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HONEY BEE COLONY LOSSES AND ASSOCIATED VIRUSES

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ABSTRACT

Recent large-scale colony losses of managed Western Honey Bee (*Apis mellifera*) have scared scientists and apiculturists alike. Indeed, the losses have cost many their means of income while threatening, deeply, the ecosystem at large. These losses are not isolated and are concurrent with losses of many wild bee populations, namely bumble bees (*Bombus spp.*). These losses have been attributed to a myriad of things including the salient 'Colony Collapse Disorder', up to 23 viruses, a fungal pathogen, several bacterial pathogens, two mite species, agricultural development and monocultures, and toxins, including pesticides and pollution. Herein, I examine apicultural practices in Kenya with a focus on hive health and pathogens in hives, and I begin to ask what role the Common Eastern Bumble Bee (*Bombus impatiens*) plays in the ecology of a honey bee virus.

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Chapter 1

Honey bee colony losses and associated viruses

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Abstract

Recent large-scale colony losses among managed Western honey bees (Apis mellifera) have alarmed researchers and apiculturists alike. Here, the existing correlative evidence provided by monitoring studies is reviewed which (i) identified members of the deformed wing virus and acute bee paralysis virus clades as lethal pathogens for entire colonies, and (ii) identified novel viruses whose impact on honey bee health remains elusive. Also discussed in this review is related evidence obtained via controlled experimental infection assays and RNAi approaches underscoring the damage inflicted by some of these viruses on individuals and colonies. The relevance of the ectoparasitic mite Varroa destructor acting as mechanical and biological virus vector for the enhanced virulence of certain viruses or mite selected virus strains is carefully considered.

Introduction

Industrial management of the Western honey bee (Apis mellifera) has a long history with honey as a source of nutrition and trade for humans [1]. In addition to income from products directly harvested from honey bees, managed colonies contributed an average of s147 million to annual crop productivity from 2002 to 2007, as they have become an important supplement to pollination services provided by native pollinators across the globe [2●●]. Indeed, managed honey bees are the single most important global commercial insect pollinator [2●●,3–5]. Despite some decreasing trends in parts of Europe and North America, the global number of managed colonies has risen by about 45% over the last 60 years [6,7]. However, this increase in the number of managed honey bee colonies does not meet the steadily increasing need for managed pollinators in agriculture [6,8–10]. Hence, large scale managed colony losses, as experienced in the recent past in some parts of the world, exacerbate the shortage of pollinators and threaten human food security especially because wild pollinators are on the decline [11,12●●,13].

Winter colony losses in general and CCD in particular

It is worth noting that the death of a honey bee colony due to natural causes, including disease, is within the scope of reasonable expectations for a living organism [14,15]. However, reoccurring unusually high winter colony losses at or above 30% in the recent past startled beekeepers and scientists alike [16–18], in part because simple, causal relationships remained elusive. The picture that emerged over the last decade is that such losses are multifactorial with weak Fall condition,

starvation, queen failure, pathogens, parasites, pesticides, and climate all playing a role. The impact of each of these factors may differ on a case-by-case basis and even regionally [15,19,20]. Often when talking about colony losses, this general phenomenon is conflated with CCD [20]. However, CCD has a very specific case definition and is mainly characterized by the presence of a live queen and a lot of capped brood, indicative of rapid loss of adult bees. Additionally, the absence of dead bees in collapsed colonies and increased pathogen incidence are diagnostic of CCD [17]. CCD sensu stricto has so far only been reported from North America where it has been identified as one of many causes of winter mortality [21–23] and likely arises from multiple etiological agents. However, it has not been cited as a significant cause of winter losses since the winter of 2006/2007 [19]. In this review we refer to winter losses of managed A. mellifera colonies as the problem of interest, though summer losses may also be substantial. Primarily, we will concisely review the role that recently discovered and emerging viruses play in observed winter colony losses.

Viruses associated with honey bees

Till date 23 viruses have been reported to infect honey bees worldwide, primarily positive-strand RNA viruses in the families Dicistroviridae and Iflaviridae [24,25 ●,26 ●●]. In the absence of the ectoparasitic mite *Varroa destructor* (hereafter referred to as *Varroa*) many honey bee pathogenic viruses only cause covert infections, which show no clinical signs and have no detectable impact on infected bees or colonies (for a definition of covert/overt virus infections see [27]). However, for acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV), black queen cell virus (BQCV), and sacbrood virus (SBV) historical reports from the pre-Varroa era on overt disease

outbreaks exist indicative of clinically significant infections in the absence of Varroa (Table 1; for historical references see [28]). However, we know from molecular data that even these four viruses can frequently be detected in seemingly healthy colonies [16,29–31], hence, also for these viruses covert infections are common. Most of the honey bee viruses might not even be honey bee specific because they infect a wide range of arthropod species [32●,33–35]. In the wake of Varroa, prevalence of virus infections has increased dramatically. This mite very quickly became established as a mechanical and biological vector of honey bee viruses as it spread though European honey bee populations since the 1980s (for a recent review see [36]). Augmented prevalence and virulence due to mite transmission was marked for members of the DWV/VDV-1 (V. destructor virus-1) and ABPV/ KBV/IAPV (Kashmir bee virus/Israeli acute bee paralysis virus) clades [37–39]). The remainder of the review is divided into the following sections: correlative evidence (prevalence studies in symptomatic, asymptomatic, and not otherwise specified colonies), positive experimental evidence (infection assays), and negative experimental evidence (RNAi approaches) for associations between viruses and colony losses with a final section on the role of Varroa in virus amplification and dissemination.

Correlative evidence for virus infections associated with colony losses

Recently, numerous prevalence studies have underscored the role of viral infections in honey bee colony losses [16,17,25 ●,26 ●●,29–31,40–45,46 ●,47]. However, the design of many of these studies was rather weak. In most cases, the study period was one year or less, colonies were sampled once or twice, and whole bee extracts of pooled bee samples were analyzed by RT-PCR for the presence of six to eight known bee viruses (ABPV, IAPV, KBV, CBPV, SBV, DWV, VDV-1, and BQCV). Diagnostic results were then related either to the health status of the

sampled colonies or to incidences of large scale colony losses in the sampled region. These studies substantiated the observation that covert virus infections are wide-spread and common in asymptomatic colonies [29–31]. At the same time they provided correlative evidence for members of the ABPV/KBV/IAPV and the DWV/VDV-1 clade being involved in colony losses, an interpretation that was later validated by studies which have improved on these previous designs by increasing the length of the sampling period [16,40], improving screening precision by analyzing specific tissues [16,47], or even using advanced genomic techniques like next generation sequencing (NGS) or microarrays for detection of novel honey bee pathogens [25●,26●●,40,46●,47].

