CHAPTER EIGHT

Emerging Viruses in Bees: From Molecules to Ecology

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Abstract

Emerging infectious diseases arise as a result of novel interactions between populations of hosts and pathogens, and can threaten the health and wellbeing of the entire spectrum of biodiversity. Bees and their viruses are a case in point. However, detailed knowledge of the ecological factors and evolutionary forces that drive disease emergence in bees and other host–pathogen communities is surprisingly lacking. In this review, we build on the fundamental insight that viruses evolve and adapt over timescales that overlap with host ecology. At the same time, we integrate the role of host community ecology, including community structure and composition, biodiversity loss, and human-driven disturbance, all of which represent significant factors in bee virus ecology. Both of these evolutionary and ecological perspectives represent major advances but, in most cases, it remains unclear how evolutionary forces actually operate across different biological scales (e.g., from cell to ecosystem). We present a molecule-to-ecology framework to help address these issues, emphasizing the role of molecular mechanisms as key bottom-up drivers of change at higher ecological scales. We consider the bee–virus system to be an ideal one in which to apply this framework. Unlike many other animal models, bees constitute a well characterized and accessible multispecies assemblage, whose populations and interspecific interactions can be experimentally manipulated and monitored in high resolution across space and time to provide robust tests of prevailing theory.

1. INTRODUCTION

The interrelationship between bees, their emerging pathogens, and ecology is a research area that is due to significant growth in years to come. Bee populations have declined significantly in recent decades, particularly in temperate zones, and this trend is thought to be at least in part attributable to the (re)emergence of pathogens including RNA viruses. Combined with their experimental tractability and ecological relevance, these circumstances make bees excellent models for investigating virus ecology. First, managed honeybees are often infested with the invasive mite, *Varroa destructor*, which vectors a wide range of viruses (de Miranda and Genersch, 2010; Martin et al., 2012; McMenamin and Genersch, 2015; Mondet et al., 2014), one of which, Deformed wing virus (DWV, family *Iflaviridae*), is thought to play a central role in colony mortality (Budge et al., 2015; Dainat et al., 2012; Di Prisco et al., 2016; Francis et al., 2013; Genersch et al., 2010; Highfield et al., 2009; Natsopoulou et al., 2017; Nazzi et al., 2012; Nguyen et al., 2011). Second, honeybee viruses are shared with sympatric wild bees; viral sequencing suggests on-going transmission between host bee species of

the same viral variants (Fürst et al., 2014; McMahon et al., 2015; Radzevičiūtė et al., 2017), likely at flowers (McArt et al., 2014), opening up the possibility to experimentally manipulate transmission in an ecologically realistic context. Third, many bee viruses have been sequenced (Chen and Siede, 2007; Genersch and Aubert, 2010) and ultrastructural features are becoming available for DWV (Škubník et al., 2017) and other bee viruses (Kalynych et al., 2016, 2017), facilitating their study. Fourth, several bee species can be reared, permitting experimental investigation of host–parasite relations in the laboratory (e.g., Graystock et al., 2016; Manley et al., 2017; McMahon et al., 2016; Meeus et al., 2014; Natsopoulou et al., 2015). For example, honeybee larvae and pupae can be reared in the lab under controlled conditions and in high numbers, and virus evolution experiments consisting of tens of passages can be completed at the whole animal scale in a matter of weeks. Furthermore, while traditional transgenic technologies such as those available to *Drosophila* have not been available in bees, the emergence of gene manipulation methodologies such as RNA interference (RNAi)-mediated gene suppression which can be used to target both bees and ectoparasitic *Varroa* mites (Garbian et al., 2012) and novel gene-editing technologies in the form of CRISPR/cas are beginning to open the door to both loss- and gain-of-function studies in nonmodel eusocial organisms, such as ants (Yan et al., 2017). The advent of second- and third-generation sequencing technologies has further facilitated genome and transcriptome-based investigations in bees (Bigot et al., 2017; Doublet et al., 2017). That bee viruses are not known to be pathogenic to humans is an added advantage in laboratory studies. Fifth, most bee species can be readily collected from flowers in the field, allowing sampling of the commoner species and determination of viral presence (reviewed in Tehel et al., 2016) and prevalence. Finally, the economic and ecological relevance of bees, as described in Section 2.1, gives significant applied value to the investigation of bee–virus interactions.

In this review, we introduce the biology of bees and their viruses, and outline how they represent an ideal system with which to explore viral emergence in a realistic ecological setting. We put forward a framework that integrates molecular- and ecosystem-level approaches to virus ecology, arguing that the greatest insights will come from tackling questions at two or more levels of biological organization. We then discuss general evolutionary and ecological principles of infectious disease, drawing from human and other well-known animal systems to identify gaps in bee virus ecology, but also to highlight areas where research into bee viruses may bring distinct

benefits. We finish by considering some critical challenges and open questions in bee virus research. Our intention in this review is to provide a basic introduction to bee virus research and a useful guide and source of ideas for forthcoming studies in this rapidly expanding field.

2. A BRIEF OVERVIEW OF BEES AND THEIR VIRUSES

2.1 Bees

The value of bees to agriculture, food security, and the wider environment through their role in pollination is indisputable. Approximately 70% of the world's most important crops are dependent to some extent on animal-mediated pollination (Klein et al., 2007), with an estimated value to the economy of US$ $235–557 \times 10^9$ at today's prices (Gallai et al., 2009; Potts et al., 2016). They comprise a range of vegetable, fruit, nut, and seed crops that are not only of high-economic value but also rich in important micronutrients otherwise in short supply in many low-income countries (Eilers et al., 2011). Perhaps not surprisingly, world agriculture has witnessed significant growth in pollinator-dependent crops over the past five decades (Aizen et al., 2009; Potts et al., 2016). With over 87% of the world's angiosperm species also dependent on animal vectors to move their pollen from anther to stigma of the same or another conspecific plant (Ollerton et al., 2011; Willmer, 2011), pollination is placed high on the list of major ecosystem services (Costanza et al., 1997).

Though many insects, such as Coleoptera, Diptera, and Lepidoptera, and a diversity of vertebrates, including bats, birds, and lizards, can effect pollination, bees are by far the most important animal pollen vectors (Willmer et al., 2017). They comprise a monophyletic group of ca. 20,000 species divided into 7 taxonomic families (Danforth et al., 2013; see Table 1) and exhibit a range of social behaviors, from solitary (ca. 90%) to eusocial, as in the honeybees (*Apis* spp.) and neotropical stingless bees (tribe Meliponini; Kocher and Paxton, 2014). All collect nectar and pollen from flowers as larval provisions for their offspring (or act as cleptoparasites of other bee species) and therefore are avid flower visitors, accounting for their important role in pollination (Willmer et al., 2017). These facts and statistics underscore the importance of bees to agricultural and natural ecosystems.

