ANNUAL REVIEWS

Annual Review of Entomology

Bee Viruses: Ecology, Pathogenicity, and Impacts

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Annu. Rev. Entomol. 2019. 64:205–26

The *Annual Review of Entomology* is online at ento.annualreviews.org

https://doi.org/10.1146/annurev-ento-011118-111942

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Keywords

bee, virus, parasite, immune mechanisms, bee management, invertebrate antiviral defense, RNAi

Abstract

Bees—including solitary, social, wild, and managed species—are key pollinators of flowering plant species, including nearly three-quarters of global food crops. Their ecological importance, coupled with increased annual losses of managed honey bees and declines in populations of key wild species, has focused attention on the factors that adversely affect bee health, including viral pathogens. Genomic approaches have dramatically expanded understanding of the diversity of viruses that infect bees, the complexity of their transmission routes—including intergenus transmission—and the diversity of strategies bees have evolved to combat virus infections, with RNA-mediated responses playing a prominent role. Moreover, the impacts of viruses on their hosts are exacerbated by the other major stressors bee populations face, including parasites, poor nutrition, and exposure to chemicals. Unraveling the complex relationships between viruses and their bee hosts will lead to improved understanding of viral ecology and management strategies that support better bee health.

1. INTRODUCTION

The field of bee viral ecology has expanded dramatically since the first published description of a bee-infecting virus in 1913 (32, 158). There is substantial knowledge regarding the different types of viruses that infect bees, the pathogenicity of these viruses and viral strains, transmission routes within and among bee species, the factors that influence viral population dynamics, and the circumstances that alter virulence [see Section 2 for an overview of these processes using deformed wing virus (DWV) as a model and **Figure 1**]. This increased understanding of bee host–virus interactions is in part due to the development of molecular and genomics tools that facilitate virus detection and quantification (76); standardized protocols for studying viruses in bee populations (40, 130); and tremendous interest from researchers, stakeholders, policy makers, and citizens in identifying the factors that shape pollinator health and drive pollinator declines. In spite of rapid advances, there are still knowledge gaps in bee viral ecology and challenges associated with translating basic science to applied, practical solutions in the field (19). Here, we summarize existing knowledge of this rapidly expanding field and highlight emerging areas of research.

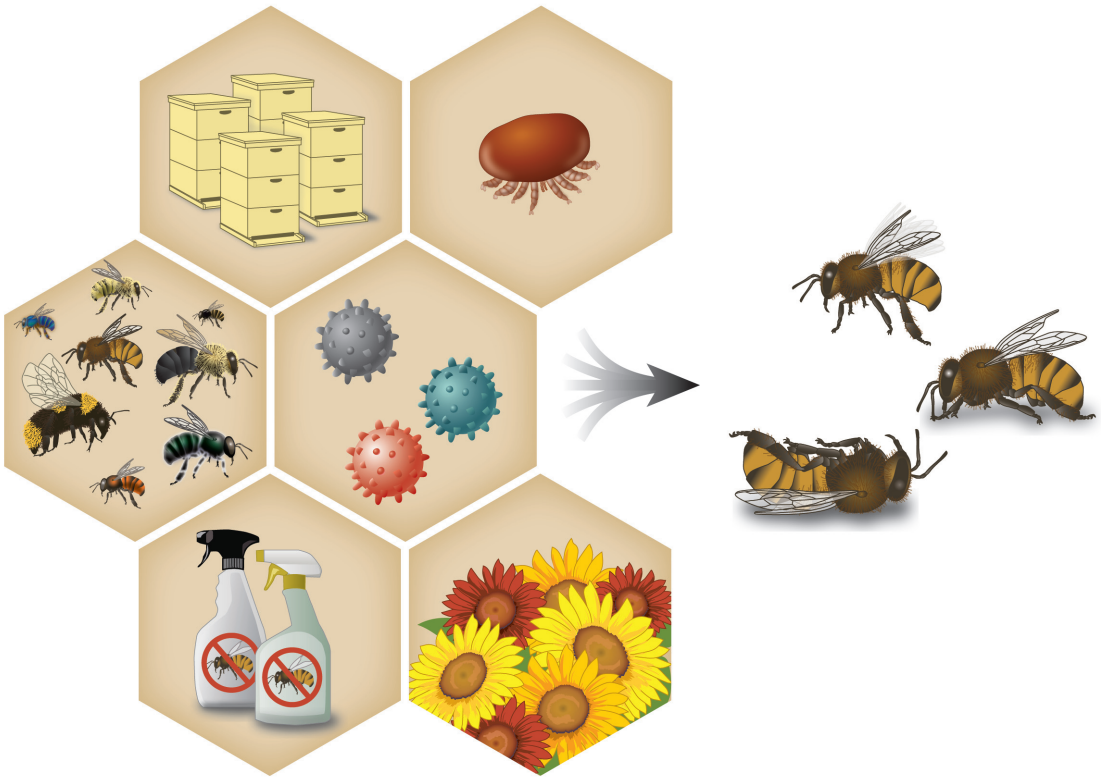

Figure 1

Multiple factors influence the transmission, infection dynamics, and pathogenicity of bee-associated viruses. Bees are host to a diversity of viral species and strains. Multiple factors determine whether virus infections in bees remain asymptomatic or result in overt symptomatic infections that cause deformity and/or paralysis or death. Exposure to viruses may be increased in areas with higher populations of bees, and many viruses are readily shared among members of a bee community that forage on common floral resources. Exposure to other parasites, pathogens, and pesticides may hinder bee antiviral defense mechanisms, while access to high-quality nutrition can improve these defenses.

2. THE COMPLEXITY OF BEE VIRUS ECOLOGY: THE CASE OF DEFORMED WING VIRUS

Deformed wing virus (DWV) is a well-studied bee virus that exemplifies the complexities of bee viral ecology (43). The western honey bee (*Apis mellifera*) is commonly infected by DWV, which may be maintained as an asymptomatic infection or result in behavioral changes (reduced activity and task performance in the colony), wing deformity, and early mortality (5, 10, 112). DWV has been detected in more than 20 bee species, and there is evidence that it replicates in several of these species and causes damaging symptoms in two bumble bee species (i.e., *Bombus terrestris* and *Bombus pascuorum*), as well as in *A. mellifera* (68, 72, 148). Below, we discuss DWV transmission, genomic variation, and the abiotic and biotic factors that influence bee host–DWV interactions and outcomes. This example illustrates the importance of integrating information from the molecular to landscape scales to achieve a comprehensive understanding of bee virus ecology.