At least two long term studies provided strong correlative evidence for IAPV and ABPV (both are members of the ABPV/KBV/IAPV clade) being involved in winter colony losses in the U.S. and Germany, respectively, [16,40] and DWV being a key factor for overwintering colony losses in Germany [16]. A recent survey of a large-scale migratory beekeeping operation in the U.S. identified for the first time a strain of aphid lethal paralysis virus (ALPV strain Brookings, *Dicistroviridae*) as a putative honey bee pathogenic virus (Table 1). However, its relevance to colony losses needs further investigation. [25●]. In addition, three novel viruses, Big Sioux river virus (BSRV), Lake Sinai virus 1 (LSV1), and Lake Sinai virus 2 (LSV2) were identified (Table 1). BSRV is a novel dicistrovirus, similar to the aphid-pathogenic virus *Rhopalosiphum padi* virus (RhPV) but sufficiently divergent to justify a new species. LSV1 and LSV2 are two novel RNA viruses showing some similarity to CBPV and presumably belonging to the *Nodavirales* superfamily. Based on the ability of these viruses to replicate in adult honey bees and their abundance compared to other significant honey bee viruses, the authors suggested that they may play a significant role in colony health (Figure 1). A comparative study on pathogen loads of CCD and non-CCD colonies revealed additional LSV variants and suggested a potential associ-

ation between LSV strain and CCD status [47]. However, the role of LSV or certain LSV variants in colony losses still needs to be established (Figure 1).

The occurrence of ALPV, LSV1, and LSV2 in the honey bee population is obviously not restricted to the U.S., as similar viruses were also identified in a Spanish honey bee colony [46●]. Most recently, two independent studies reported Tobacco ring spot virus (TRSV) and Turnip ring spot virus (TuRSV), two plant pathogenic viruses of the family *Secoviridae*, to be associated with honey bees [26●●,46●]. TuRSV was proposed to be passively present in bees due to the ingestion of contaminated pollen [46●].

However, TRSV was shown to replicate in nearly every bee tissue and elevated virus levels were found in weak colonies [26 ●] (Table 1). This novel and surprising finding of TRSV replicating in honey bees caused some debate [48,49] although this is not the first example of a plant virus replicating in both plants and insects. So far, such viruses have been described from the RNA virus families *Bunyaviridae*, *Nodaviridae*, and *Rhabdoviridae* which are vectored by insects via persistent propagative transmission mode [50,51,52●,53]. However, TRSV is a member of the genus *Nepovirus* which is not transmitted through insects but through pollen [54]. It is long since known that honey bees mediate transmission of pollen-borne plant pathogenic viruses between infected and non-infected plants [55,56]. Hence, pollen collecting honey bees are exposed to plant viruses and it is conceivable that especially honey bee larvae fed with plant virus contaminated pollen might get infected and might then show infection in several tissues of the adult bee. The recent discovery of such an infection is likely for lack of looking for it. Hence, further investigations are necessary to unravel whether the TRSV–honey bee interaction is indeed another example of viral host range spanning the plant and animal kingdom and if so, it would be worthwhile to search for other similar relationships.

Experimental evidence for virus infections causing death of bees by infection assays (positive evidence)

Surveillance studies are at a disadvantage compared to controlled exposure bioassays because the former can only generate correlative evidence, as opposed to the causal relationship demonstrated by the latter. During the infancy of honey bee virology, around 50 years ago, it was via experimental infection that scientists identified ABPV and CBPV as etiological agents of bee paralysis [57]. Additionally, the authors describe not only plasticity of symptomology depending on route of transmission (injection versus ingestion), but they also noted the existence of covert infections caused by these commonly lethal viruses [57,58]. While years following led to discovery of novel viruses, it was not until Varroa began infesting the European honey bee that we began to see their potential lethality when contributing to Parasitic Mite Syndrome (PMS) [59–65]. In fact, it was in Varroa infested colonies in the 1980s that symptomatic DWV infections were noticed for the first time (i.e. crippled wings), driving the identification of this typically asymptomatic virus. However, as a factor within PMS, DWV has quickly become tightly associated with colony collapse (Figure 1), and is therefore one of the most well-characterized honey bee viruses [28,66].

This unique relationship has led to quite a few controlled experimental infections of bees of varying ages with DWV in order to elucidate its mechanism of virulence and whether transmission route affects virulence. Indeed, it has been shown that mimicking mite-vectored trans-mission by injecting isolated DWV particles into virus-free white-eyed pupae, results in

dose-dependent mortality of pupae and emergence of adults with deformed wings [67] which are also not viable [68]. Furthermore, it has been demonstrated that when a feeding Varroa mite is actively infected with DWV, and therefore acting as a biological vector, a bee is more likely to acquire a clinically significant DWV infection from the transmitting mite [69].

Transmitting DWV to adult bees via injection, thus mimicking vectoral DWV transmission by phoretic mites, resulted in a systemic infection which included the nervous system when at least 10E+08 virus particles were injected [67] which may lead to cognitive impairment and learning deficits [70]. It is not clear, how-ever, whether this latter effect was due to the virus or other components that may have been present in the whole bee extract used in this study. Therefore, this link between covert DWV infection and cognitive malfunctioning has yet to be adequately demonstrated. Recent studies have demonstrated the role of Varroa and the route of transmission in the evolution of virulence in DWV.

In 2011, Moore and colleagues began to clear up just how Varroa was augmenting the clinical severity of DWV infections in honey bees when they demonstrated that only certain variants of the DWV sequence space pre-dominated in mite-infested colonies. Specifically, variants of the DWV quasi-species that had VDV-1 capsid proteins and DWV non-capsid proteins prevailed [71]. In 2014, Ryabov along with Moore and colleagues demonstrated that augmentation of DWV titers by Varroa mites is clearly due to facilitation of this specific virulent variant. A follow-up injection assay showed that it was the injection of the virus directly into the hemolymph, thus mimicking virus inoculation by the mite, that selected for this recombinant [72●●]. It is conceivable, then, that these virulent DWV variants may be the precipitating factor for PMS.

It is possible that even oral transmission of DWV plays a significant role in increased mortality of larvae/pupae and the emergence of deformed adults, though the study did not evaluate this as no

dose information was provided [73●]. However, the role of oral transmission seems to be substantiated by the infection of mid-gut epithelium, particularly in mite infested colonies suffering from PMS, which is conceivably DWV/VDV-induced rather than mite-induced [37,67]. In fact, highly infected brood are preferentially cannibalized by workers, who then are likely to become infected given that the pupae being cannibalized have even higher viral titers than deformed adults [74 ●●,75]. Despite the restriction of infection in the gut to the mid-gut epithelium [67], the threat that cannibalism of infected pupae poses to the adult must be further evaluated.

For members of the ABPV clade, especially for IAPV and ABPV, good correlative evidence exist that they are involved in large scale colony losses [40] or winter losses [16] (Figure 1). Like DWV, these viruses are also effectively transmitted by Varroa [59,60,76] (Figure 1). ABPV was first isolated and described 50 years ago after adult bees were observed darkening in pigment following infection with some unknown agent [57]. We now know that IAPV, first described in 2007, causes similar symptoms. However, it was also shown that IAPV-injected adult bees die within 4 days, while oral transmission resulted in death after only 10 days [77]. Two recent studies [78 ●●,79 ●] aimed at a better and more detailed understanding of how IAPV infections damage honey bees.