Pollination is considered as an ecosystem service under threat because of the perceived decline of animal pollinators across the world, particularly bees (Brown and Paxton, 2009; Potts et al., 2016; Vanbergen and IPI Initiative, 2013). Though the number of colonies of the most important commercial pollinator, the Western honeybee (*Apis mellifera*), has increased exponentially

Table 1 The Bees, an Ecologically and Economically Important Monophyletic Group of Approximately 20,000 Insect Species Within the Order Hymenoptera (Danforth et al., 2013; Hedtke et al., 2013; Michener, 2007)

Family	Melittidae	Andrenidae	Halictidae	Colletidae	Stenotritidae	Apidae	Megachilidae
No. of described species	200	>2900	>4300	>2500	21	>5700	>4000
Distribution	Absent from Australia and S. America, well represented in southern Africa	Absent from Australia; otherwise worldwide	Worldwide	Worldwide, well represented in Southern Hemisphere	Australia only	Worldwide, well represented in the neotropical and oriental regions	Worldwide
Social organization	Solitary	Solitary	Solitary to eusocial, including socially polymorphic spp.; two independent origins of sociality	Solitary	Solitary (?)	Solitary to eusocial, including advanced eusocial spp., e.g., honey bees; two independent origins of sociality	Solitary
Ecological traits	Fossorial nesting; often annual	Fossorial nesting; often annual	Fossorial nesting; often annual; several cleptoparasitic spp.	Fossorial nesting; often annual	Fossorial nesting; annual (?)	Nesting below and above-ground and in self-made nests; advanced eusocial spp. are perennial; many cleptoparasitic spp.	Nesting above-ground in cavities using collected plant material; often annual; some cleptoparasitic spp.

Continued

Table 1 The Bees, an Ecologically and Economically Important Monophyletic Group of Approximately 20,000 Insect Species Within the Order Hymenoptera (Danforth et al., 2013; Hedtke et al., 2013; Michener, 2007)—cont'd

Family	Melittidae	Andrenidae	Halictidae	Colletidae	Stenotritidae	Apidae	Megachilidae

A maximum-likelihood estimate of phylogenetic relationships among bee species based on multiple DNA sequences (after Hedtke et al., 2013); bees arose from within the sphecid wasps. (?) Poorly studied, social and ecological traits unknown.

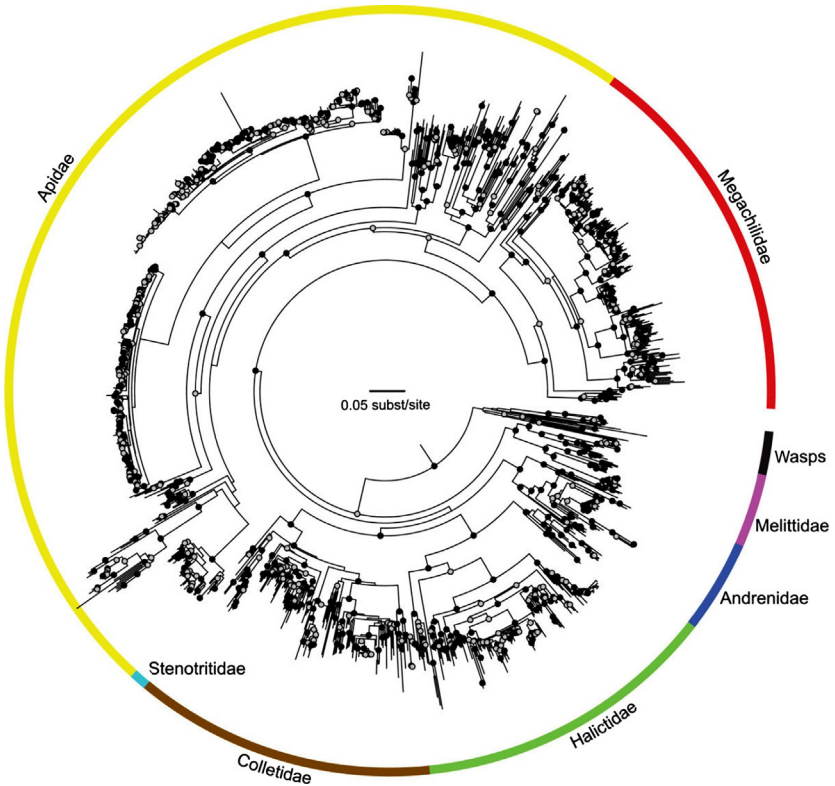

Figure reproduced in original form from Hedtke, S.M., Patiny, S., Danforth, B.N., 2013. The bee tree of life: a supermatrix approach to apoid phylogeny and biogeography. BMC Evol. Biol. 13, 138, (Publisher: Biomed Central) under a CC-BY-2.0 licence.

over the past 50 years (Potts et al., 2016)—reflecting the profitability of beekeeping and the wider socioeconomic climate (Moritz and Erler, 2016; Moritz et al., 2010)—this belies the high mortality of colonies at c.30% per year, particularly over winter, in Northern temperate regions (Neumann and Carreck, 2010). These losses are generally compensated for by beekeepers multiplying colonies in the subsequent season. The exotic ectoparasitic mite *Varroa destructor* and the viruses it transmits, particularly DWV, are considered the major cause of elevated honeybee mortality (Budge et al., 2015; Dainat et al., 2012; Di Prisco et al., 2016; Francis et al., 2013; Genersch et al., 2010; Highfield et al., 2009; Nazzi et al., 2012; Natsopoulou et al., 2017; Nguyen et al., 2011). DWV was present in honeybee populations prior to the arrival of *V. destructor*, but its epidemiology has altered dramatically since the global spread of the mite from southeast Asia since the 1950s (Martin et al., 2012; Oldroyd, 1999; Wilfert et al., 2016). Prior to *V. destructor*, DWV was associated with largely asymptomatic infections with occasional outbreaks of acute disease (Forsgren et al., 2012). Few other bee species are managed, though one, the largely European bumble bee *Bombus terrestris*, has been increasingly employed for glasshouse pollination since 1990 (Velthuis and van Doorn, 2006), and various other bumble bee species are now domesticated and commercialized as pollinators, along with some solitary bee species (Aizen et al., 2016). There is conclusive evidence for a decline of other, unmanaged bee species (often referred to as "wild bees") in Europe (Biesmeijer et al., 2006; Fitzpatrick et al., 2007; Nieto et al., 2014) and North America (Bartomeus et al., 2013; Cameron et al., 2011), which are increasingly acknowledged to play an important role in crop pollination and flowering plant biodiversity (Garibaldi et al., 2013). Degradation and loss of habitat is thought to be the major cause for the decline of wild bees in these regions (Winfree et al., 2009), though increasingly the role of pesticides (especially neonicotinoids; see Rundlöf et al., 2015; Woodcock et al., 2016, 2017) and pathogens, including viruses that have spilled over from managed honeybees (Fürst et al., 2014; McMahon et al., 2015; Radzevičiūtė et al., 2017), have been highlighted as potentially important drivers of decline. These different stressors may act synergistically in contributing to pollinator losses (Potts et al., 2016).