There are several routes of DWV transmission that can influence DWV abundance and virulence. In *A. mellifera* colonies, DWV is transmitted vertically (i.e., from queen or drone to offspring) and horizontally via trophallaxis and shared food resources (30, 163). In addition, DWV can be vectored by *Varroa destructor*, an important honey bee parasite whose abundance in colonies is strongly correlated with winter losses in temperate regions (53, 71, 114, 149). DWV levels in bees parasitized by *Varroa* are significantly higher than levels of bees infected by other routes (16, 136, 162); the mechanisms underlying these increased levels are an area of active research (4, 85). *Varroa* appear to benefit from this interaction because they produce more offspring while feeding on DWV-infected pupae: High DWV titers reduce immune signaling and the melanization response in bees, and it is hypothesized DWV-infected bees are not able to heal mite feeding sites as readily, allowing the female mite and her offspring continued easy access to food (49).

Several studies indicate that *Varroa* mites can facilitate transmission of specific DWV sequence variants or strains in *A. mellifera* (also see Section 3.2). A phylogeographic analysis of DWV suggests that DWV was originally present in most *A. mellifera* populations worldwide (though not in Australia; see 132), but the spread of *Varroa* mites from their original host (*A. cerana*) in Southeast Asia to *A. mellifera* led to a change in the associated DWV populations (159). This model is supported by studies in Hawaii, where DWV viral diversity decreased in Hawaii with the introduction of *Varroa* (96, 138).

In controlled laboratory experiments with *A. mellifera*, when DWV was introduced by injection or *Varroa* feeding versus oral infection, specific variants reached higher levels [i.e., DWV-A/VDV-1 (DWV-B) recombinants] (107, 136). These results are intriguing, and future studies are required to determine whether these variants exhibit greater overall fitness, or whether they experience greater fitness in the context of this specific transmission route (i.e., direct injection into hemolymph), or whether amplification of particular variants after *Varroa*-transmission events are simply stochastic events. The duration of selection pressure by *Varroa* may also influence the population structure of DWV. After introduction of *Varroa* mites into New Zealand, DWV virus abundances were not positively correlated with *Varroa* infestation levels at the colony level but were positively associated with the time since *Varroa* introduction (106). Similarly, DWV levels were low in *Varroa*-parasitized bees sampled from an isolated population located on an island off the coast of Brazil, which the authors suggest is due to the lack of a virulent DWV strain in this population (17). Therefore, it is possible that continuous selection for specific virus strains in the presence of *Varroa* may result in amplification of virulent viral strains that may also be transmitted by other routes of infection.

DWV abundance in *A. mellifera* can be influenced by a number of biotic and abiotic factors. Exposure to neonicotinoid pesticides can compromise activation of immune pathways and result

in increased DWV load (50) (see discussion in Section 7). Coinfections with other pathogens or parasites can either enhance or inhibit DWV infections (57, 150). Consumption of a poor-quality diet (e.g., sugar water) can result in greater DWV levels compared to bees fed higher-quality, pollen-containing diets (47, 150). Similarly, *Varroa*-exposed pupae fed pollen after emergence exhibited increased survival compared to *Varroa*-exposed pupae fed sugar water after emergence (6). Moreover, the apolar fractions of pollen extracts seemed to provide greater benefit for bees than the polar fractions, which suggests that the lipids in pollen are an important macronutrient influencing immunity (6). Recent studies suggested that at least some bee species selectively forage on pollen to achieve a preferred protein:lipid ratio in their diets (152–154). However, the relationship between DWV infection and nutrition requires further investigation because other studies demonstrated that DWV abundance was greater in pollen-fed bees compared to bees fed sucrose-only diets, which could reflect reduced immunocompetence or increased tolerance (2).

Outside of the colony, DWV can be transmitted via coforaging and exposure to DWV deposited on flowers by infected bees, possibly via contact with virus-contaminated bee feces, floral nectaries, or bee-carried pollen (84, 97, 144). High *A. mellifera* colony densities (64) and *Varroa* infestations in honey bee colonies (138) can lead to increased prevalence of DWV in surrounding bee populations. Therefore, beekeeper management practices also influence viral transmission. However, it is important to note that viral transmission between bees is bidirectional, and thus increasing levels of viruses in wild bees may contribute to increasing levels in *A. mellifera* (99, 101, 144).

In summary, as one of the most well-studied bee viruses, DWV illustrates the complex web of interactions, from the molecular to ecological level, that influences the spread of DWV among bee populations and the degree of its impacts on individual hosts. Below we discuss bee host–virus interactions at these different levels of biological organization in more detail.

3. DIVERSITY OF BEE VIRUSES

3.1. Classes of Bee Viruses

Over the last decade, our understanding of the diversity of viruses infecting bee species has grown dramatically, owing to rapid improvements in and increasing accessibility of next-generation sequencing approaches. In the past, isolating, identifying, and evaluating the prevalence and distribution of new viruses was a laborious process, involving electron microscopy and antibody-mediated detection (7, 8, 32). Next-generation sequencing techniques have facilitated high-throughput sequencing of transcriptomes, metagenomes, and viromes (isolates of encapsulated viruses) from relatively small amounts of material (76). These methods coupled with the increasing amount of genomic data in publicly accessible databases [e.g., NCBI nr (National Center for Biotechnology Information non-redundant database)] have enhanced analysis of omics studies and facilitated the discovery of new virus genomes. Moreover, bioinformatics approaches that facilitate identification of viral sequences based on their sequence characteristics have been developed; indeed, employing these tools allowed the identification of 1,445 RNA virus sequences from over 220 invertebrate species (143). Thus, it is now possible to screen for viruses in samples obtained from multiple species of bees—and their parasites—from across the world efficiently and rapidly.