Pupae injected with 10E+04 genome equivalents of IAPV showed a heterogeneous pattern of symptoms. Compared to controls (PBS (phosphate buffered saline) injected), IAPV injected pupae either stopped developing without any further symptoms or showed darkening of body parts [78●●]. It is uncertain however whether the darkening is due to virus induced tissue damage and necrosis, or melanization of tissue as a result of host immune response. Remarkably, studies suggest that heterogeneous pathology is dependent not just on IAPV strain diversity, but also on

colony- dependent or patriline-dependent differences in susceptibility of the bees to IAPV infection [77,80●●]. As with DWV, IAPV has been found in healthy colonies despite being associated also with collapsing colonies [40].

Honey bee colonies might not only suffer from collapse when mortality rates of brood and adult bees increase due to virus infection but also when virus infections negatively affect bee behavior and, thus, colony performance. This aspect has been studied in adult forager bees injected with as little as 44 copies of IAPV and equipped with radio frequency identification (RFID) tags. While no mortality was observed, homing ability was significantly reduced at 2 and 3 days post-infection. Indeed, only between 2.3% and 0% of IAPV infected foragers found their way back, compared to 50% of those injected with PBS [79 ●]. It is reasonable to suspect that a considerable proportion of foragers not returning to the hive may have detrimental effects on the colony and contribute to weakening and eventually collapse of the entire colony.

Experimental evidence for the impact of viruses on honey bees by treatment with dsRNA (negative evidence)

RNA interference (RNAi) and Dicer, a multi-domain enzyme with RNase III activity, are key regulators in invertebrate antiviral immunity and are crucial for sup-pressing viruses producing double-stranded RNA (dsRNA) intermediates. Dicer acts as a sensor of viral dsRNA. After binding and cleaving of virus-derived dsRNA molecules into siRNAs, it delivers these siRNAs to the host's RISC complex where they then can target viral RNA for inhibition [81]. Therefore, in honey bee virology RNAi approaches with experimentally delivered virus-specific dsRNAs can

be used to investigate the relation between certain viruses and symptoms observed in individual bees and bee colonies.

There is only one study applying RNAi approaches to DWV infected bees. Honey bee larvae orally infected in laboratory experiments with high enough DWV titers either died during larval development or developed into adult bees exhibiting wing deformities [73●]. Preventive feeding of DWV-dsRNA revealed a more than 300-fold reduction in DWV levels in adult bees (21 days post egg hatching and infection) relative to bees developed from larvae that were fed with virus or with virus and GFP-dsRNA. In addition, significantly reduced frequencies of wing deformities relative to other virus-fed treatments were observed [73 ●] confirming that DWV is the etiological agent of the deformed wing syndrome.

For IAPV infections, data obtained through RNAi approaches at colony level are available. Maori and co-workers [82] used queen-right mini colonies with approximately 200 worker bees and experimentally infected these colonies by feeding IAPV in sucrose solution. By day 8 post infection, bee mortality in the non-infected control group was approximately 25% while around 80% of IAPV-infected bees had died. Preventive feeding of IAPV-specific dsRNA preparations (calculated as approximately 1 mg per bee) for three days prior to virus infection significantly reduced bee mortality in IAPV-infected bees to a level not significantly different from control mortality. Hence, ingesting IAPV-dsRNA obviously triggered an antiviral immune response in honey bees and prevented the bees from succumbing to a subsequently initiated IAPV-infection under laboratory conditions. This promising laboratory effect was then evaluated under natural bee keeping conditions using a total of 160 bee hives in Florida (USA) and Pennsylvania (USA) [83]. However, no significant correlation between in-creased colony or individual mortality and IAPV infection was observed in this study and, hence, it was difficult to conclusively assess the

effect of dsRNA treatment on IAPV infection and colony survival. However, indirect measures suggested that IAPV-dsRNA treatment had some positive effect at the colony level.

A more recent study [80●●] suggested the existence of an IAPV-encoded viral suppressor of RNAi (VSR). In laboratory experiments with IAPV-infected caged bees it was demonstrated that silencing this putative viral interference protein via RNAi through feeding of VSR-specific siRNA resulted in a remarkable reduction in IAPV replication within 24 hours post treatment. The authors concluded that the studies on IAPV inhibition through RNAi rein- force the therapeutic potential of carefully designed siR- NAs for treatment of viral infections in honey bees.

Hence, the laboratory studies so far implied that there is a curative effect of virus-specific dsRNA on the severity and outcome of infections with the corresponding virus. However, a more recent study demonstrated that any dsRNA might serve as a viral pathogen associated molecular pattern (PAMP) in honey bees triggering an antiviral response that controls virus infection in general [84 ●●]. Therefore, approaches using virus-specific dsRNAs might not be suitable to prove the role of a specific virus or viral protein in a certain context but rather the involvement of viruses in a more general sense. Furthermore, the use of dsRNA as a management strategy for viruses in the field might rather not help to control a specific virus but may activate a nonspecific antiviral immunity resulting in a general positive effect at the colony level as observed by Hunter and co-workers [83].

Impact of V. destructor

It is without question that Varroa infestation poses the most serious threat to the Western honey bee colonies and that this is related to the mite's ability to vector virus infections or to exacerbate preexisting infections [39]. Virus infections of honey bees became a serious health problem for entire colonies only after Varroa started to infest honey bee colonies. Varroa theoretically can take up any virus present in bee hemolymph and, hence, can mechanically vector any virus back into the hemolymph when feeding on the next bee or pupa. Therefore, it is not surprising that since the introduction of Varroa the prevalence of honey bee viruses and infections in-creased. However, for most viruses, conclusive evidence linking them to colony losses is still lacking despite the nearly ubiquitous presence of the virus vector Varroa in the honey bee population. Only members of the ABPV clade and the DWV clade are thought to play a major role in colony losses in the presence of mite infestation.

Overt DWV infections, the development of the deformed wing syndrome, and colony collapse due to the PMS are closely associated with the vectoral transmission of DWV by Varroa (Figure 1). It is widely accepted by now that Varroa serves as a biological vector of DWV, and thereby plays a crucial role in the virulence of DWV. The selection of an especially virulent variant of DWV through Varroa has been anticipated and discussed for some time. Primarily, because it became obvious that DWV replication in the mite is necessary and sufficient for enhanced virulence of DWV as well as the development of de-formed wings [69,85]. A recent study in Hawaii found that the introduction of Varroa to a naive population greatly reduced the strain diversity of DWV while increasing the prevalence of infection [86]. These results supported the hypothesis that Varroa facilitates the dominance of certain strains which might be associated with

increased viral virulence. Some hallmarks of such a virulent variant may have been identified recently [71,72●●]. The described variants were characterized by the capsid protein coding region of VDV-1 (a member of the DWV clade originally isolated as virus replicating in Varroa [87]) and the coding region for the non-structural proteins from DWV. How-ever, the question of whether this variant was selected by amplification in the mite or by transmission route was not fully solved. Both of these studies further our understanding of the molecular mechanisms that underlie the in-creased virulence of DWV in the presence of Varroa.