2.2 Bee Viruses

Because of their potential impact on apiculture, honeybee viruses have been intensely studied for many decades. At least 18 different RNA and DNA viruses had been identified using classical virological methods (Chen and

Siede, 2007). These viruses, reviewed in detail by Chen and Siede (2007) as well as by de Miranda et al. (2010a,b) and de Miranda and Genersch (2010) for individual viral groups, are predominantly positive single-stranded (+ss) RNA viruses, many of them belonging to the Picornavirales (picorna-like viruses). With the availability of next-generation sequencing, several additional RNA viruses, such as the Lake Sinai viruses (LSV) (assumed family *Noraviridae*), have been described (McMenamin and Genersch, 2015; Mordecai et al., 2016; Remnant et al., 2017; Runckel et al., 2011). We can expect many more to be added to this list as next-generation and third-generation sequencing becomes more widespread in the field of epidemiology (Bigot et al., 2017). The challenge with this powerful approach to virus discovery will in future lie first in determining whether these viruses in fact infect the bees they were isolated from, and second whether these viruses are virulent, i.e., whether they negatively affect the purported host's fitness. The first challenge can, at least partially, be addressed by sequencing host small RNAs; viral short RNA fragments show a peak at a characteristic length (21 bp in the case of bees), indicating that the host's antiviral RNAi system has attacked an actively replicating RNA virus (Obbard et al., 2009). The second issue is more difficult to address and will require a combination of detailed lab experiments, field/semifield studies and modeling to assess the cost of such infections to honeybees, and potentially other bee species. Importantly, it is now clear that many viruses previously considered "honeybee viruses" are in fact multihost pathogens that infect other bees and potentially even phylogenetically more distant groups (Manley et al., 2015; McMahon et al., 2015) (Table 2). Whether the arrival of the *V. destructor* mite in *A. mellifera* has been a driver of viral emergence in non-*Apis* bees represents a major outstanding question in bee virus ecology, although the positive correlation in virus prevalence and sequence identity between *Apis* and *Bombus* populations (Fürst et al., 2014; McMahon et al., 2015; Radzevičiūtė et al., 2017) indicates frequent and ongoing transmission between bee species. Regardless of virus origins, this means that for common bee viruses, including, e.g., DWV, black queen cell virus (BQCV), or acute bee paralysis virus (ABPV), the pathogen's evolutionary ecology has to be studied within a multihost pathogen framework.

2.3 Deformed Wing Virus

DWV is one of the most prevalent viruses in the western honeybee *A. mellifera*, which when combined with the vector *V. destructor* is a leading

Table 2 A List of Bee-Associated Viruses Sequenced to Date (All Positive Single-Stranded RNA Viruses), Their Families, and References

Family	Virus	Abbr.	References
Dicistroviridae	Acute bee paralysis virus[a]	ABPV	Bailey et al. (1963) and de Miranda et al. (2010a)
	Aphid lethal paralysis virus	ALPV	Runckel et al. (2011)
	Big Sioux River virus	BSRV	Runckel et al. (2011)
	Black queen cell virus	BQCV	Bailey and Woods (1977) and Leat et al. (2000)
	Kashmir bee virus[a]	KBV	Bailey and Woods (1977) and de Miranda et al. (2004)
	Israeli acute paralysis virus[a]	IAPV	Maori et al. (2007)
Iflaviridae	Deformed wing virus[b]	DWV	de Miranda and Genersch (2010)
	Kakugo virus[b]	KV	Fujiyuki et al. (2004)
	Sacbrood virus	SBV	Bailey and Fernando (1972) and Ghosh et al. (1999)
	Slow bee paralysis virus	SBPV	Bailey and Woods (1974) and de Miranda et al. (2010b)
	Varroa destructor virus-1[b]	VDV-1	Ongus et al. (2004)
Secoviridae	Tobacco ringspot virus	TRSV	Li et al. (2014)
Unassigned, related to *Nodaviridae* and *Tombusviridae*	Chronic bee paralysis virus	CBPV	Bailey et al. (1963), Olivier et al. (2008), and Ribière et al. (2010)
	Halictus scabiosae Adlikon virus	HsAV	Bigot et al. (2017)
	Lake Sinai virus 1 and 2[c]	LSV	Runckel et al. (2011)

[a]ABPV/IAPV/KBV.
[b]DWV/KV/VDV-1.
[c]LSV-1/LSV-2.
Note: Several viruses are so closely related that it is yet to be determined if they should be classified as different species.
Adapted from Manley, R., Boots, M., Wilfert, L., 2015. Emerging viral disease risk to pollinating insects: ecological, evolutionary and anthropogenic factors. J. Appl. Ecol. 52, 331–340.

cause of overt symptomatic infections typified by adult bees displaying crumpled wings and malformed bodies (Ball and Allen, 1988; Gisder et al., 2009; Möckel et al., 2011; Shen et al., 2005; Yue and Genersch, 2005). DWV is now known to comprise of at least three distinct genotypes (Mordecai et al., 2015) that are differentially virulent in honeybees (McMahon et al., 2016; Ryabov et al., 2014) and able to recombine in nature (Moore et al., 2011; Wang et al., 2013; Zioni et al., 2011). Interestingly, DWV is also known to naturally infect the managed species *Apis cerana* and has been detected in the nonmanaged species *Apis dorsata* and *Apis florea* (Zhang et al., 2012). Outside of honeybees, DWV has been found widely in bumblebees, including solitary bees and wasps (reviewed in Gisder and Genersch, 2017) and there is evidence that it can actively replicate in several *Bombus* and solitary bee species (Fürst et al., 2014; Genersch et al., 2006; Graystock et al., 2016; Levitt et al., 2013; Radzevičiūtė et al., 2017).

2.4 Black Queen Cell Virus

BQCV (family *Dicistroviridae*) was originally described from *A. mellifera* as the disease agent of decomposed black-patched queen pupae (Bailey and Woods, 1977) but has since been found to frequently infect workers. Alongside DWV, it is one of the most prevalent viruses of *A. mellifera*. As with DWV, BQCV is found frequently in non-*Apis* bees (McMahon et al., 2015; Radzevičiūtė et al., 2017; Singh et al., 2010), and there is evidence of active infection in at least one *Bombus* species (Peng et al., 2011) and several other solitary bees (Radzevičiūtė et al., 2017), although greater knowledge concerning its pathological effects on *Apis* and non-*Apis* bees is needed.

2.5 Acute Bee Paralysis Virus

ABPV (family *Dicistroviridae*) and its close relatives Kashmir bee virus (KBV) and Israeli acute bee paralysis virus (IAPV) are virulent and widespread viruses in *A. mellifera*. The arrival of the *V. destructor* mite may have increased the prevalence of ABPV in *A. mellifera* (Genersch and Aubert, 2010), but its high virulence (leading to reduced survival of infected pupae) could explain why ABPV is detected less frequently than DWV in *V. destructor*-infested colonies (Schroeder and Martin, 2012; Sumpter and Martin, 2004). Only a little is known about the ABPV-complex in non-*Apis* bees. For example, when orally infected with IAPV and KBV, *B. terrestris* microcolonies were seen to suffer slower colony establishment or lower offspring production (Meeus et al., 2014), while in the field, IAPV and KBV have been detected

in non-*Apis* hymenopterans (Singh et al., 2010). Meanwhile, ABPV has been detected in *Bombus* species in the field as viable infections (Bailey and Gibbs, 1964). A UK survey showed that ABPV is significantly more prevalent in *Bombus lapidarius* than in other common bumblebee species and even than in *A. mellifera*, and can occur in bumblebees at putatively high viral loads (McMahon et al., 2015). Such species prevalence patterns might indicate significant variation in host susceptibility to viral infection (Ruiz-González et al., 2012).

2.6 Slow Bee Paralysis Virus

The situation for Slow bee paralysis virus (SBPV) (family *Iflaviridae*) is less complete, although both field (Carreck et al., 2010) and laboratory experiments (Santillán-Galicia et al., 2014) suggest that it can be transmitted between honeybees via *V. destructor* mites and that it may also be more virulent than DWV. With respect to wild bees, a recent laboratory study in *B. terrestris* found the virulence of SBPV infection to be condition (starvation) dependent (Manley et al., 2017), but virtually nothing is known of SBPV epidemiology. As with ABPV, SPBV is frequently detected in bumblebees (McMahon et al., 2015; Parmentier et al., 2016), and recent studies have confirmed its presence in several non-*Bombus* bees including *Melipona* (Ueira-Vieira et al., 2015) and *Anthophora* (Radzevičiūtė et al., 2017).