Several new viruses and viral families have been identified in populations of bees and their parasites using next-generation sequencing approaches, and undoubtedly these numbers will continue to increase. These viruses and viral families were reviewed in 2018 (101), and thus we briefly summarize them here, with an aim to demonstrate the diversity of virus genome types that have been identified thus far (see **Supplemental Table 1** for a listing of bee-associated viruses identified before early 2018).

Supplemental Material >

The majority of characterized bee viruses have positive-sense single-stranded RNA genomes [(+)ssRNA], and many are in the order Picornavirales (23, 32). These include the common bee viruses in the family *Dicistroviridae* [Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV), acute bee paralysis virus (ABPV), and black queen cell virus (BQCV)] or in the *Iflaviridae* family [DWV, Kakugo virus, Varroa destructor virus-1/DWV-B, sacbrood virus (SBV), and slow bee paralysis virus]. Additional well-characterized (+)ssRNA viruses include the Lake Sinai viruses, which are in the *Sinaivirus* genus (38, 101).

From 2015 to 2018, sequencing studies identified sequences corresponding to (+)ssRNA viruses in several other families and genera (see **Supplemental Table 1**). These include the *Tymoviridae* (42, 68, 141), *Secoviridae* (68, 141), *Nodaviridae* (68), and *Flaviviridae* (131) families; the *Sobemovirus* and *Negevirus* genera (141); the new genus *Halictivirus* (12); and a Nora-like virus (131).

Supplemental Material >

Viruses in the *Tymoviridae* and *Secoviridae* families are typically plant-associated viruses. While it is possible that the detection of these viruses is simply due to the presence of the virus in pollen samples and not active infection of the bees, another virus in the family *Secoviridae*, Tobacco ringspot virus (TRSV), infects and replicates in *A. mellifera* (91). Thus, a fruitful area of future research would be to determine the extent to which plants and bees share similar viruses, serve as hosts to the same viruses, and/or serve as vectors of plant viruses (see, for example, 18, 33).

Sequences corresponding to negative-sense single-stranded viruses [(−)ssRNA] in the family *Bunyaviridae* (131, 140, 141), the order Mononegavirales (140, 141), and the family *Orthomyxoviridae* (141) have been detected in bees. Additionally, viruses from the family *Rhabdoviridae*, in the order Mononegavirales, were identified (88, 131, 141).

Likewise, contigs with similarity to double-stranded RNA (dsRNA) viruses in the families *Partitiviridae* (68) and *Totiviridae* (141) were identified in 2018. While *Partitiviridae* viruses have been identified primarily in plants and fungi (116), a metagenomics analysis of viruses in more than 220 invertebrate species identified many partitiviruses (143).

Finally, two double-stranded DNA (dsDNA) viruses have been described, Apis mellifera filamentous virus (70, 77) and Osmida cornuta nudivirus (OcNV) (141). Two different viral sequences corresponding to single-stranded DNA (ssDNA) viruses from the *Circoviridae* family have been identified (68), as well as viral sequences corresponding to the *Parvoviridae* family (141).

Thus, next-generation sequencing approaches have dramatically enhanced identification of viruses associated with bees and their parasites and expanded the types of viruses detected in bee populations. Identification of new virus genomes necessitates future studies aimed at further characterizing these viruses, including determining their impacts on bee hosts [including potential positive impacts (133)] and their transmission routes, among different bee species, parasites, and plant species.

3.2. Viral Variants, Quasispecies, and Strains

With their large population sizes, fast replication rates, high mutation rates (some RNA viruses have mutation rates more than one million–fold higher than their host), and ability to recombine, viruses can rapidly accumulate sequence variation (3, 87). Indeed, RNA virus populations are often referred to as quasispecies (or mutant swarms), where a single host contains a diversity of viral sequence variants. These sequence variants exhibit differing degrees of fitness and also may complement or interfere with one another. Select variants remain robust across hosts; these are termed master sequences or consensus sequences and are typically considered to represent distinct viral strains. A quasispecies within a host contains a so-called cloud of mutants that diverge from this master sequence.

Viral variants have been studied most extensively for DWV, although they have been described in other common bee viruses as well (31, 41). For DWV, sequence variation has been used to trace its spread across continents (see Section 2 and Reference 159). Furthermore, it is possible to identify variants shared by wild bees and managed honey bees that distinguish viral populations associated with different apiaries at a local (90, 144) or national (67) scale. While these studies evaluated variation in key viral genes [e.g., *RNA dependent RNA polymerase* (*RdRp*)], whole-genome comparisons indicate that sequence variations occur throughout the genome (35, 37, 101).

Master sequences or strains have been identified for DWV: DWV-A, DWV-B, and DWV-C (83). Thus far, DWV-C has been identified only in samples collected in 2006–2007 from six colonies (83, 108), and thus it is unclear how widespread this variant might be; indeed, analysis of 168 publicly accessible RNA sequencing data sets on *A. mellifera* did not detect definitive DWV-C sequences (34). DWV-B was originally referred to as VDV-1, but subsequent analysis indicated that it is a variant of DWV, sharing 84% sequence identity to DWV-A (124). DWV-B was originally detected in European *A. mellifera* populations (124) but has recently been identified in *A. mellifera* populations in the United States (134) and Africa (103, 123). Additionally, controlled laboratory studies indicate that direct injection of DWV into pupal hemolymph, by either *Varroa* mite vectors or experimental introduction, can result in recombinant DWV-A/B viruses that can reach high levels (100, 136).

The population dynamics, prevalence, and virulence of DWV variants in wild bee and managed *A. mellifera* populations are an area of active investigation. These processes are undoubtedly strongly influenced by the mode of transmission. Although DWV-A/B recombinants are readily found in DWV-injected or *Varroa*-infested bees in controlled laboratory studies (100, 107, 136), a study of DWV-infected colonies in Germany found no evidence of recombinants (111). Similarly, recombinants were identified in only 21 of 168 RNA-sequencing data sets containing DWV sequences (34). It may be that under controlled laboratory settings or with a given mode of transmission (such as introduction through injection or feeding *Varroa*), certain variants have a selective advantage and can replicate to high levels, but under other conditions (colonies in the field) or routes of transmission (oral, mating, or vertical), these variants do not have a selective advantage and thus do not reach high levels in the field and cannot persist.

