JD: **Gene expression in honey bee (*Apis mellifera*) larvae exposed to pesticides and Varroa mites (*Varroa destructor*)**. *J Insect Physiol* 2012, **58**:1042-1049.

36. •• Schmehl DR, Teal PEA, Frazier JL, Grozinger CM: **Genomic analysis of the interaction between pesticide exposure and nutrition in honey bees (*Apis mellifera*)**. *J Insect Physiol* 2014, **71**:177-190.
Demonstrated a strong genome-wide association between genes involved in responses to pesticide and diet.

37. Johnson RM, Mao WF, Pollock HS, Niu GD, Schuler MA, Berenbaum MR: **Ecologically appropriate xenobiotics induce cytochrome P450s in *Apis mellifera***. *PLoS One* 2012, **7**.

38. •• Mao W, Schuler MA, Berenbaum MR: **Honey constituents up-regulate detoxification and immunity genes in the western honey bee *Apis mellifera***. *Proc Natl Acad Sci U S A* 2013, **110**:8842-8846.
Demonstrated that key secondary plant compounds found in honey triggers increased expression of detoxification genes.

39. Mao WF, Schuler MA, Berenbaum MR: **CYP9Q-mediated detoxification of acaricides in the honey bee (*Apis mellifera*)**. *Proc Natl Acad Sci U S A* 2011, **108**:12657-12662.

40. McDonnell CM, Alaux C, Parrinello H, Desvignes JP, Crauser D, Durbesson E, Beslay D, Le Conte Y: **Ecto- and endoparasite induce similar chemical and brain neurogenomic responses in the honey bee (*Apis mellifera*)**. *BMC Ecol* 2013, **13**.

41. Schwarz RS, Evans J: **Hologenome theory and the honey bee pathosphere**. *Curr Opin Insect Sci* 2015.

42. Cornman SR, Schatz MC, Johnston SJ, Chen YP, Pettis J, Hunt G, Bourgeois L, Elsik C, Anderson D, Grozinger CM *et al.*: **Genomic survey of the ectoparasitic mite *Varroa destructor*, a major pest of the honey bee *Apis mellifera***. *BMC Genomics* 2010, **11**.

43. Chen YP, Pettis JS, Zhao Y, Liu XY, Tallon LJ, Sadzewicz LD, Li RH, Zheng HQ, Huang SK, Zhang X *et al.*: **Genome sequencing and comparative genomics of honey bee microsporidia, *Nosema apis* reveal novel insights into host–parasite interactions**. *BMC Genomics* 2013:14.

44. Cornman RS, Chen YP, Schatz MC, Street C, Zhao Y, Desany B, Egholm M, Hutchison S, Pettis JS, Lipkin WI *et al.*: **Genomic analyses of the microsporidian *Nosema ceranae*, an emergent pathogen of honey bees**. *PLoS Pathogens* 2009, **5**.

45. Runckel C, DeRisi J, Flenniken ML: **A draft genome of the honey bee trypanosomatid parasite *Crithidia mellificae***. *PLoS One* 2014, **9**.

46. Schwarz RS, Bauchan GR, Murphy CA, Ravoet J, de Graaf DC, Evans JD: **Characterization of two species of trypanosomatidae from the honey bee *Apis mellifera*: *Crithidia mellificae* Langridge and McGhee, 1967 and *Lotmaria passim* *n. gen., n. sp***. *J Eukaryot Microbiol* 2015 http://dx.doi.org/10.1111/jeu.12209.

47. Chan QWT, Cornman RS, Birol I, Liao NY, Chan SK, Docking TR, Jackman SD, Taylor GA, Jones SJM, de Graaf DC *et al.*: **Updated genome assembly and annotation of Paenibacillus larvae, the agent of American foulbrood disease of honey bees**. *BMC Genomics* 2011, **12**.

48. Cornman RS, Bennett AK, Murray KD, Evans JD, Elsik CG, Aronstein K: **Transcriptome analysis of the honey bee fungal pathogen, *Ascosphaera apis*: implications for host pathogenesis**. *BMC Genomics* 2012, **13**.