Although ABPV and its close relatives are also associated with Varroa and colony losses, a similarly fatal triangular relationship between virus, Varroa, and honey bees could not develop. Members of the ABPV clade, when injected into pupae by mites, rapidly kill the infected pupa there-by preventing the developing bee from emerging and interrupting the reproduction of the mite which is trapped in the capped cell. Therefore, although mite transmitted ABPV/IAPV/KBV infections are lethal and contribute to colony losses (Figure 1), mite transmission of this virus clade is a dead-end hampering any further virulence evolution [38].

Conclusion

This review is not an exhaustive review on colony losses but rather collects on different levels recent evidence for the involvement of viruses in colony losses (Figure 1). Hence, we did not include all factors involved in the collapse of honey bee colonies such as non-viral patho-gens, metazoan parasites other than Varroa, pesticides, malnutrition, climate, and beekeeping practice although we are aware of the relevance of these factors. It is important to point out, that THE colony loss or THE honey bee decline does not exist. Instead, the phenome- non of colony losses,

although globally observed and reported, has different dimensions, reasons, and key players varying by region and time. It is also important to note that large scale colony losses are not happening for the first time but rather similar losses have been described in the historical past. This recurrence far from detracts from the importance of studying honey bee health, but rather increases the interest from the perspective of not only agriculture, but also basic science and ecology. Indeed, this is the first time in history that honey bee colony losses have attracted so much attention which has in turn boosted scientific interest and research in the field of honey bee diseases. Much of this research is interesting in its broader applicability in terms of potential control strategies for viral infections.

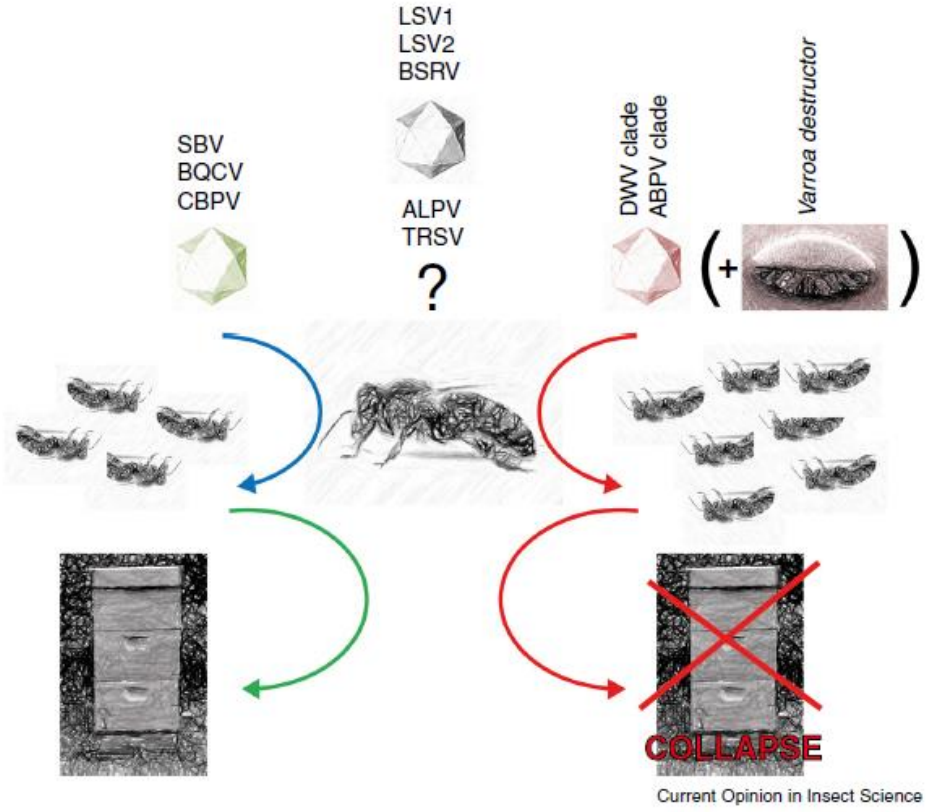

Figure 1. Honey Bees and Their Associated Viruses

Viruses affecting honey bees. Till date, 23 viruses have been found to infect honey bees. Although most bee viruses cause covert infections, some viruses do cause visible symptoms and death of individual bees (e.g. SBV, BQCV, and DWV). However, colony losses due to these viruses are rather rare. Members of the DWV and ABPV clade not only kill individual bees but have been related to the collapse of entire colonies, especially in the presence of the ectoparasitic mite Varroa. Recently, three novel bee viruses were discovered (LSV1, LSV2, and BSRV) and two known viruses were described for the first time to infect honey bees (ALPV, TRSV). The exact impact of these five viruses on individuals and colonies is still elusive (illustrated by the question mark) and awaits final experimental proof or disproof.

Table 1. Common and Emergin Honey Bee Viruses

Virus	**Family**	***Varroa* Vector Status**	**Symptoms of Overt Infection**	**Reference**
*ABPV clade	*Dicistroviridae*	++	– Paralysis – Darkened cuticle pigment – Impaired cognition and homing ability – Mortality (adult and immature bees) – Colony collapse	[77,82]
» ALPV	*Dicistroviridae*	Unknown	– Unknown	[25]
*BQCV	*Dicistroviridae*	+	– Pale-yellowish, leathery cuticle of capped larva – Failure of larva to pupate – Sac-like appearance – Mortality (of larvae) – Deceased larvae and walls of cell turn black	[24]
» BRSV	*Dicistroviridae*	Unknown	– Unknown	[25]
*DWV clade	*Iflaviridae*	+++	– Deformed wings – Learning deficits – Discoloring – Shortened and bloated abdomens – Mortality (adult and immature bees) – Colony collapse	[37]
» LSV1/2	*Nodaviridae*	Unknown	– Unknown	[25]
*SBV	*Picornavirales* (super family)	++	– Pale-yellowish, leathery cuticle of capped larva – Failure of larva to pupate – Sac-like appearance – Mortality (potentially of adults, certainly capped larvae) – Dead larva becomes dark, brittle scale	[24]
» TRSV	*Secoviridae*	+	– Winter colony collapse?? (correlative only!!)	[26]

Table 1 This list gives a summary of the most common (designated with *) and recently emerging (designated with ») honey bee viruses, whether they are vectored by Varroa, and their symptoms. If Varroa has been shown to be a significant biological vector, the virus was designated with (+++). If the virus is frequently associated with Varroa but the mite has not been determined to be a biological vector, the vector status was designated with (++). Finally, if the virus is sporadically associated with tissues of the mite, or the vectoring status is in question but possible, it was designated with (+). The final column is a reference

where more details can be found about the virus, including symptomology, the evidence of its association with Varroa, prevalence data, association with colony collapse, among other things.

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. Bloch G, Francoy TM, Wachtel I, Panitz-Cohen N, Fuchs S, Mazar A: **Industrial apiculture in the Jordan valley during Biblical times with Anatolian honeybees.** *Proc. Natl. Acad. Sci. USA* 2010, **107**:11240-11244.

2. Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, Kremen C, Carvalheiro LG, Harder LD, Afik O, et al.: **Wild pollinators enhance fruit set of crops regardless of honey bee abundance.** *Science* 2013, **339**:1608-1611.