DWV-B may be increasing in prevalence in managed *A. mellifera* bee populations. In the United States, DWV-B prevalence increased dramatically from 2 infected apiaries of 75 tested in 2010 to 161 infected apiaries of 603 tested in 2016 (134). In 23 colonies in Germany, Natsopoulou et al. (111) found high levels of DWV-B but low levels of DWV-A in 2016, suggesting that DWV-B is replacing DWV-A in that area. However, analysis of DWV sequences from 168 transcriptome studies deposited in public databases found that DWV-A was more prevalent than DWV-B, and DWV-B was more abundant than DWV-A in only 2 cases where DWV-A/B recombinants were found (34). In studies with European *A. mellifera*, DWV-B infections were associated with higher mortality in cages (100) and colonies (111) than DWV-A infections. Further analysis is needed to evaluate the dynamics of DWV-A and DWV-B in bee populations, their relative pathogenicity, and the factors (sequence variation, replication rates, and/or transmission dynamics) that may underpin these processes.

Fully exploring the associations between virus strains, recombinant viruses, virus infection levels, *Varroa* infestation, and bee health at both the individual and colony levels will require next-generation sequencing of viromes at a much larger scale (across more colonies, genotypes of bees, ecological regions, and levels of *Varroa* infestation). Indeed, it is quite clear that multiple factors can influence virus abundance and pathogenesis, regardless of the viral strain (see Section 2).

4. TRANSMISSION OF VIRUSES IN BEE COMMUNITIES

Bee viruses are readily transmitted within and between host species (148). Thus, while most bee viruses were originally identified and studied in *A. mellifera* and other *Apis* species (1, 165), they have been detected in a range of other bee species [spanning the families Andrenidae, Halictidae, Apidae, and Megachilidae (148)] as well as in other insects [cockroaches, small hive beetles, ants, and wax moths (90)]. In addition, many bee-associated viruses are found and can replicate in *Varroa destructor* mites (51, 73). Next-generation sequencing approaches have been used to evaluate the viriomes of non-*Apis* species (68, 140, 141), including *Varroa* mites (89). These studies have identified new viruses—many of which are also present in *A. mellifera* (see **Supplemental Table 1**).

Supplemental Material >

There are many routes of bee virus transmission within and among bee species in a community. Infected bees can deposit viruses on flowers as they forage, and these viruses can then infect other bees that visit these flowers (reviewed in 84, 98, 148). Similarly, other studies suggest that *Varroa*-infested honey bee foragers can also deposit *Varroa* on flowers and thus transmit this parasite (and the viruses it carries) to foragers from other colonies (127). Likewise, bumble bee–associated mites can be transferred to new bumble bee hosts via flowers (142), although whether these mites transmit viruses remains to be determined. Viruses can also be transmitted when a bee or infected nest parasite enters another nest or colony. Bees can enter other colonies by accidental drifting (where they return to the wrong colony), during robbing (where they actively seek out and invade weak colonies to obtain food resources), or when they enter non-natal colonies as social parasites to gain reproductive success (13, 45, 63, 66).

How this broad cross-species transmission and infectivity influences bee-associated viral community dynamics is an active area of investigation. When sequences from DWV-infected managed *A. mellifera* and wild bees are compared, the sequence variation is associated with geographic location rather than host species (67, 90, 144), suggesting that these variants are freely shared among coforaging bees and that they do not appear to be host-species-specific variants. However, despite the fact that viruses can be transmitted bidirectionally between species, managed *A. mellifera* bees likely serve as an important source for viruses within a bee community, due to their large population sizes/densities and the role of *Varroa* mites in amplifying viral populations. Indeed, increasing *A. mellifera* colony density is associated with increased *Varroa* levels in *A. mellifera* colonies (119) and increased virus prevalence in the associated wild bee community (64), which is consistent with the fact that DWV prevalence and abundance are correlated with *Varroa* populations (138). However, transmission dynamics will be greatly influenced by floral community traits and plant–pollinator networks (reviewed in 98). Finally, there is almost certainly a seasonal influence on viral prevalence and transmission. In *A. mellifera* colonies in temperate regions, *Varroa* populations and associated viral populations rise in the fall (74, 92, 149), and thus fall-foraging bee species (such as bumble bee queens preparing for overwintering) near apiaries are likely exposed to higher levels of viruses than spring-foraging species (such as *Osmia* bees).

Similarly, how this broad cross-species transmission and infectivity influences the health of bee populations remains to be fully examined. As discussed in Section 5, the transmission routes, transmission quantities, and physiological state of the bee greatly influence the ability of a virus to establish an acute or chronic infection and the overall impact of the virus on its host. For example, feeding low quantities of virus to adult bees typically does not establish chronic infections that lead to clear negative effects on bees (104, 156). Thus, pollen foragers themselves (including fall-foraging bumble bee queens) may not become infected by virus-contaminated pollen they collect. However, bees collect pollen to feed to developing larvae, which are likely more susceptible to infection. Examining the impact of feeding virus-infected pollen collected from the field on developing brood is a fruitful area of future study.

Thus, while it is clear that multiple viruses can be easily shared by multiple hosts within a bee community, numerous questions remain. These include understanding what properties allow viruses to infect different host species, whether there is selection for specific strains to infect specific hosts, what the main routes of transmission are in the field, and what impact these viruses have on bee populations and communities, which may be differentially susceptible to virus infection. Fully understanding viral-host dynamics within these communities can improve our ability to manage these viral infections. For example, while it may be possible to reduce viral prevalence in the landscape by managing *Varroa* populations in *A. mellifera* colonies (92) other studies have demonstrated that even with *Varroa* control, DWV reaches high prevalence in *A. mellifera* colonies by the end of the summer in temperate regions (95, 113). Moreover, the transmission dynamics of many viruses, including LSV2, are not associated with *Varroa* mite infestation levels (74, 88, 89, 149). Additional management strategies at the landscape scale—such as adjusting the plant community to make the plant–pollinator network more modular—may more effectively limit virus transmission (65).