2.7 Sacbrood Virus

Sacbrood virus (SBV) (family *Iflaviridae*) is another common and globally prevalent virus of honeybees, which leads to queen larvae failing to pupate and acquiring a sac-like appearance. Although there is less evidence that *V. destructor* plays a role in SBV transmission, correlative studies have found SBV to be positively associated with *V. destructor*-infested honeybee colonies (Mondet et al., 2014). Although some studies have detected SBV in non-*Apis* bees, including bumblebees (Gamboa et al., 2015; McMahon et al., 2015; Singh et al., 2010) and the solitary bee *Andrena vaga* (Ravoet et al., 2014), it would appear that SBV may be more strictly associated with honeybees (Gisder and Genersch, 2017).

2.8 Chronic Bee Paralysis Virus

Chronic bee paralysis virus (CBPV) is an intriguing RNA virus because its genome consists of two separate positive-stranded RNA molecules. Its classification is unclear, although it shares characteristics with the plant virus

family *Tombusviridae* and the *Nodaviridae* (Ribière et al., 2010). Much like SBV, CBPV can exert strong pathogenic effects, including trembling and clustering of crawling bees, although its frequency of detection is generally lower than several of the other common viruses discussed earlier. It is, though, apparently increasing in prevalence in US colonies (Traynor et al., 2016). Many of the symptoms associated with CBPV infection match the descriptions of "Isle of Wight Disease," which caused mass honeybee die-offs in Britain at the turn of the 20th century (Ribière et al., 2010). CBPV is thought to be able to infect non-*Apis* bees and other hymenopterans such as ants (Celle et al., 2008).

2.9 Other Viruses

In addition, a number of other viruses have recently been discovered in honeybees and some wild bees, including LSV, Big Sioux River virus (BSRV), aphid lethal paralysis virus (ALPV) (Runckel et al., 2011), tobacco ringspot virus (TRSV) (Li et al., 2014), and a novel "Halictivirus," Halictus scabiosae Adlikon virus (HsAV) (Bigot et al., 2017). Their pathogenicity across different bee species, including at the colony level in *A. mellifera* and other eusocial species, remains targets of ongoing research.

2.10 Bee Virus Evolutionary Ecology

For multihost pathogens including many bee-associated viruses, virus ecology in any one host will depend on the transmission networks between hosts, which in turn are determined by the frequency of transmission events within and between species. A species' transmission potential is determined by its abundance, the prevalence of disease within the species, and by its infectivity, i.e., how many infective particles it sheds (Streicker et al., 2013). In bees, between-species infectivity will also depend on realized transmission opportunities, as between-species transmission will depend on direct or indirect contacts, e.g., via contaminated flowers or through activities such as drifting of workers between colonies or robbing of food reserves from one colony by members of another colony and even another species (Manley et al., 2015). The evolutionary trajectory of a virus will in turn be determined by the different adaptive landscapes each host represents, as well as by the frequency and length of time each host is encountered by a viral isolate. This can result both in the evolution of viral generalists and in the diversification of viral specialists, depending on the underlying epidemiology and genetic differences in the hosts (see Rigaud et al., 2010 for a review of these ideas).

Viral ecology and evolution are in fact inseparable. RNA viruses are among the fastest evolving entities, with evolutionary rates of 10^{-3}–10^{-5} substitutions per site per year (Holmes, 2008); DWV, for example, has a mean evolutionary rate of 1.35×10^{-3} per site per year, with 10–15 substitutions expected per genome per year (Mordecai et al., 2015). For these viruses—in contrast to their eukaryotic hosts—the ecological and evolutionary timescales thus overlap, making virus evolution integral to any study of virus ecology. This rapid evolution allows RNA viruses to adapt quickly to novel host environments, having the highest risk factor for disease emergence among all classes of pathogen (Dobson and Foufopoulos, 2001; Taylor et al., 2001). Another key aspect to viral evolution is recombination or reassortment. These processes, exemplified by influenza viruses (Tumpey et al., 2005), but also common in nonsegmented viruses (Eden et al., 2013), can enable viruses to dramatically change their epidemiology and host range, being frequently associated with disease emergence such as in the recent case of H1N1 Swine flu (Dawood et al., 2009). In bee viruses, recombination between genotypes of DWV has been shown to be a common occurrence (McMahon et al., 2016; Moore et al., 2011; Wang et al., 2013; Zioni et al., 2011), with recombinants potentially under selection by transmission via the ectoparasitic viral vector *V. destructor* (Ryabov et al., 2014). Studying genome evolution is thus integral to understanding viral ecology in bees and the potential of these diseases to emerge in novel host species.

3. A UNIFIED MOLECULE-TO-ECOLOGY APPROACH TO BEE VIRUS RESEARCH

As we introduced above, evolution plays a fundamental role in virus ecology. Evolution is governed by conditions in the proximate environment of any organism, which in the case of a pathogen is the host. Both host and pathogen evolution are driven by environmental factors that influence population-scale processes, but the temporal and spatial scales at which hosts and pathogens operate can differ by several orders of magnitude. For example, RNA virus populations are defined by step-wise infection processes mediated by immune molecules and cell-level interactions inside individual hosts. In contrast, host population dynamics play out over much greater temporal and spatial scales. In the past these multiple spheres of host–pathogen activity have often been studied separately. Mechanistic interactions between pathogens and hosts have fallen largely in the domains of molecular and cell biology, while evolutionary focus has been more

concerned with host-level outcomes and epidemiology. But as has recently been argued, a unification of these approaches can be beneficial for explaining variation in infectious disease traits between hosts (Hall et al., 2017). We argue, as others have recently (Hedrick, 2017), that such a unified framework is necessary to understand and ultimately predict the emergence, severity, and spread of infectious diseases.

For example, ecological considerations in the host can influence pathogen molecular adaptation because host immune response has been shaped by evolutionary events of the past, such as historical population exposure to disease. Such events can vary considerably between closely related host species, or even between populations of the same species (Black, 1992). Such variation can influence the ways in which hosts can respond to pathogens, in turn leading to significantly altered infection outcomes (Hedrick, 2011; Laayouni et al., 2014). At the most basic level, a host's immune response determines the likelihood and type of infection, which as discussed earlier can be species- or population-specific (Taylor et al., 2001; Woolhouse et al., 2005). Whether a host displays resistance or tolerance toward a novel pathogen can influence pathogen load and duration (acute vs chronic), infection success, and therefore onward transmission. Other direct consequences of infection and immune activation include factors such as host mortality, morbidity, and behavior (e.g., avoidance/fever), which can shape the probability and type of contact between infected and susceptible individuals. Furthermore, ecological considerations beyond the level of individual immunity can influence the dynamics of transmission, including host sociality, population demography (Faria et al., 2014), and density (Anderson and May, 1979); metapopulation structure; multispecies community composition (Johnson et al., 2015); biodiversity (Keesing et al., 2010); vector ecology (Randolph, 2001; Randolph and Rogers, 2000); and (human-driven) disturbance (Cable et al., 2017; Lafferty, 2009; Rogers and Randolph, 2000).