●● This is a brilliant study putting the role of honey bees and wild pollinators into perspective. Honey bees are indispensable for commercial pollination because they can be managed and moved. However, wild pollinators are better pollinators - if they are present.

3. Morse RA, Calderone NW: **The value of honey bee pollination in the United States.** *Bee Culture* 2000, **128**:1-15.

4. Aizen MA, Garibaldi1 LA, Cunningham SA, Klein AM: **How much does agriculture depend on pollinators? Lessons from long-term trends in crop production.** *Ann. Bot. (Lond.)* 2009, **103**:1579–1588.

5. Allsopp MH, de Lange WJ, Veldtman R: **Valuing insect pollination services with cost of replacement.** *PloS ONE* 2008, **3**:e3128.

6. Aizen MA, Harder LD: **The global stock of domesticated honey bees is growing slower than agricultural demand for pollination.** *Curr. Biol.* 2009, **19**:915-918.

7. vanEngelsdorp D, Meixner MD: **A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them.** *J. Invertebr. Pathol.* 2010, **103** S80-S95.

8. Gallai N, Salles J-M, Settele J, Vaissiere BE: **Economic valuation of the vulnerability of world agriculture confronted with pollinator decline.** *Ecol. Econ.* 2009, **68**:810-821.

9. Eilers EJ, Kremen C, Smith Greenleaf S, Garber AK, Klein AM: **Contribution of pollinator-mediated crops to nutrients in the human food supply.** *PLoS ONE* 2011, **6**:e21363.

10. Aizen M, Garibaldi L, Cunningham S, Klein A: **Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency.** *Curr. Biol.* 2008, **18**:1572-1575.

11. Goulson D, Hanley ME, Darvill B, Ellis JS: **Biotope associations and the decline of bumblebees (*Bombus* spp.).** *J. Insect Conserv.* 2006, **10**:95-103.

12. Burkle LA, Marlin JC, Knight TM: **Plant-pollinator interactions over 120 years: Loss of species, co-occurrence and function.** *Science* 2013, **339**:1611-1615.

●● In this study, historical data sets from the late 1800s and contemporary data from 2009-2010 were analyzed to quantify the degree to which global change over the past 120 years disrupted plant-pollinator interactions. Although plant-pollinator interactions turned out to have been quite flexible, the authors also found a reduced redundancy in network structure, weakened interaction strengths, and a decline in the quantity and quality of pollinator services.

13. Cameron SA, Loziera JD, Strange JP, Koch JB, Cordes N, Solter LF, Griswold TL: **Patterns of widespread decline in North American bumble bees.** *Proc. Natl. Acad. Sci. USA* 2011, **108**:662–667.

14. Dukas R: **Mortality rates of honey bees in the wild.** *Insect. Soc.* 2008, **55**:252-255.

15. Genersch E: **Honey bee pathology: Current threats to honey bees and beekeeping.** *Appl. Microbiol. Biotechnol.* 2010, **87**:87-97.

16. Genersch E, von der Ohe W, Kaatz H, Schroeder A, Otten C, Büchler R, Berg S, Ritter W, Mühlen W, Gisder S, et al.: **The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies.** *Apidologie* 2010, **41**:332-352.

17. vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, Frazier M, Frazier J, Cox-Foster D, Chen Y, et al.: **Colony collapse disorder: a descriptive study.** . *PLoS ONE* 2009, **4**:e6481.

18. Potts SG, Roberts SPM, Dean R, Marris G, Brown MA, Jones HR, Neumann P, Settele J: **Declines of managed honey bees and beekeepers in Europe.** . *J. Apicult. Res.* 2010, **49**:15-22.

19. Ratnieks FLW, Carreck NL: **Clarity on honey bee collapse?** *Science* 2010, **327**:152-153.

20. Williams GR, Tarpy DR, vanEngelsdorp D, Chauzat M-P, Cox-Foster DL, Delaplane KS, Neumann P, Pettis JS, Rogers REL, Shutler D: **Colony Collapse Disorder in context.** *Bioessays* 2010, **32**:845-846.

21. vanEngelsdorp D, Hayes Jr. J, Underwood RM, Pettis J: **A survey of honey bee colony losses in the U.S., fall 2007 to spring 2008.** *PLoS ONE* 2008, **3**:e4071.

22. vanEngelsdorp D, Hayes Jr. J, Underwood RM, Pettis JS: **A survey of honey bee colony losses in the United States, fall 2008 to spring 2009.** *J. Apic. Res.* 2010, **49**:7-14.

23. vanEngelsdorp D, Underwood R, Caron D, Hayes J: **An estimate of managed colony losses in the winter of 2006-2007: A report commissioned by the apiary inspectors of America.** *Am. Bee J.* 2007, **147**:599-603.

24. Chen Y-P, Siede R: **Honey bee viruses.** *Adv. Virus Res.* 2007, **70**:33–80.

25. 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**:e20656.

● This study idenfied novel viruses associated with honey bees and further emphasizes the importance of employing state-of-the-art techniques to identify novel pathogens.

26. Li JL, Cornman RS, Evans JD, Pettis JS, Zhao Y, Murphy C, Pen 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**:e00898-00813.

●● A very interesting paper showing a pollen-borne plant virus replicating in adult honey bee tissues. Although this paper was controversially discussed (see [47, 48]), TRSV might become another example of a virus with a host range spanning the plant and animal kingdoms, and another virus poentially threatening honey bee colonies.

27. Hails RS, Ball BV, Genersch E: **Infection strategies of insect viruses.** In *Virology and the honey bee.* Edited by Aubert M, Ball BV, Fries I, Moritz RFA, Milani N, Bernardinelli I: European Communities; 2008:255-275.

28. Ball BV, Bailey L: **Viruses**. In *Honey bee pests, predators and diseases.*, edn 3rd. Edited by Morse RA, Flottum K: A.I. Root; 1997:11-31.

29. Tentcheva D, Gauthier L, Zappulla N, Dainat B, Cousserans F, Colin ME, Bergoin M: **Prevalence and seasonal variations of six bee viruses in *Apis mellifera* L. and *Varroa destructor* mite populations in France.** *Appl. Environ. Microbiol.* 2004, **70**:7185-7191.

30. Baker A, Schroeder D: **Occurrence and genetic analysis of picorna-like viruses infecting worker bees of *Apis mellifera* L. populations in Devon, South West England.** *J. Invertebr.Pathol.* 2008, **98**:239–242.

31. Gauthier L, Tentcheva D, Tournaire M, Dainat B, Cousserans F, Colin ME, Bergoin M: **Viral load estimation in asymptomatic honey bee colonies using the quantitative RT-PCR technique.** *Apidologie* 2007, **38**:426-435.

32. 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.

- This study convincingly shows that viruses once thought to be bee-specific are not even specific to the pollinator community. Hence, other arthropod species may be involved in disease transmission to and from bees and in pollinator populations. Importantly, it also highlights the complicated nature of viral ecology in general and raises interesting questions relevant to the broader field of viral ecology.

33. 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**:e14357.