5. PATHOLOGICAL IMPACTS OF BEE VIRUSES ON THEIR HOSTS

The effects of viruses on bee hosts vary with each specific virus–host pair and range from asymptomatic or covert infections to symptomatic infections that cause deformity and/or paralysis or death. The impact of a particular virus, or virus strain, on the host varies with dose, as well as the host's genotype, developmental stage, and physiological state. Additional factors, including other pathogens, nutritional status, microbiome, and exposure to environmental chemicals, also influence pathological impacts of viral infection (for examples and reviews, see Sections 2 and 7 and References 22, 46, 59, 102, 115). To date, the majority of studies have focused on *A. mellifera* host–virus interactions, although knowledge of the impacts of bee viruses on other bee species is growing (101).

The effects of virus infection on individual *A. mellifera* bees were originally investigated by Bailey, Ball, and others, who defined symptoms associated with several common honey bee viruses, including a so-called hairless or greasy phenotype associated with chronic bee paralysis virus; wing deformity caused by DWV; paralysis associated with IAPV, ABPV, and KBV; and complications during larval development associated with SBV and BQCV (reviewed in 32). To date, the majority of studies have been performed using viruses isolated from naturally infected bees and/or pupae or viruses amplified in laboratory-reared pupae (54, 100). While these studies have provided valuable information on how bees respond to viruses at the molecular and physiological level (see Section 6), propagation of virus isolates in cultured cells and the development of infectious molecular clones will greatly improve our ability to examine specific virus–host interactions (27, 75, 86), including characterization of variant-specific differences in pathogenicity (see Section 3).

In non–*A. mellifera* bees, viral infections have been associated with deformed wings (72) and reduced reproductive output (104). However, feeding viruses to caged *A. mellifera*, *Megachile rotundata*, and *Colletes inaequalis* resulted in high mortality in *A. mellifera* but not in the other two species, which may indicate differential susceptibility or pathogenicity in different bee hosts (54). Alternatively, this may have been due to different ages of the bees infected (see 24), or it may indicate that the viruses are more adapted to the honey bee hosts from which they were obtained. Additionally, the mode of viral introduction (oral infection versus injection) and quantities introduced can greatly influence infection dynamics and pathogenicity (104, 156).

For social bees such as *A. mellifera*, where there are hundreds or thousands of individuals in a colony, the social structure can mitigate the impacts of different stressors, including viral infections. However, this social structure can also be undermined if key individuals or sufficient numbers of

individuals are impacted, even if the effects on each individual are quite subtle (reviewed in 9). For example, one of the best-characterized stress-induced behavioral changes affecting honey bee colony health is accelerated behavioral maturation of workers, such that they transition from the nursing (brood care) to the foraging behavioral state more rapidly and become what are referred to as precocious foragers (9). This accelerated maturation could benefit the colony by removing infected individuals from the brood nest (and thereby potentially reducing pathogen transmission) and could also benefit the pathogen by enhancing horizontal transmission to other colonies or bee species. However, precocious foragers also are not as effective as typical foragers and have shorter life spans (128). Indeed, accelerated behavioral maturation, reduced foraging activity, and reduced longevity have been demonstrated for adult worker honey bees experimentally infected with DWV (10, 112). Moreover, worker bee pupae that were experimentally subjected to increased *Varroa* parasitization—and thus expected to have higher DWV levels as adults—exhibited reduced activity and increased mortality and never became foragers (5).

Erratum >

If the foraging force in an *A. mellifera* colony is reduced by attrition, this will, in turn, stimulate younger bees to initiate foraging precociously to ensure a colony has adequate nutritional resources (128). This increased mortality and rapid turnover of the adult bee populations can destabilize the colony demographics (for example, there may not be a sufficient number of adult bees to rear enough brood to replace them) and lead to colony collapse (9). Indeed, at the colony level, several studies have correlated the presence and/or abundance of pathogens, including (+)ssRNA viruses, with reduced colony population size, colony collapse disorder, and colony deaths (31, 151). Modeling the impacts of viruses and other stressors on colony population and demographics, as well as experimental validation of those models, will be an important aspect of understanding the relative impacts and effects of these stressors on colony survival of *A. mellifera* and other social bee species.

6. MOLECULAR RESPONSES OF BEES TO VIRAL INFECTIONS

The pathological impacts of viruses on bees are governed by the intricate balance between host-defense and virus-counterdefense mechanisms, which coevolve and occur at the cellular and molecular levels. Bees use a suite of antiviral defense mechanisms, including autophagy, apoptosis, eicosanoid biosynthesis, endocytosis, melanization, the JAK/STAT (Janus kinase/signal transducer and activator of transcription), Toll, NFκB (nuclear factor κB), JNK (c-Jun N-terminal kinase), and MAPK (mitogen-activated protein kinase) pathways, and RNA interference (RNAi) (see 20 for a detailed review). Below, we focus on RNA-mediated antiviral responses and the role of metabolism in modulating virus–bee interactions, discuss transcriptomic responses to viruses and identification of potential biomarkers of pathogenic viral infections, and highlight emerging areas of investigation.

6.1. Viral dsRNA-Triggered Antiviral Responses in Bees, Including Sequence-Specific RNAi and Non-Sequence-Specific Pathways

Most replicating viruses produce long dsRNA molecules (e.g., the replicative intermediate forms of both positive- and negative-sense ssRNA viruses, polycistronic mRNAs, and genomic RNAs with secondary structure). These long dsRNAs are recognized as non-self, virus-associated molecular patterns (VAMPS) by host proteins involved in antiviral defense, including pathogen recognition receptors. The primary dsRNA-triggered insect antiviral defense mechanism is the RNAi small interfering RNA (siRNA) pathway (for detailed reviews, see 22, 52, 117). In brief, this pathway is triggered by cytosolic, virally produced dsRNA, which is the substrate of Dicer, an

endonuclease that cleaves long dsRNAs into 21–23–base pair siRNAs that are subsequently incorporated into the multi-protein RNA-induced silencing complex (RISC). The RISC uses Argonaute-bound siRNAs to target complementary RNAs, including viral RNAs, for cleavage, thus limiting virus replication.