49. Qin X, Evans JD, Aronstein KA, Murray KD, Weinstock GM: **Genome sequences of the honey bee pathogens *Paenibacillus* larvae and *Ascosphaera apis***. *Insect Mol Biol* 2006, **15**:715-718.

50. Cornman RS, Boncristiani H, Dainat B, Chen YP, vanEngelsdorp D, Weaver D, Evans JD: **Population-genomic variation within RNA viruses of the Western honey bee, *Apis mellifera*, inferred from deep sequencing**. *BMC Genomics* 2013, **14**.

51. Moran NA: **Genomics of the honey bee microbiome**. *Curr Opin Insect Sci* 2015 http://dx.doi.org/10.1016/j.cois.2015.04.003.

52. Chen YP, Siede R: **Honey bee viruses**. In *Advances in Virus Research*, vol. 70. Edited by Maramorosh K, Shatkin AJ, Murphy FA. Elsevier Academic Press; 2007:34-80.

53. Chen J, Evans J: **Historical presence of Israeli acute paralysis virus in the United States**. *Am Bee J* 2007, **147**:1027-1028.

54. Chen YP, Pettis JS, Corona M, Chen WP, Li CJ, Spivak M, Visscher PK, DeGrandi-Hoffman G, Boncristiani H, Zhao Y *et al.*: **Israeli acute paralysis virus: epidemiology, pathogenesis and**

implications for honey bee health. *PLoS Pathog* 2014, **10**:e1004261.

55. •• Runckel C, Flenniken ML, Engel JC, Ruby JG, Ganem D, Andino R, DeRisi JL: **Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, Nosema, and Crithidia**. *PLoS One* 2011:6.
Demonstrated the power of using a metagenomics approach to identify pathogens involved in declines of honey bees or other species.

56. Ravoet J, Maharramov J, Meeus I, De Smet L, Wenseleers T, Smagghe G, de Graaf DC: **Comprehensive bee pathogen screening in Belgium reveals *Crithidia mellificae* as a new contributory factor to winter mortality**. *PLoS One* 2013, **8**.

57. Li JL, Cornman RS, Evans JD, Pettis JS, Zhao Y, Murphy C, Peng WJ, Wu J, Hamilton M, Boncristiani HF *et al.*: **Systemic spread and propagation of a plant-pathogenic virus in European honeybees, *Apis mellifera***. *Mbio* 2014, **5**.

58. de Miranda JR, Genersch E: **Deformed wing virus**. *J Invertebr Pathol* 2010, **103(Suppl1)**:S48-S61.

59. Kuster RD, Boncristiani HF, Rueppell O: **Immunogene and viral transcript dynamics during parasitic Varroa destructor mite infection of developing honey bee (*Apis mellifera*) pupae**. *J Exp Biol* 2014, **217**:1710-1718.

60. •• Ryabov EV, Wood GR, Fannon JM, Moore JD, Bull JC, Chandler D, Mead A, Burroughs N, Evans DJ: **A virulent strain of deformed wing virus (DWV) of honeybees (*Apis mellifera*) prevails after Varroa destructor-mediated, or in vitro, transmission**. *PLoS Pathog* 2014, **10**:e1004230.
Used transcriptome sequencing to demonstrate the certain viral strains are preferentially transmitted by Varroa, and these strains are particular virulent.

61. •• Martin SJ, Highfield AC, Brettell L, Villalobos EM, Budge GE, Powell M, Nikaido S, Schroeder DC: **Global honey bee viral landscape altered by a parasitic mite**. *Science* 2012, **336**: 1304-1306.
Used molecular approaches to demonstrate that strain adundance in viral populations in honey bees change dramatically once Varroa mites are introduced.

62. Nazzi F, Brown SP, Annoscia D, Del Piccolo F, Di Prisco G, Varricchio P, Della Vedova G, Cattonaro F, Caprio E, Pennacchio F: **Synergistic parasite–pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies**. *PLoS Pathog* 2012, **8**:e1002735.

63. Mondet F, de Miranda JR, Kretzschmar A, Le Conte Y, Mercer AR: **On the front line: quantitative virus dynamics in honeybee (*Apis mellifera* L.) colonies along a new expansion front of the parasite Varroa destructor**. *Plos Pathog* 2014, **10**.