34. Genersch E, Yue C, Fries I, de Miranda JR: **Detection of *Deformed wing virus*, a honey bee viral pathogen, in bumble bees (*Bombus terrestris* and *Bombus pascuorum*) with wing deformities.** *J. Invertebr. Pathol.* 2006, **91**:61-63.

35. Fürst MA, McMahon DP, Osborne JL, Paxton RJ, Brown MF: **Disease associations between honey bees and bumblebees as a threat to the wild pollinators.** *Nature* 2014, **506**:364-366.

36. Rosenkranz P, Aumeier P, Ziegelmann B: **Biology and control of *Varroa destructor*.** *J. Invertebr. Pathol.* 2010, **103**:S96-S119.

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

38. de Miranda JR, Cordoni G, Budge G: **The Acute bee paralysis virus - Kashmir bee virus - Israeli acute paralysis virus complex.** *J. Invertebr. Pathol.* 2010, **103**:S30-S47.

39. Genersch E, Aubert M: **Emerging and re-emerging viruses of the honey bee (*Apis mellifera*).** *Vet. Res.* 2010, **41:54**.

40. Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, Moran NA, Quan P-L, Briese S, Hornig M, Geiser DM, et al.: **A metagenomic survey of microbes in honey bee colony collapse disorder.** *Science* 2007, **318**:283-287.

41. Soroker V, Hetzroni A, Yakobson B, David D, David A, Voet H, Slabezki Y, Efrat H, Levski S, Kamer Y, et al.: **Evaluation of colony losses in Israel in relation to the incidence of pathogens and pests.** *Apidologie* 2011, **42**:192-199.

42. Bacandritsos N, Granato A, Budge G, Papanastasiou I, Roinioti E, Caldon M, Falcaro C, Gallina A, Mutinelli F: **Sudden seaths and colony population decline in Greek honey bee colonies.** *J. Invertebr. Pathol.* 2010, **105**:335-340.

43. Meixner MD, Francis RM, Gajda A, Kryger P, Andonov S, Uzunov A, Topolska G, Costa C, Amiri E, Berg S, et al.: **Occurrence of parasites and pathogens in honey bee colonies used in a European genotype-environment interactions experiment.** *J. Apicult. Res.* 2014, **53**:215-229.

44. Berenyi O, Bakonyi T, Derakhshifar I, Köglberger H, Nowotny N: **Occurence of six honeybee viruses in diseased Austrian apiaries.** *Appl. Environ. Microbiol.* 2006, **72**:2414-2420.

45. Nielsen SL, Nicolaisen M, Kryger P: **Incidence of acute bee paralysis virus, black queen cell virus, chronic bee paralysis virus, deformed wing virus, Kashmir bee virus and sacbrood virus in honey bees (*Apis mellifera*) in Denmark.** *Apidologie* 2008, **39**:310-314.

46. Granberg F, Vicente-Rubiano M, Rubio-Guerri C, Karlsson OE, Kukielka D, Belák S, Sánchez-Vizcaíno JM: **Metagenomic detection of viral pathogens in Spanish honeybees: Co-**

infection by Aphid lethal paralysis, Israel acute paralysis and Lake Sinai viruses. *PLoS ONE* 2013, **8**:e57459.

- This study supports the findings reported in [25] and demonstrates that ALPV and LSV are not restricted to the U.S. but are also present in honey bees in Europe. This further emphasizes the importance of studies employing techniques to identify novel pathogens.

47. Cornman RS, Tarpy DR, Chen Y, Jeffreys L, Lopez D, Pettis JS, vanEngelsdorp D, Evans JD: **Pathogen webs in collapsing honey bee colonies.** *PLoS ONE* 2012, **7**:e43562.

48. Miller WA, Carrillo-Tripp J, Bonning BC, Dolezal AD, Toth AL: **Conclusive evidence of replication of a plant virus in honeybees is lacking.** *mBio* 2014, **5**:e00985-00914.

49. Li JL, Cornman RS, Evans JD, Pettis JS, Zhao Y, Murphy C, Peng WJ, Wu J, Hamilton M, Boncristiani HF, et al.: **Reply to "Conclusive evidence of replication of a plant virus in honeybees is lacking".** *mBio* 2014, **5**:e01250-01214.

50. Ammar E-D, Tsai C-W, Whitfield AE, Redinbaugh MG, Hogenhout SA: **Cellular and molecular aspects of Rhabdovirus interactions with insect and plant hosts.** *Ann. Rev. Entomol.* 2004, **54**:447-468.

51. Whitfield AE, Ullman DE, German TL: **Tospovirus-thrips interactions.** *Annu. Rev. Phytopathol.* 2005, **43**:459-489.

52. Mann KS, Dietzgen RG: **Plant rhabdoviruses: new insights and research needs in the interplay of negative-strand RNA viruses with plant and insect hosts.** *Arch. Virol.* 2014, **159**:1889-1900.

- A comprehensive review of negative-strand RNA plant viruses that are transmitted by insects and also infect these vector insects, bringing to light the prevalence of cross-kingdom viral infection, a poorly understood phenomenon.

53. Power AG: **Insect transmission of plant viruses: a constraint on virus variability.** *Curr. Opin. Plant Biol.* 2000, **3**:336-340.

54. Card SD, Pearson MN, Clover GRG: **Plant pathogens transmitted by pollen.** *Austral. Plant Pathol.* 2007, **36**:455-461.

55. Childress AM: **Bee-mediated transmission of blueberry leaf mottle virus via infected pollen in highbush blueberry.** *Phytopathology* 1987, **77**:167-172.

56. Bristow PR, Martin RR: **Transmission and the role of honey bees in field spread of blueberry shock ilarvirus, a pollen-borne virus of highbush blueberry.** *Phytopathology* 1999, **89**:124-130.

57. Bailey L, Gibbs AJ, Woods RD: **Two viruses from adult honey bees (*Apis mellifera* Linnaeus).** *Virol.* 1963, **21**:390-395.

58. Bailey L: **The purification and properties of chronic bee-paralysis virus.** *J. Gen. Virol.* 1968, **2**:251-260.

59. Allen M, Ball BV, White RF, Antoniw JF: **The detection of acute paralysis virus in *Varroa jacobsoni* by the use of a simple indirect ELISA.** *J. Apicult. Res.* 1986, **25**:100-105.

60. Ball BV: **The association of *Varroa jacobsoni* with virus diseases of honey bees.** *Exp. Appl. Acarol.* 1983, **19**:607-613.

61. Ball BV: **Acute paralysis virus isolates from honeybee, *Apis mellifera,* colonies infested with *Varroa jacobsoni.*** *J. Apicult. Res.* 1985, **24**:115-119.

62. Ball BV: ***Varroa jacobsoni* as a virus vector.** In *Present status of varroatosis in Europe and progress in the varroa mite control.* Edited by Cavalloro R; 1989:241-244.

63. Ball BV: **Honey bee viruses: a cause for concern?** *Bee World* 1996, **77**:117-119.

64. Ball BV: **The damaging effects of *Varroa jacobsoni*.** In *Living with Varroa*. Edited by Matheson A: International Bee Research Association; 1993:9-16.