There is substantial evidence that bees use the siRNA pathway to combat viral infections. Multiple laboratory-based studies have shown that administration of virus-sequence-specific dsRNAs or siRNAs reduces viral load in *A. mellifera* (31, 48, 94) and *Bombus terrestris* (26). At the colony level, deep sequence analysis of *A. mellifera* samples obtained from collapsing colonies identified an abundance of virus-specific siRNAs (29). In addition, transcriptome analyses indicate RNAi is used in antiviral defense because transcript levels of key RNAi enzymes (i.e., Dicer and Argonaute-2) are increased in IAPV-infected honey bees (69), honey bees infected with a model virus (i.e., Sindbis-GFP) (21), and IAPV-infected bumble bees (157), though not in DWV-infected honey bees (136). In contrast, transcriptional regulation of the RNAi machinery has not been observed in virus-infected *Drosophila melanogaster* (55, 166).

Since treatment with IAPV-specific dsRNA reduced levels of IAPV in caged *A. mellifera* bees, it was hypothesized that this method could be used to reduce viral infections in colonies in the field (94). Initial field studies demonstrated that feeding *A. mellifera* colonies IAPV-specific dsRNA resulted in increased honey production and larger colony size, although posttreatment viral burden was not assessed (79). While therapeutic dsRNA is potentially a promising avenue to explore, commercial development of dsRNA and siRNAs as antiviral treatments requires evaluating strategies for delivery and treatment efficacy and safety, as well as the possibility of unintentional biological consequences (25, 28, 81, 105). Furthermore, additional studies have found global transcriptional changes in response to dsRNA exposure, which have unknown consequences for bee health (21, 62).

Intriguingly, antiviral immune responses in bees also include non-sequence-specific dsRNA-triggered mechanisms that reduce virus abundance in *A. mellifera* and *B. terrestris* (21, 62, 118, 129). Such a general antiviral response to dsRNA may be an evolutionary adaptation to limit virus transmission within bee colonies composed of tens to thousands of bees living in extremely close proximity, since it has not been observed in solitary insects, including *D. melanogaster* and mosquitoes (137).

Dicer likely plays a role in both sequence- and non-sequence-specific dsRNA-triggered antiviral immunity, as it is a member of a larger family of dsRNA-sensing proteins (i.e., DExD/H-box helicase), including those involved in triggering the primarily general dsRNA-triggered mammalian antiviral response (i.e., the interferon response) (44). Further investigation of dsRNA-mediated antiviral responses in bees may reveal additional evolutionarily conserved mechanisms in other organisms and may help develop safe and effective strategies for using therapeutic dsRNA treatment in the field.

6.2. Role of Metabolic Pathways in Mediating Bee–Virus Interactions

The relationship between cell metabolism, nutritional status, and antiviral defense in bees requires further exploration. Bees consuming higher-protein diets have lower viral loads (47). Moreover, several transcriptome studies identified differential expression of genes involved in metabolic processes in virus-infected honey bees (21, 31, 164). Additionally, studies have indicated that ATP-sensitive inwardly rectifying potassium (K_{ATP}) channels play a role in limiting viral infection: These channels respond to metabolic changes in the cell (e.g., the relative levels of ATP and ADP) and may provide a clear mechanistic link between cell metabolism and antiviral defense in honey bees (122). These changes in metabolic function may be either the result of the hosts' antiviral

response or an energetic consequence of virus infections, which routinely include over one billion virus genome equivalents per individual bee—even in asymptomatic bees.

6.3. Transcriptional-Level Biomarkers of Bee Health

The relative role of bee immune pathways in the context of specific viral infections has been investigated primarily at the transcriptional level (21, 31, 49, 58, 62, 69, 113, 135, 164). Remarkably—and despite the fact that experiments were performed at both the individual bee and colony levels and varied in the purity and strain of virus inoculum, the route of virus infection, tissue(s) examined, postinfection assay time, and bee developmental stage—genes in the Toll, Imd, JAK/STAT, JNK, heat shock response, and RNAi pathways are consistently induced. For example, several studies have observed increased expression of antimicrobial peptides (AMPs), including hymenoptaecin and apidaecin, which are indicative of Toll and Imd pathway activation (21, 58, 69, 135, 136). Although the function of AMPs, which are best characterized for their ability to interact with and disrupt bacterial membranes, in virus-infected bees remains unknown, they may also interact with enveloped viruses and/or play immunomodulatory roles. While much remains unknown regarding the role of well-recognized proteins in bee antiviral defense, there are also numerous, uncharacterized genes that are differentially expressed in virus-infected bees that may be important in currently unrecognized immune mechanisms that may be conserved in other species.

Identifying transcriptional changes (biomarkers) that are consistently associated with damaging levels of viruses in colonies may help monitor the impacts of viruses in the field and identify colonies or populations of bees that are at risk (164). These transcriptional changes can include antiviral responses or downstream physiological or behavioral changes. Candidate biomarkers of bee health may include genes such as *PGRP-2*, *hymenoptaecin*, and *cytochrome P450*s (e.g., 314A1, GB45725; 6AS10, GB48738; 6AS10, GB40285), because several studies have documented greater expression in response to viruses and/or *Varroa* mites (21, 31, 58, 69, 164). Furthermore, five independent honey bee virus–infection studies detected differential expression of the gene *lethal(2)essential for life-like*, which encodes a small heat shock protein (21). Functions of this protein in *Drosophila* include insulin signaling, life span regulation (61), and muscle development and performance (160). These putative functions of *lethal(2) essential for life-like* in metabolism and muscle performance are intriguing in the context of a recent study demonstrating cardiac function in *A. mellifera* is related to virus levels (121).