All of these factors combine to form multiple scales of biological organization within which pathogens, including bee viruses, must operate and evolve (Fig. 1). There are a number of areas in bee virus research that can benefit from a unified approach, with the added potential for such a framework in bees to serve as a general model for research in virus ecology. Key questions from these areas include: (Fig. 1(1)) What are the molecular features of the virion that drive viral pathogenesis? Molecular features of virion tertiary structure such as the VP3 domain of the DWV virion (indicated with an arrow) can play a role in directing cell entry and pathogenesis; (Fig. 1(2)) How do structural components mediate cell entry, and how do viral

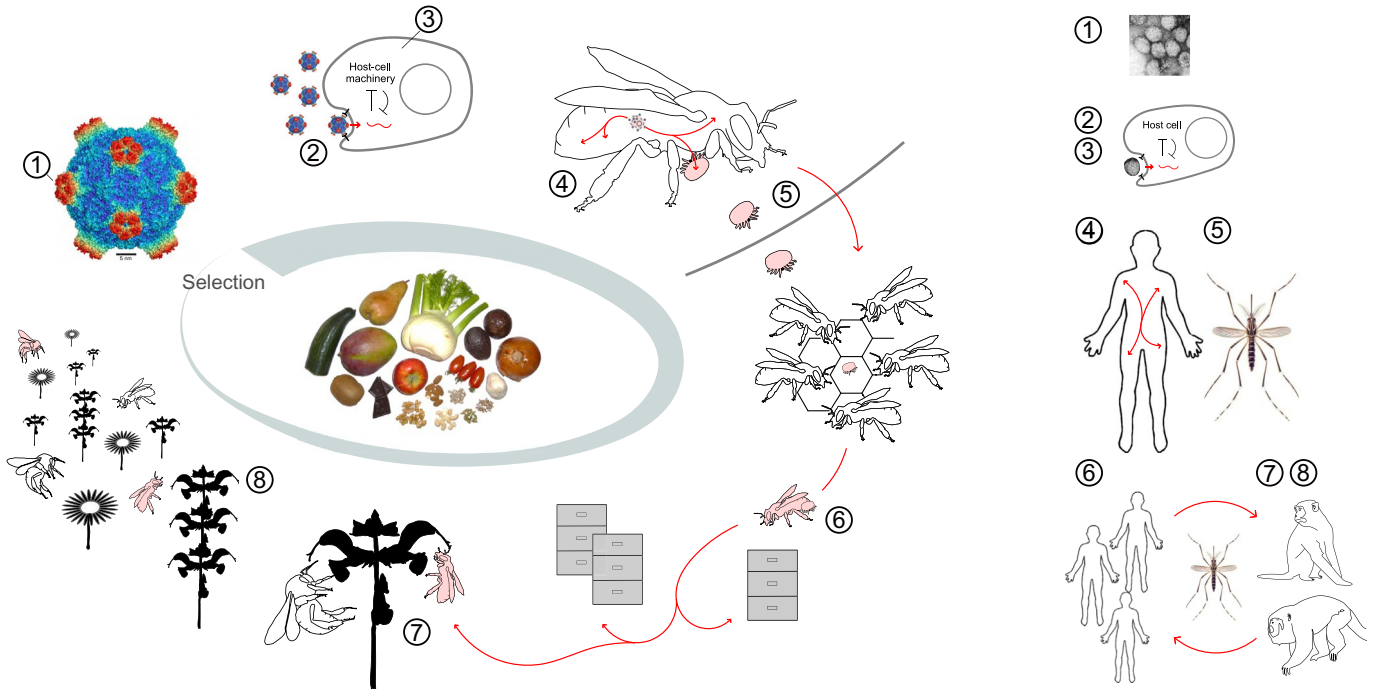

Fig. 1 *Left*: A molecule-to-ecology framework for bee–virus research. The framework integrates multiple levels of biological organization, demonstrated here by a series of levels (1–8). The scheme is intended to stimulate cross-disciplinary approaches to the study of bee viruses. The *gray ellipse* represents the strength of direct selection acting on viruses, which is reduced at higher levels of biological organization. Readers are referred to the main text (Section 3) for a detailed description of each level. Popular foods that depend on pollinators for successful fruit set are pictured in the center of the figure. *Right*: Equivalent steps in the molecule-to-ecology framework for human RNA viral diseases, such as the arboviruses Dengue virus (DENV) and Yellow fever virus (YFV) (family *Flaviviridae*). The viruses are transmitted by the mosquito *Aedes aegypti* both within and between human and monkey populations such as New World howler monkeys (YFV) and Old World rhesus macaques (DENV). *DWV image adapted from Škubník, K., Nováček, J., Füzik, T., Přidal, A., Paxton, R.J., Plevka, P., 2017. Structure of deformed wing virus, a major honey bee pathogen. Proc. Natl. Acad. Sci. U.S.A. 114, 3210–3215 under a CC BY-NC-ND-4.0 licence, (Publisher: National Academy of Sciences).*

receptors in host cells vary with host taxonomy and evolutionary history to dictate host susceptibility and virulence? (Table 1); (Fig. 1(3)) How does a virus suppress host antiviral defense and subvert host-cell machinery to its own benefit, and how important is population- and species-level variation in host immunity in mediating infections and virulence?; (Fig. 1(4)) How do viruses spread inside a host and are infections associated with chronic asymptomatic infections or severe acute infections? How are these infection outcomes related to mechanisms at steps 1–3? Furthermore, when two or more viral genotypes coinfect a host cell, does recombination lead to increased evolutionary scope?; (Fig. 1(5)) Does the *Varroa* mite not only vector virus but also act as an intermediary host, imposing its own selection on a virus? Furthermore, does the vector impact virus adaptation by allowing hemolymph-to-hemolymph indirect transmission. What impact does such adaptation in one host have on spillover and virulence in other hosts?; (Fig. 1(6)) How readily is virus transmitted between honeybee colonies and (Fig. 1(7)) between host bee species at flowers or via other routes (e.g., robbing)?; (Fig. 1(8)) How do pollinator-flower networks influence virus transmission, and what impact do emerging viruses have on stability of host populations, and the ecosystem service of pollination provision? Integrating across these questions will allow a far deeper understanding of the ecological and evolutionary forces driving viral emergence, with the prospect of developing models that can help to predict viral emergence under future socioeconomic and societal scenarios. In the next sections, we describe interactions between bees and their viruses at different levels within the molecule-to-ecology framework.

3.1 Host–Virus Molecular Mechanisms

At the most basic level, we do not know the mechanistic basis of cell entry and infection for any bee–virus system. Yet this knowledge is crucial for understanding the type and extent of molecular compatibility between a virus and a novel immune system or transmission route. This is relevant for understanding possible levels of maladaptation between novel host–virus combinations, and the ensuing impact on virus adaptation to a host's immune system, virulence evolution and transmission.