65. Ball BV, Allen ME: **The prevalence of pathogens in honey bee (*Apis mellifera*) colonies infested with the parasitic mite *Varroa jacobsoni*.** *Annals Appl. Biol.* 1988, **113**:237-244.

66. Bailey L, Ball BV: *Honey Bee Pathology*. New York, London: Academic Press; 1991.

67. Möckel N, Gisder S, Genersch E: **Horizontal transmission of deformed wing virus (DWV): Pathological consequences in adult bees (*Apis mellifera*) depend on the transmission route.** *J. Gen. Virol.* 2011, **92**:370-377.

68. Yang X, Cox-Foster D: **Effects of parasitization by *Varroa destructor* on survivorship and physiological traits of *Apis mellifera* in correlation with viral incidence and microbial challenge.** *Parasitology* 2007, **134**:405-412.

69. Gisder S, Aumeier P, Genersch E: **Deformed wing virus (DWV): viral load and replication in mites (*Varroa destructor*).** *J. Gen. Virol.* 2009, **90**:463-467.

70. Iqbal J, Mueller U: **Virus infection causes specific learning deficits in honeybee foragers.** *Proc. R. Soc. B* 2007, **274**:1517-1521.

71. Moore J, Jironkin A, Chandler D, Burroughs N, Evans DJ, Ryabov EV: **Recombinants between *Deformed wing virus* and *Varroa destructor virus-1* may prevail in *Varroa destructor*-infested honeybee colonies.** *J. Gen. Virol.* 2011, **92**:156-161.

72. 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 Path.* 2014, **10**:e1004230.

●● This paper describes the characterization of a virulent DWV variant characterized by the capsid protein coding region of VDV-1 and the coding region for the non-structural proteins from DWV. The authors demonstrate that route of transmission has serious and relevant impacts on infection and virulence. It highlights the importance of studying in detail how these viruses are being transmitted and how transmission routes impact virulence and strain identity/diversity.

73. Desai SD, Eu Y-J, Whyard S, Currie RW: **Reduction in deformed wing virus infection in larval and adult honey bees (*Apis mellifera* L.) by double-stranded RNA ingestion.** *Insect Mol. Biol.* 2012, **21**:446-455.

● This study provides further evidence that DWV infection causes pupal death and/or wing deformities. Feeding larvae with DWV-dsRNA (RNAi approach) prior to an oral infection with DWV reduced larval/pupal mortality and the frequency of wing deformities compared to infected but untreated control larvae. The study therefore not only further implicates DWV as the etiological agent of deformed wings and PMS, but also provides proof of concept for the use of RNAi technology as a research tool, and perhaps even a clinical tool in honey bees.

74. Schöning C, Gisder S, Geiselhardt S, Kretschmann I, Bienefeld K, Hilker M, Genersch E: **Evidence for damage-dependent hygienic behaviour towards *Varroa destructor* parasitised brood in the western honey bee, *Apis mellifera*.** *J. Exp. Biol.* 2012, **215**:264-271.

●● This study convincingly shows that bees engaged in hygienic behavior preferentially remove brood infested by mites carrying replicating DWV thus confirming that mites acting as biological vector of DWV are more damaging than mites just mechanically transmitting DWV. This study also points toward DWV as being the etiological agent of the purported PMS.

75. Chen YP, Higgins JA, Feldlaufer MF: **Quantitative real-time reverse transcription-PCR analysis of Deformed wing virus infection in the honeybee (*Apis mellifera* L.).** *Appl. Environ. Microbiol.* 2005, **71**:436-441.

76. Di Prisco G, Pennachio F, Caprio E, Boncristiani HF, Evans JD, Chen Y: ***Varroa destructor* is an effective vector of Israeli acute paralysis virus in the honeybee, *Apis mellifera*.** *J. Gen. Virol.* 2011, **92**:151-155.

77. Maori E, Lavi S, Mozes-Koch R, Gantman Y, Edelbaum O, Tanne E, Sela I: **Isolation and characterization of IAPV, a dicistrovirus affecting honeybees in Israel: evidence for intra- and inter-species recombination.** *J. Gen. Virol.* 2007, **88**:34-28-3438.

78. 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.

●● This study used injections of IAPV into honey bee pupae reared *ex situ* under laboratoy conditions to elucidate the interactions between IAPV and honey bees. Based on their results they suggest that there is genotypic variation in virus susceptibility and reaction towards virus infection between bees and colonies. Selective breeding for virus resistance would thus be possible.

79. Li Z, Chen Y, Zhang S, Chen S, Li W, Yan L, Shi L, Wu L, Sohr A, Su S: **Viral infection affects sucrose responsiveness and homing ability of forager honey bees, *Apis mellifera* L.** *PLoS ONE* 2013, **8**:e77354.

● This paper provides good evidence for sublethal effects of IAPV. It shows that IAPV infection impairs homing ability of forager bees which might in turn negatively affect colony performance and survival.

80. 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.

●● In this study considerable genetic variation between strains of IAPV was demonstrated and it was suggested that these differences may account for the difference in virulence properties and severity of disease manifestations among IAPV strains. Most importantly, the authors identified a putative immune-suppressive protein, VSR (viral suppressor of RNAi) encoded by IAPV and demonstrated that silencing expression of this protein could effectively inhibit replication of IAPV and confer significant antiviral activity in honey bees. This study not only

begins to hint at options for clinical control of infection, it also opens the way for basic science aimed at describing honey bee viruses in the detail that we understand human viruses.

81. Kingsolver MB, Huang Z, Hardy RW: **Insect Antiviral Innate Immunity: Pathways, Effectors, and Connections.** *J. Mol. Biol.* 2013, **425**:4921-4936.

82. 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.

83. 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 Path.* 2010, **6**:e1001160.

84. Flenniken ML, Andino R: **Non-specific dsRNA-mediated antiviral response in the honey bee.** *PLoS ONE* 2013, **8**:e77623.

●● The authors provide convincing evidence that in honey bees any or most dsRNAs might serve as a viral pathogen associated molecular pattern (PAMP). Hence, dsRNA, be it virus specific or not, might trigger an antiviral response that controls virus infection in honey bees. These results are important to consider when interpreting results obtained with virus specific RNAi approaches against viruses in honey bees.

85. Yue C, Genersch E: **RT-PCR analysis of *Deformed wing virus* in honeybees (*Apis mellifera*) and mites (*Varroa destructor*).** *J. Gen. Virol.* 2005, **86**:3419-3424.

86. 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.

87. Ongus JR, Peters D, Bonmatin J-M, Bengsch E, Vlak JM, van Oers MM: **Complete sequence of a picorna-like virus of the genus *Iflavirus* replicating in the mite *Varroa destructor*.** *J. Gen. Virol.* 2004, **85**:3747-3755.

ACADEMIC VITA

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Relevant Information:
Languages: English, German (intermediate)
Citizenship: United States

Career Objective:
To graduate from Penn State having produced honors and Master's theses and finish producing two peer-reviewed publications. The experience I gain in earning Bachelor's and Master's degrees of Science will prepare me pursue a Doctor of Philosophy degree, in a field related to immunology and infectious disease, disease dynamics, and entomology. With my education and lab experience, I intend to obtain a research position at an academic institution or a government research laboratory.