Additionally, studies examining transcriptional responses of *A. mellifera* collected from colonies have shown a negative correlation between levels of *vitellogenin* and *Varroa* mite infestation levels, which are positively correlated with DWV loads and colony mortality (36, 145, 164). Vitellogenin serves many functions in *A. mellifera* honey bee workers, including roles in nutrition, immunity, and longevity (for a review, see 125). Levels of *vitellogenin* are strongly associated with behavioral maturation, and reduction in *vitellogenin* levels leads to precocious foraging. Thus, reduced *vitellogenin* levels may be indicative of physiological stress and accelerated behavioral maturation. If enough workers are affected by virus infection to the point where their behavioral maturation is accelerated, this can destabilize the colony demography and lead to colony collapse (see Section 5).

Importantly, understanding the biological role(s) of genes that are potential biomarkers of bee health is required to interpret the meaning of variation in relative expression levels. High expression levels of particular genes could be indicative of colonies or populations that have high, damaging viral infection levels and are at risk, and thus should be treated to reduce viral titers (controlling *Varroa* mite levels, for example) or to mitigate the impacts of viruses (such as nutritional supplementation). Alternatively, higher expression levels of key antiviral genes may characterize a colony or population that has high immunocompetence and can effectively combat a

viral infection and thus may be a desirable trait that could be incorporated as a selection criterion in bee breeding programs. Moreover, depending on the biological role of the gene, it may be preferable to monitor or select for reduced or intermediate expression.

6.4. Beyond Transcriptomics

Knowledge of the molecular mechanisms mediating bee–virus interactions has been derived primarily from transcriptome analyses and thus is largely correlational. To fully understand these mechanisms, functional analyses are required, including RNAi-mediated knock-down of specific transcripts, CRISPR (clustered regularly interspaced short palindromic repeats)-mediated mutations of key genes, and chemical inhibition of target processes. For example, using an RNAi approach to reduce the expression of *dorsal-1A/NFkB* in honey bees demonstrated this signaling pathway was responsible for inducing the expression of *Amel\102*, an immune gene that is likely central to melanization and encapsulation that exhibits reduced expression in the context of DWV infection (50). An RNAi approach was also utilized to demonstrate that the antiviral genes *dicer* and *MF116383*, which encodes a putative serine/threonine cyclin-dependent kinase, are involved in reducing levels of a model virus (Sindbis-GFP) (21). Similarly, chemical manipulation demonstrated that a K_{ATP} channel serves to reduce levels of viruses (122).

In addition, alternative splicing, epigenetic regulation, and transgenerational immune priming are areas of research that are underexplored in the context of virus infection in bees (39, 62, 69, 78). Future experiments examining these processes both within individual bee species and across species will enhance our understanding of the impacts of viruses on bees and host responses to viruses, as well as elucidate coevolutionary adaptations.

7. ENVIRONMENTAL CHEMICALS AFFECTING HOST–VIRUS INTERACTIONS

The outcome of bee virus infections is influenced by abiotic factors, including environmental chemicals. Bees are exposed to chemicals through their diets, as both nectar and pollen contain a diverse array of micronutrients and secondary plant compounds (154). As key pollinators of agricultural crops, bees are exposed to agrochemicals by direct contact with chemicals in the air or on the surface of a flower or indirectly via consumption of contaminated floral resources (146) and by contact with contaminated wax within the hive (110). In addition, managed *A. mellifera* are exposed to in-hive chemicals that beekeepers use to control parasites, including *Varroa* mites (110, 120). Here, we discuss studies examining the impacts of these different types of chemicals on bee–virus interactions.

7.1. Phytochemicals

Phytochemicals, which are present in nectar and pollen, can affect bee health either positively or negatively (80). Recent research examining the potential beneficial impact of phytochemicals, as immune stimulants that contribute to reducing pathogen burden, determined that one of the seven tested (i.e., 0.16 ppm thymol) reduced the natural levels of DWV infection when newly emerged bees were fed and returned to the colony for seven days (126). However, similar results were not obtained in cage studies nor with other pathogens (126). Phytochemical exposure also correlated to increased antimicrobial peptide (AMP) production (93, 126), which is an indication of activation of immune signaling. However, although several studies have determined that AMPs may exhibit increased expression in the context of virus infection, their role in antiviral defense is unknown (see Section 6).

7.2. Agrochemicals

Agrochemicals include grower-applied pesticides, herbicides, fungicides, and adjuvants (82). The chemicals of greatest concern to honey bee health are insecticides, which are designed to interfere with specific processes and pathways in insects (e.g., developmental or neurological systems) (82). However, other agrochemicals may have negative impacts. For example, inert ingredients used in formulations to enhance the effectiveness of the active ingredient have negative effects on bees (109) that include increasing viral-induced mortality (60). Herbicides—which are designed to inhibit plant growth—limit the availability of floral resources and adversely affect bee nutritional status (14), thus indirectly influencing the outcome of bee virus infections (46). The degree to which different chemicals impact bee health varies depending on the particular bee species and their exposure level, which can range from significant to undetectable depending on the geographic region and crop system (reviewed in 102).

There are few studies that directly examine the influence of chemicals on virus infections in bees (50, 60, 122). These studies have found that exposure to diverse chemical classes—neonicotinoids, organosilicones, and K_{ATP} channel agonists—can increase viral levels (50, 60, 122), but the underlying mechanisms are largely uncharacterized. Understanding these mechanisms may provide greater insight into bee antiviral immune responses and improve our ability to manage agrochemical use in the field to minimize off-target impacts on bees and other insect populations while maintaining their effectiveness in managing pests (11).

One potential mechanism by which insecticide exposure can increase viral titers was identified for neonicotinoids, which have been extensively studied in the context of bee health (161). Bees naturally infected with DWV and orally and topically exposed to neonicotinoids (i.e., clothianidin or imidacloprid) exhibited a dose-dependent increase of DWV (50). Similarly, sublethal, though not necessarily field-relevant, doses of thiacloprid increased BQCV levels and larval mortality (56). In contrast, treatment with the organophosphate chlorpyrifos did not alter DWV abundance in honey bees (50). Further investigation determined that the Toll immune pathway was inhibited by neonicotinoid exposure (50). Specifically, neonicotinoid treatment caused increased expression of a dorsal/NFκB inhibitor (i.e., *Amel\LRR*) (50). This key immune pathway can also be impaired by other factors (including *Varroa* mites; see Section 2).