Only recently have the fine-detailed structures of some bee viruses been elucidated, with SBPV (Kalynych et al., 2016) and more recently DWV (Škubník et al., 2017) now characterized to less than 4 Å. Both of these iflaviruses contain similar surface topologies, with capsids containing

protrusions of the so-called P-domain, which is derived from the VP3 protein subunit. While the P-domain differs in sequence identity between SBPV and DWV, both viruses contain eight highly conserved residues. They are shared with other iflaviruses and potentially involved in receptor-binding or catalytic disruption of the host-cell membrane, enabling the RNA genome to be transported into the cell cytoplasm (Škubník et al., 2017). Further evidence of involvement of the VP3 protein is suggested by interactions between the RNA genome and residues from the VP3 subunit. Unfortunately, the mechanistic basis of cell entry for iflaviruses—including DWV—is currently unknown. This represents a key area for future research, particularly for improving understanding of the potential similarities in host-mediated viral entry mechanisms between bee species. If mammalian picornaviruses are anything to go by (McKnight and Lemon, 2017), it is likely that at least some aspects of host cell entry mechanisms are conserved across different iflaviruses and bee-host species. One obvious first point of difference to explore between mammalian–picornavirus and invertebrate–iflavirus interactions is the key role for the RNAi machinery in bee antiviral defense, as has been extensively demonstrated in flies (Ding and Voinnet, 2007). However, a role for canonical immune pathways in antiviral defense has also been highlighted in insects (reviewed in Merkling and van Rij, 2013), with NF-κB signaling in the Toll pathway being implicated in honeybee antiviral defense against DWV (Di Prisco et al., 2013).

Although these represent relevant targets for forthcoming studies in bee–virus interactions, a robust approach requires exploration of the repertoire of mechanisms found at sequential steps in the infection process of the host (Ebert et al., 2016): from external membrane interactions at the gut or cuticle (which critically, are irrelevant in the case of *Varroa*-vectored viruses delivered directly into hemolymph) to host-cell transcriptional manipulation mechanisms, and eventually packaging and transport of virions to sites of transmission. A complete overview also requires characterization of potential points of difference between species, and identification of forms of selection pressure that may vary across stages of infection and transmission routes. Such processes can shape pathogen adaptation in various ways (Schmid-Hempel and Ebert, 2003). Depending on the mechanism involved, they could have potential repercussions for relevant disease traits, such as specificity and pathogenesis in novel hosts. Although it is hard to make general predictions, a possibility is that virus adaptation to vector-mediated transmission in honeybees may result in highly specific modifications to a small subset of host–virus mechanisms relating to virus replication.

From a mechanistic point of view, it is difficult to hypothesize what the consequences of vector-adaptation might be for wild bees exposed to viruses via the gut. Host specialization could lead to increased virulence in nonadapted hosts (Ebert, 1998), but it is also conceivable that relaxed selection on gut membrane and milieu associations leads to reduced infectiousness of a virus.

3.2 Between-Host Transmission and Impact of Novel Vectors

Transmission opportunities and routes play a key role in epidemiology. Disease emergence is generally facilitated by a change in host–parasite ecology (Woolhouse, 2002) that leads to novel transmission opportunities between species. Changes in host species range or biological niche, for example, through invasions or through global change, can bring potential host species into contact. In the case of bee diseases, globalization and the transport of nonnative bee species have led to the spread and emergence of disease. For example, the use of nonnative commercially produced bumble bees has led to the spread of nonnative infectious diseases or variants in South America (Maharramov et al., 2013; Schmid-Hempel et al., 2014) and Japan (Goka et al., 2006). The global transportation of honeybees has not only led to the spread of infectious diseases such as *Nosema ceranae* (Klee et al., 2007) but also to the emergence of the ectoparasitic mite *V. destructor* (Oldroyd, 1999) and the emergence of DWV as a global pandemic (Wilfert et al., 2016).

From human medicine, agriculture, and wildlife biology, it is clear that the emergence of novel transmission routes can have drastic effects on epidemiology, even though our understanding of the underlying theory in this area is not yet clearly developed. Most extant cases of novel transmission routes can be traced to human activities and have led to disease emergence or reemergence, beginning with diseases acquired during livestock domestication such as measles (Pearce-Duvet, 2006). For oral-fecal transmitted diseases, the introduction of novel food sources, or the contamination of existing ones can lead to the establishment of a novel transmission route as exemplified by the variant form of Creutzfeldt–Jakob disease in humans which arose as a result of consuming prion-contaminated beef (Collinge et al., 1996). In bees a parallel can be drawn with flowers, which may act as important points of interspecific transmission. Indirect vectors are also key transmission routes for emerging pathogens. For example, the use of unsterilized needles in health clinics is thought to have been a major contributor to the early spread of HIV (Faria et al., 2014). But biotic vectors have played additional major roles in disease emergence. Avian malaria in

Hawaii increased significantly following the introduction of malaria-carrying *Culex quinquefasciatus* mosquitoes (Woodworth et al., 2005), while the global expansion of rats and their *Yersinia pestis* carrying fleas (*Xenopsylla cheopis*)—or even human lice and fleas—could have led to the Black Death in Europe in the 14th century (Dean et al., 2018; Stenseth et al., 2008).

In the case of the honeybee and *V. destructor*, the situation is slightly different because viruses such as DWV were already present in bees prior to the arrival of the mite vector (Forsgren et al., 2012). As discussed in the introduction, this hemolymph-feeder has led to the emergence of a novel transmission route for bee viruses, leading to a dramatic increase in titer and prevalence of DWV-linked disease (de Miranda and Genersch, 2010; Martin, 2001; Martin et al., 2012; McMahon et al., 2016; Mondet et al., 2014; Wilfert et al., 2016). *V. destructor* was originally an ectoparasite of the honeybee *A. cerana*, native to south and east Asia, where it causes little damage to its host (Nazzi and Le Conte, 2016; Rosenkranz et al., 2010). Within the last century, *A. mellifera* colonies were brought from Europe to E. Asia, sympatric with *A. cerana*, which allowed *V. destructor* to successfully switch host to *A. mellifera* (Nazzi and Le Conte, 2016; Rosenkranz et al., 2010). Mite-infested colonies of *A. mellifera* were unwittingly returned to Europe, whereupon *V. destructor* dispersed into neighboring colonies and the mite was subsequently transported with *A. mellifera* colonies more or less worldwide. As a consequence, mites are nowadays found on all major land masses harboring *A. mellifera* except Australia, which maintains strict quarantine regulations. Mites feed on tissue of *A. mellifera* adults and pupae, from which they can acquire numerous host viruses that they can transmit with high efficiency to a subsequent host (Bowen-Walker et al., 1999; Gisder et al., 2009, Di Prisco et al., 2011), though they are only able to successfully reproduce on *A. mellifera* pupae.

An obvious question that arises and can be experimentally addressed in bees is: what impact did the introduction of indirect transmission have on virus evolution? Due to the nature of the *V. destructor* life cycle in the honeybee host, predictions can be made about the potential impact of the mite on virus virulence evolution (Fig. 2). Specifically, the developing honeybee host pupa should remain alive until close to the completion of metamorphosis to provide sufficient time for successful mite reproduction, including offspring mating. For optimal transmission, any virus found in a mature and mated daughter mite will hold a significant selective advantage over a virus found in an immature or unmated daughter mite—placing a cost on virus virulence that impacts honeybee pupae before mites can mate (Martin, 2001).