64. Muli E, Patch H, Frazier M, Frazier J, Torto B, Baumgarten T, Kilonzo J, Kimani JN, Mumoki F, Masiga D *et al.*: **Evaluation of the distribution and impacts of parasites, pathogens, and pesticides on honey bee (*Apis mellifera*) populations in East Africa**. *PLoS One* 2014, **9**.

65. Otterstatter MC, Thomson JD: **Does pathogen spillover from commercially reared bumble bees threaten wild pollinators?** *PLoS One* 2008, **3**.

66. Szabo ND, Colla SR, Wagner DL, Gall LF, Kerr JT: **Do pathogen spillover, pesticide use, or habitat loss explain recent North American bumblebee declines?** *Conserv Lett* 2012, **5**:232-239.

67. Levitt AL, Singh R, Cox-Foster DL, Rajotte E, Hoover K, Ostiguy N, Holmes EC: **Cross-species transmission of honey bee viruses in associated arthropods**. *Virus Res* 2013, **176**:232-240.

68. •• Singh R, Levitt AL, Rajotte EG, Holmes EC, Ostiguy N, Vanengelsdorp D, Lipkin WI, Depamphilis CW, Toth AL, Cox-Foster DL: **RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-apis hymenopteran species**. *PLoS One* 2010, **5**.
Used molecular approaches to demonstrate that viruses are readily transmitted between populations of bees.

69. Zhang X, He SY, Evans JD, Pettis JS, Yin GF, Chen YP: **New evidence that deformed wing virus and black queen cell virus are multi-host pathogens**. *J Invertebr Pathol* 2012, **109**:156-159.

70. Furst MA, McMahon DP, Osborne JL, Paxton RJ, Brown MJ: **Disease associations between honeybees and bumblebees as a threat to wild pollinators**. *Nature* 2014, **506**:364-366.

71. McMahon DP, Furst MA, Caspar J, Theodorou P, Brown MJ, Paxton RJ: **A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees**. *J Anim Ecol* 2015, **84**:615-624.

72. Spivak M, Reuter GS: **Resistance to American foulbrood disease by honey bee colonies *Apis mellifera* bred for hygienic behavior**. *Apidologie* 2001, **32**:555-565.

73. Huang Q, Kryger P, Le Conte Y, Moritz RFA: **Survival and immune response of drones of a Nosemosis tolerant honey bee strain towards *N. ceranae* infections**. *J Invertebr Pathol* 2012, **109**:297-302.

74. Huang Q, Kryger P, Le Conte Y, Lattorff HMG, Kraus FB, Moritz RFA: **Four quantitative trait loci associated with low *Nosema ceranae* (Microsporidia) spore load in the honeybee *Apis mellifera***. *Apidologie* 2014, **45**:248-256.

75. Huang Q, Lattorff HMG, Kryger P, Le Conte Y, Moritz RFA: **A selective sweep in a microsporidian parasite Nosema-tolerant honeybee population, *Apis mellifera***. *Animal Genetics* 2014, **45**:267-273.

76. Arechavaleta-Velasco ME, Alcala-Escamilla K, Robles-Rios C, Tsuruda JM, Hunt GJ: **Fine-scale linkage mapping reveals a small set of candidate genes influencing honey bee grooming behavior in response to varroa mites**. *PLoS One* 2012:7.

77. Tsuruda JM, Harris JW, Bourgeois L, Danka RG, Hunt GJ: **High-resolution linkage analyses to identify genes that influence varroa sensitive hygiene behavior in honey bees**. *PLoS One* 2012, **7**.

78. Behrens D, Huang Q, Gessner C, Rosenkranz P, Frey E, Locke B, Moritz RFA, Kraus FB: **Three QTL in the honey bee *Apis mellifera* L. suppress reproduction of the parasitic mite Varroa destructor**. *Ecol Evol* 2011, **1**.

79. Locke B, Forsgren E, de Miranda JR: **Increased tolerance and resistance to virus infections: a possible factor in the survival of varroa destructor-resistant honey bees (*Apis mellifera*)**. *PLoS One* 2014:9.

80. Nino EL, Jasper C: **Developing successful honey bee breeding programs: optimizing breeding practices and maximizing beekeeper satisfaction**. *Curr Opin Insect Sci* 2015.