7.3. Beekeeper-Applied Pesticides

Beekeepers routinely use acaricides to control *V. destructor* mite populations in managed *A. mellifera* colonies. Since uncontrolled mite infestations are primary factors associated with colony deaths in temperate regions (53, 71, 114, 149), acaricides (e.g., tau-fluvalinate, thymol, coumaphos, formic acid, and amitraz) are frequently used. Indeed, a 2010 survey of ~900 North American honey bee colonies found acaricides in the wax of 98% of the colonies (110).

Acaricide exposure has been shown to impact the expression of *A. mellifera* immune genes, though not in a uniform manner. For example, in one study, thymol or coumaphos treatment resulted in reduced expression of two immune genes (i.e., *DSC37* and *BASK*) (15), whereas in another study, genome-wide expression changes associated with coumaphos or tau-fluvalinate exposure showed increased immune gene expression (139). However, as noted in Section 6.3, differential expression of immune genes could be indicative of either immunocompetence or infection levels.

Few studies have examined viral levels or effects of viral infections in bees treated with acaricides. In the case of Boncristiani et al. (15), while acaricide treatment of colonies resulted in reduced immune gene expression, there was no significant impact on viral titers (note that these

colonies were in a region that had no *Varroa* mites). However, experiments have demonstrated that virus-infected bees treated with an acaricide [amitraz or its primary metabolite *N*-(2,4-dimethylphenyl)-*N*-methylformamidine] had higher mortality than untreated virus-infected bees, suggesting interactions between viruses and acaricides (121).

However, the ability of acaricides to reduce *Varroa* populations in bee colonies—and thereby significantly reduce viral loads—suggests that, overall, beekeepers benefit from acaricide treatment. Monitoring of acaricide-treated and untreated honey bee colonies over the course of a typical beekeeping season in Sweden (March–October) determined that early (i.e., at least six weeks prior to the end of the brood rearing period) treatment with the pyrethroid tau-fluvalinate (i.e., Apistan strips) reduced *Varroa* mite infestation, which in turn resulted in a 1,000-fold reduction in DWV abundance (92). Furthermore, treated colonies survived the winter, whereas 50% of untreated colonies died. These results illustrate that acaricide treatment can be an effective control for DWV and likely other mite-vectored viruses. However, since DWV was still present in treated colonies, albeit at lower levels than untreated colonies, it can clearly be maintained via other transmission routes (e.g., oral). Thus, using acaricides within an integrated pest management (IPM) context (147) would reduce negative impacts on individual bees while still ensuring that *Varroa* populations—and the viruses they transmit—remain low.

7.4. Integrated Pest Management

Understanding the mechanistic basis for why some environmental chemicals, but not others, have direct impacts on bee–virus interactions is an emerging area of research. Adopting an IPM approach, where the use of agrochemicals and beekeeper-applied acaricides is minimized and any chemical application approaches are adjusted to reduce exposure of bees, can reduce negative impacts of agrochemical use on bee populations while also reducing the likelihood of selection for insecticide-resistant pest populations and ensuring the long-term efficacy of the pesticide (11, 102).

8. CONCLUSIONS

Bee viral ecology is a rapidly expanding field that encompasses genomics, physiology, behavior, community ecology, and evolutionary biology. Aided by the development of new genomics tools and increasing interest from scientists, stakeholders, policy makers, and the public, the number of studies of viruses in managed and wild bee populations has increased dramatically in recent years (148). These studies have elucidated the diversity of viral species, strains, and variants that infect bees; the complex transmission routes of these viruses within and among bee species; and the intricate molecular and physiological responses of bees to viruses. These studies have also demonstrated that multiple abiotic and biotic factors can influence the ability of the bee host to resist or tolerate a viral infection. However, the vast majority of these studies are correlative, and the underlying mechanistic processes remain to be discovered. Why are certain viral strains more pathogenic? How can the same viral species infect and replicate in such a wide array of bee hosts? Why have bees evolved antiviral response mechanisms that are triggered by non-sequence-specific dsRNA? How do nutrition, metabolism, and the immune system interact to determine the outcomes of viral infections? Why do some environmental chemicals positively or negatively influence the outcomes of viral infections, while others do not? As Karl von Frisch, who received the 1973 Nobel Prize in Physiology or Medicine for his research on decoding the dance language of honey bees, observed about his work, "Thus we see, after traveling a long way, that we have not reached the end of the road but stand instead at the threshold of new problems" (155, p. 96). Bee

viral ecology is similarly filled with intriguing puzzles and mysteries, which await investigation by an integrated and collaborative community of researchers.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The Flenniken laboratory is supported by the National Science Foundation CAREER Program, the United States Department of Agriculture National Institute of Food and Agriculture, Agriculture and Food Research Initiative (USDA-NIFA-AFRI) program, Montana Department of Agriculture Specialty Crop Block Grant Program, the National Institutes of Health IDeA Program COBRE grant GM110732, Hatch Multistate Funding (NC-1173), and Project *Apis m*.

The Grozinger laboratory is supported by the National Science Foundation, United States Department of Agriculture National Institute of Food and Agriculture, Agriculture and Food Research Initiative (USDA-NIFA-AFRI) program, USDA's Animal and Plant Inspection Service (USDA-APHIS), USDA's Foundation for Food and Agricultural Research, and Hatch Multistate Funding (NC-1173).

We are grateful to Eliza Jewett-Hall, Annual Reviews, for assistance with the graphical design in **Figure 1**, and Dr. Francesco Nazzi (University of Udine) for providing a thoughtful review of the completed manuscript. We would like to thank members of the Flenniken laboratory (Alex McMenamin and Dr. Katie Daughenbaugh) and Dr. Marie Pizzorno (Bucknell University) for reviewing this manuscript prior to publication, as well as members of the Grozinger laboratory and the Center for Pollinator Research's Colloquium on Integrative Pollinator Ecology for helpful comments and discussions.

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Annual Review of Entomology

Volume 64, 2019

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Errata

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