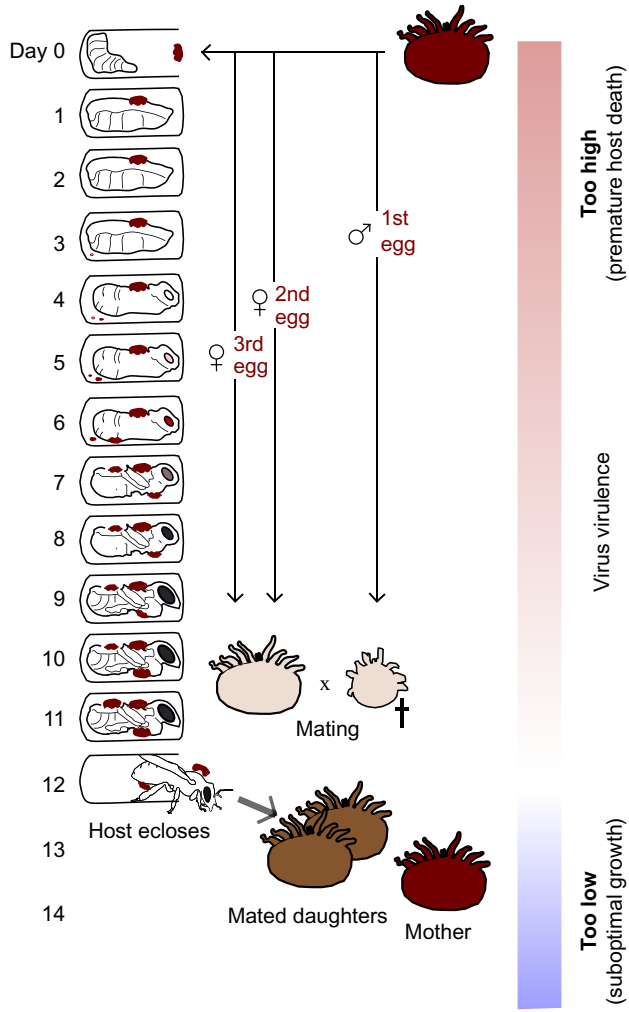

Fig. 2 Hypothetical impact of indirect vector transmission on virus virulence evolution in *A. mellifera*. The *V. destructor* reproductive life cycle is shown, with mites depicted at various developmental stages. Corresponding stages of honey bee pupal development (in days post-capping) are shown to the left of the figure. A mother mite lays maximally three eggs that can mature to adulthood before hosts emerge from the brood cell. Virulence should evolve to an intermediate level that maximizes transmission (*white region*). Too virulent (*red region*) and the virus will have suboptimal transmission due to high-host mortality preventing mite mating and host emergence (eclosion). Not virulent enough (*blue region*) and the virus will have suboptimal transmission due to low growth (fewer transmission units).

On the other side, viruses replicating too slowly and with delayed virulence effects will hold a selective handicap because fewer transmission units will be found in mated mites. We hypothesize that the evolution of virus virulence shifted following the arrival of *V. destructor*, with viruses such as DWV evolving a level of virulence in pupae (and likely also in adults) that optimizes the number of transmission units passed to uninfected susceptible vectors, and ultimately hosts. Addressing this issue in bees could provide key insight into how anthropogenic disturbance can drive pathogen adaptation in unpredictable ways, leading to unintended cascade effects across ecological communities.

3.3 Multihost–Multipathogen Community Ecology

A second interesting question that arises from the observation that viruses were already present in bees prior to the arrival of the mite vector, is: what impact did this new transmission route in one species have on other bee species? Addressing this question requires basic knowledge about host–virus dynamics in bee assemblages. Unfortunately, even knowledge of the species that are primary or natural hosts for the many so-called bee viruses is lacking, and this is partly attributable to a general lack of research in non-*Apis* species (Brown and Paxton, 2009; McMahon et al., 2015; Tehel et al., 2016). What can be stated with some degree of confidence is that the increased abundance and prevalence of vectored viruses in honeybees has resulted in increased virus transmission opportunities between bee species.

Which bee species are more or less susceptible to infection is not known, but in other host–virus systems (e.g., bats), there is some correlation between phylogeny and viral host range, with closely related host species more likely to share viruses (Faria et al., 2013; Streicker et al., 2010). Such relationships cannot be drawn from the current bee–virus literature due to lack of data, and anyhow do not necessarily reveal the reservoir host. However, we do know that some bee viruses are widely distributed across host bee species (e.g., DWV and BQCV) and that others have a narrower host range (e.g., SBV) (Gisder and Genersch, 2017; Tehel et al., 2016). Although *Bombus* species—which are phylogenetically close to *Apis* (Table 1)—have often been found to harbor viruses that are also in honeybees, these patterns most likely reflect investigator effort rather than real biological pattern. Clearly, more information on the primary or natural hosts is needed for understanding the historical exposure and future risks posed by RNA viruses to different populations or species of bee.

Nonetheless, some information and putative predictions can be made. Bee assemblages are integrated into complex multihost flower-visitor networks. Due to their inherent complexity, the relationship between pollinator networks and virus transmission both within and among bee species is unknown. Laboratory and semifield studies using bee pathogens, some including viruses, have demonstrated that transmission via flowers can occur (Dürrer and Schmid-Hempel, 1994), that flower visiting can lead to the movement of transmission stages from flower-to-flower by vectoring flower visitors (Graystock et al., 2015), and that different floral structures may alter the likelihood of transmission (Dürrer and Schmid-Hempel, 1994; McArt et al., 2014). Real field-scale studies have also shown that flower-visitor networks have some explanatory power when mapped onto patterns of parasite abundance across host species (Ruiz-González et al., 2012). However, flower visitation is not the only route of transmission for bee viruses. Nectar robbing of honeybee hives by other honeybees, bumble bees, or wasps—all of which are common events—and social parasitism and nectar robbing in bumble bees (Goulson et al., 2017), among others, are likely to play some, as yet unquantified role in viral transmission. Finally, in the social bees, transmission within nests is likely to be a major, again relatively unquantified mode of transmission (but see Otterstatter and Thomson, 2007; Rutrecht and Brown, 2008). Thus, while quantifying transmission via flower-visitor networks is important, it is also a key to determine what proportion of the patterns in viral prevalence across host species it can actually predict.

Quantifying these networks, however, is itself hugely challenging. Networks have a significant temporal component, which has been largely ignored by current snapshot studies (e.g., Ruiz-González et al., 2012). This temporal component is a combination of seasonal population dynamics and demographics, with different potential host and vector species being present at different times of year, and at different abundances across the year, including changes in the species presence and abundance of flowers themselves. While, in temperate regions, we know a lot about these changes individually, as yet nobody has mapped them onto each other at an annual scale. This is a major challenge for bee–virus biologists. Similar patterns are doubtless present in the tropics, but as yet no studies of transmission have occurred in this biome. In addition to simple changes in population abundance, the presence of different sexes and castes of bee, and other flower visitors, also change across the year. If sexes or castes vary in their resistance to viruses, which may be expected in social species given that for most of the year

worker bees are the major component of the population, this could also have significant impacts on transmission networks. Finally, bees and other flower visitors, like wasps, exist in social and nonsocial forms (Table 1). As noted earlier, due to transmission within colonies, this could have dramatic impacts on transmission patterns via flowers through mass action effects. If pathogen prevalence builds up in colonies across the year (e.g., Rutrecht and Brown, 2008), then the contribution of social insects to transmission networks will also increase, and most likely disproportionately to the contribution of other nonsocial flower visitors.

The quantification of directionality in floral transmission networks may enable the identification of reservoir hosts, which would play a central role in transmission. It is at this point that the realms of ecology and evolution overlap again for these RNA viruses. If reservoir hosts exist in bee–virus systems, then we should expect adaptation of the virus to this reservoir host, with concomitant effects on infectivity and virulence within and across species (reviewed by Rigaud et al., 2010). Such reservoirs may be particularly important in temperate systems, where host population sizes decrease dramatically over winter for the majority of bee species.