Academic Background:

2013- Present **Master of Science, Entomology**
Expected Graduation Date: Summer 2015
Adviser: Dr. Christina Grozinger
The Pennsylvania State University, University Park, PA 16802

2011- Present **Bachelor of Science, Immunology and Infectious Disease,**
Expected Graduation Date: May 2015
The Pennsylvania State University, University Park, PA 16802

Employment Experience:

May 2013 - Present **Master of Entomology Degree Student,** under Dr. Christina Grozinger

Research Area:

- Host-pathogen interaction with honey bees as a model system
    - Differences in how hive body-types are occupied by feral swarms in Kenyan populations
        - Differences in colony health in different hive types
    - Specifically, the prevalence of two viruses as a function of hive type, season and mite loads in Kenyan populations

    - The variance of one virus as a function of hive type, season, and mite loads in Kenyan populations
- Viral ecology of Deformed Wing Virus
    - The role of the common eastern bumble bee in the ecology and evolution of this honey bee virus
    - The potential for vertical transmission of this virus in bumble bees

June 2014 – August 2014 **Research Intern,** Dr. Elke Genersch and Gillian Hertlein

Research Area:

- Immune response in honey bee larvae to *Paenibacillus larvae*, the bacterial etiological agent in American Foulbrood disease.
    - Rearing and infection of larvae with spores of multiple strains.
    - Assessing the mortality of infected.
    - Confirming infection with culture and molecular techniques.
    - Assessing immune response.
- Isolating a defensin-homolog from royal jelly
    - Extracting whole protein via acid/base chemistry
    - Using SDS-PAGE to fraction the components
    - Re-naturing protein of interest on gel
    - Eluting protein from gel and testing for biological activity

May 2012 – May 2013 **Undergraduate Research Assistant,** With Dr. Fabio Manfredini

Research Area:

- Behavioral observations of agonistic and non-agonistic interactions within couples of fire ant queens during colony foundation.
- Screening of viral-loads in laboratory-infected fire ant queens in order to discern the functional effect of two RNA viruses on social rank.
- Antibacterial response (or defense).
    - In assistance to Federico Capa, a PhD student under Dr. Manfredini's guidance for the Summer of 2012:
        - Compared susceptibility to an immune challenge in different social castes among honey bees and paper wasps

Laboratory/Molecular Techniques:

- Conventional PCR
- Quantitative Real Time PCR
- DNA Extractions:
    - From insect tissues
    - Plasmid extraction using Miniprep Quiagen kit
- RNA Extractions from insect tissues:
    - Quiagen RNeasyMinikit
    - Trizol reagent RNA extraction

    - Quiazol Mini Kit
- cDNA Syntheses
- Conducted bioassays to determine worker attractiveness (retinue) to male chemical extracts.
    - Chemical extraction of pheromones from insect tissues via organic solvents, set up cages with live honey bees, performed behavioral observations.
- Bacterial injections in larvae
- Additional experience with beekeeping and establishment of wasp laboratory colonies.
- Gel Electrophoresis
    - Agarose gels
    - Polyacrylamide gels: native and denaturing
- Interpreting DNA sequence data
    - Resolving said sequences by examination of forward and reverse Sanger traces.
    - Utilizing National Center for Biotechnology Information (NCBI) databases.
- Designed and implemented assay for viral ecology
    - Included maintaining and handling small groups of bumble bees
- Reared honey bee larvae under laboratory conditions
- Bacteria culture techniques to confirm infection
- Designed and implemented protocol for protein isolation from royal jelly
    - Whole protein extraction from suspension
    - SDS-PAGE to fraction and isolate
    - Renaturing protein

August 2009-August 2011 **Worked in the Relocation Department for GlaxoSmithKline**
200 North 16th Street, Philadelphia, PA 19102

Duties included administrative work such as coordinating departmental meetings, managing the departmental filing system, maintaining correspondences with third party service companies, coordinated departmental move such that productivity suffered little to no loss, constructing a manual for use of Microsoft Office 2007,taught the members of the department new skills in said program, maintained office supply cabinet which was crucial for supplying the relocating employees with assimilation materials, processed expense reports.

Publications

McMenamin A., and Genersch E. (2015) "Honey bee (Apis mellifera) colony losses and associated viruses". *Current Opinion in Insect Science.* http://dx.doi.org/10.1016/j.cois.2015.01.015

McMenamin A., Mumoki, F., Kilonzo, J., Mweu, B., Baumgarten, T., Frazier, M., Patch, H., Torto, B., Masiga, D., Tumlinson, J., Grozinger, C., Muli, E. "The impact of season and hive type on honey bee (Apis mellifera) colony behavior and health in Kenya". (2015, in preparation)

Teaching Experience:

Fall 2014 Teaching assistant, ENT 313, **Introduction to Entomology**
Gave lectures, helped design and implement laboratory sessions, quizzes and an exam for students. Guided students in producing term papers and assisted students individually on a need-basis

Spring 2014 Teaching assistant, ENT 313, **Introduction to Entomology**
Gave lectures, helped design and implement laboratory sessions, quizzes and an exam for students. Guided students in producing term papers with presentation portion

Fall 2013 Teaching assistant apprentice, ENT 313, **Introduction to Entomology**
Gave lectures, helped design and implement laboratory sessions, quizzes and an exam for students

Service:

August 2012 - May 2013 Vice President for the Biomedical Sciences club at the Pennsylvania State University

Current and Previous Grants and Awards:

Fall 2014-Spring 2015. Impacts of cross-species transmission of bee viruses on virulence. **Barry M. Goldwater Scholarship.** $7,500.

Summer 2014. Assessing immune response to Paenibacillus larvae in honey bee larvae. PI: Dr. Elke Genersch. **International Research and Travel Grants.** $3,350

Summer 2014. Impacts of cross-species transmission of bee viruses on virulence. PI: C.M. Grozinger. **College of Agricultural Sciences Student Research Grant**. $3,000

Fall 2013. Impacts of season and management practices on pathogen load in Kenyan honey bees. PI: C.M. Grozinger. **Dutch Gold Honey Undergraduate Scholarship**. $2,000

Summer 2013. Impacts of season and management practices on pathogen load in Kenyan honey bees. PI: C.M. Grozinger. **Summer Discovery Grant**. $3,000

Outreach:

Great Insect Fair	Bee Nutrition and 'Buzzkill' game, The Pennsylvania State University
	13 September 2014
Great Insect Fair	The Pollinator Booth, The Pennsylvania State University
	5 October 2013
Queen Rearing Workshop	Center for Pollinator Research, The Pennsylvania State University
	June 2013
Arbor Day	Pollinator Booth, The Pennsylvania State University Arboretum.
	April 2013
Spring Creek Festival	Millbrook Marsh Nature Center, State College, Pennsylvania
	1 June 2013
Discover Penn State Program	The Pennsylvania State University
	March 2013
Career Exploration	The Pennsylvania State University
	3 November 2012

References:

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