81. Tarpy DR, Nielsen R, Nielsen DI: **A scientific note on the revised estimates of effective paternity frequency in Apis**. *Insectes Sociaux* 2004, **51**:203-204.

82. Mattila HR, Seeley TD: **Genetic diversity in honey bee colonies enhances productivity and fitness**. *Science* 2007, **317**:362-364.

83. Gempe T, Hasselmann M, Schiott M, Hause G, Otte M, Beye M: **Sex determination in honeybees: two separate mechanisms induce and maintain the female pathway**. *Plos Biol* 2009, **7**.

84. •• Schulte C, Theilenberg E, Muller-Borg M, Gempe T, Beye M: **Highly efficient integration and expression of piggyBac-derived cassettes in the honeybee (*Apis mellifera*)**. *Proc Natl Acad Sci U S A* 2014, **111**:9003-9008.
Demonstrated the feasibility of creating transgenic strains of bees.

85. Gaj T, Gersbach CA, Barbas CF 3rd: **ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering**. *Trends Biotechnol* 2013, **31**:397-405.

86. Rangberg A, Mathiesen G, Amdam GV, Diep DB: **The paratransgenic potential of *Lactobacillus kunkeei* in the honey bee *Apis mellifera***. *Benef Microbes* 2015:1-11.

87. •• Garbian Y, Maori E, Kalev H, Shafir S, Sela I: **Bidirectional transfer of RNAi between honey bee and varroa destructor: varroa gene silencing reduces varroa population**. *Plos Pathog* 2012, **8**.
Demonstrated that dsRNA fed to honey bees could be transmitted to ectoparasites (Varroa) and reduce expression of the targeted Varroa gene and reduce Varroa levels.

88. •• Paldi N, Glick E, Oliva M, Zilberberg Y, Aubin L, Pettis J, Chen YP, Evans JD: **Effective gene silencing in a microsporidian parasite associated with honeybee (*Apis mellifera*) colony declines**. *Appl Environ Microbiol* 2010, **76**:5960-5964.

Demonstrated that dsRNA fed to honey bees could be transmitted to endoparasites (Nosema) and reduce expression of the targeted Nosema gene and reduce Nosema levels.

89. Hunter W, Ellis J, Vanengelsdorp D, Hayes J, Westervelt D, Glick E, Williams M, Sela I, Maori E, Pettis J *et al.*: **Large-scale field application of RNAi technology reducing israeli acute paralysis virus disease in honey bees (*Apis mellifera*, Hymenoptera: Apidae)**. *PLoS Pathogens* 2010, **6**.

90. •• Maori E, Paldi N, Shafir S, Kalev H, Tsur E, Glick E, Sela I: **IAPV, a bee-affecting virus associated with Colony Collapse Disorder can be silenced by dsRNA ingestion**. *Insect Mol Biol* 2009, **18**:55-60.
Demonstrated that dsRNA fed to honey bees could be reduce viral titers.

91. Scott JG, Michel K, Bartholomay LC, Siegfried BD, Hunter WB, Smagghe G, Zhu KY, Douglas AE: **Towards the elements of successful insect RNAi**. *J Insect Physiol* 2013, **59**:1212-1221.

92. •• Nunes FMF, Aleixo AC, Barchuk AR, Bomtori AD, Grozinger CM, Simoes ZLP: **Non-target effects of green fluorescent protein (GFP)-derived double-stranded RNA (dsRNA-GFP) used in honey bee RNA interference (RNAi) assays**. *Insects* 2013, **4**:90-103.
Demonstrated that dsRNA from a non-honey bee gene can produce off-target effects in honey bees.

93. Lundgren JG, Duan JJ: **RNAi-based insecticidal crops: potential effects on nontarget species**. *Bioscience* 2013, **63**:657-665.

94. Doke MA, Frazier M, Grozinger CM: **Overwintering honey bees: biology and management**. *Curr Opin Insect Sci* 2015. in this issue.

95. Biddinger DJ, Rajotte EG: **Integrated pest and pollinator management — adding a new dimension to an accepted paradigm**. *Curr Opin Insect Sci* 2015. in this issue.