A final but important consideration for natural populations of bees is that other stressors such as energetic stress and biocides can act synergistically with pathogens to negatively affect both individual and colony-level fitness (Bryden et al., 2013; Di Prisco et al., 2013; Doublet et al., 2015; Manley et al., 2017; Potts et al., 2016). These should also be taken into consideration as potential ecosystem-level factors that can further shape virus transmission and evolutionary trajectories across pollinator networks.

4. EVOLUTIONARY AND ECOLOGICAL PRINCIPLES OF DISEASE EMERGENCE: RELEVANCE FOR BEE RESEARCH

In the final section of this review, we shift emphasis to a discussion of general principles in disease ecology and their relevance to emerging bee viruses. We focus on insights derived from well-known study systems as well as theoretical developments. We pay particular attention to three different but overlapping topics in the molecule-to-ecology framework: (i) molecular mechanisms as determinants of pathogen life history, (ii) virulence as a balance between within- and between-host selection, and (iii) ecosystem disturbance as a driver of emergence.

4.1 Host–Pathogen Molecular Interactions Determine Virus Life History Attributes

Obtaining molecular-level mechanistic details of how pathogens interact with their hosts is essential for understanding key life history parameters of infection such as pathogen survival, which is achieved by evading the host's immune system (Schmid-Hempel, 2009), and pathogen growth, which is achieved by extracting resources from the host for replication. Properties such as host immune evasion and replication are often associated with damage to the host (pathogenesis). For example, HCV cell entry exploits a single phospholipid-modifying enzyme from the host, PLA2G16, which is recruited to the site of viral capsid attachment. This host-exploiting mechanism is used by diverse picornaviruses to enable genome delivery into mammalian host cell (Staring et al., 2017). Remarkably, the 2A protein of some picornaviruses is even derived from homologues of *PLA2G16* (Hughes and Stanway, 2000), suggesting these viruses have coopted host proteins into their genomes to accelerate host cell entry. Immune evasion mechanisms may also be passive, as in the case of HIV, where an extremely high mutation rate is sufficient to keep ahead of the human adaptive immune system (Rambaut et al., 2004). Or viruses may passively evade host immunity by entering a phase of latency, which could evolve as a successful evolutionary strategy under stable host population conditions (Sorrell et al., 2009). Virus latency is common in alphaherpesviruses such as Marek's disease virus (MDV) of poultry, which typically cause mild, chronic infections. Interestingly, human-driven disturbance in the form of widespread vaccination and agricultural intensification is thought to have driven the emergence of highly virulent viruses and a reduced role for latency in the infection cycle of the virus (Osterrieder et al., 2006; Trimpert et al., 2017).

Unlike vertebrates, insects do not have an adaptive immune system, outwardly making host immune interactions a comparatively simpler process to understand. Despite this, the precise mechanisms involved in host immune evasion remain poorly characterized in insect viruses, although some progress has been made in *Drosophila*. Here, evidence points to the exploitation of clathrin-mediated phagocytosis during cell entry in *Drosophila C virus* (DCV) (Bonning and Miller, 2010). DCV (family *Dicistroviridae*) is a relative of the widespread bee viruses BQCV and ABPV. In such viruses, translation is mediated by two IRESs (internal ribosomal entry sites) as opposed to one in other Picornavirales (such as DWV). Dicistrovirid IRESs fool the host ribosome by binding the 40S and 60S ribosomal subunits where the tRNAs and mRNA would normally be found (Pestova et al., 2004).

To counter this, insects including bees (Brutscher and Flenniken, 2015) employ RNAi as a key antiviral defense mechanism, which targets viral RNAs for intracellular destruction (in contrast to mammals; Bogerd et al., 2014; Seo et al., 2013; although see Li et al., 2016). Unsurprisingly, insect viruses have in turn evolved silencers of host RNAi. In DCV, a suppressor protein binds long dsRNAs, which prevents their destruction by inhibiting the enzyme dicer from processing dsRNAs into siRNAs. Interestingly, micro-RNAs (miRNAs) are not manipulated by DCV. This is interesting from a pathogen life-history perspective because miRNAs are required for normal insect development. The restricted targeting of dsRNA by DCV suggests that insect viruses have evolved sophisticated mechanisms to facilitate long-lasting persistent infections (van Rij et al., 2006). How the arrival of the *V. destructor* mite may have affected these carefully orchestrated host-manipulation mechanisms in bees (if present) represents an interesting open question, because a persistent infection strategy that is advantageous under stable host population conditions may suddenly become disadvantageous in the presence of a biotic vector. A further point here is that *V. destructor* mites vector virus directly into the host hemolymph during host feeding, thus side-stepping the ventricular and lower gut barrier, where environmental (e.g., gut microbiota composition), physical (e.g., cell lumen) as well as innate immune defenses can combine to impose major obstacles to infection (Engel et al., 2016; Kwong and Moran, 2016).

4.2 Virulence as a Balance Between Within- and Between-Host Selection

In animal systems, virulence is typically defined as the negative impact of a pathogen on host fitness. In natural coadapted host–pathogen systems, a well-established body of theory and empirical research places transmission between hosts as a key consideration in virulence evolution (Schmid-Hempel, 2011). Virulence is hypothesized to evolve to a level that maximizes R_0, the basic reproductive number of a pathogen, which must be above 1 for an infection to spread in a host population. However, selection at the within-host cellular level can shape and even be in conflict with selection for between-host transmission (Alizon et al., 2011; Mideo et al., 2008). Clearly, both levels are essential: while a pathogen must be able to infect a novel host, its fitness is zero unless it can also successfully invade other hosts. While the validity of the trade-off framework is generally supported by theory and experimental research (reviewed in Cressler et al., 2016), it is questionable whether a strict two-dimensional trade-off between transmission

and virulence is realistic in most cases (Ebert and Bull, 2003), for example, in pathogens entering novel host populations (Weiss, 2002). As discussed in the previous section, a focus on the mechanism of pathogenesis, which is inherently system-specific, can provide significant benefits to the study of virulence evolution (Frank and Schmid-Hempel, 2008). In particular, the link between pathogenesis, the underlying process by which virulence is generated, and its role in host immune evasion needs to be known so as to predict the evolutionary trajectory of virulence—up, down, or stationary (Schmid-Hempel, 2009). And because a coevolved link between virulence and transmission is absent in emerging diseases, understanding the mechanisms that enable pathogens to survive and grow in potentially novel hosts (e.g., non-*Apis* bees) or host conditions (e.g., *V. destructor*-vectored conditions) is critical to explaining virus virulence. We have hypothesized that significant shifts in selection accompanied the arrival of the *V. destructor* mite (Fig. 2), potentially resulting in conditions that favored the emergence of differentially virulent viruses (McMahon et al., 2016). But the effect on other bee species of honeybee/*V. destructor*-driven changes in virus adaptation and prevalence remains unclear. More certain is the effect of increased prevalence of *V. destructor*-vectored viruses, which may enhance the frequency of encounters between viruses and a higher diversity of novel insect hosts, making epidemics in novel species potentially more likely.

In such cases, virulence is an incidental property resulting from (i) mechanistic and infection cycle similarities between the original and novel host environment and (ii) direct selection on pathogens to adapt to the immune system of the novel host. The strength of indirect selection at the population-level will initially be minor. This arises because host–pathogen interactions are sequential: selection to avoid host clearance precedes selection for optimal transmission to an uninfected host. Indeed, in the original formulation of the trade-off model, it is only indirect selection that results from between-host competition between pathogens that determines optimal population-level virulence (Anderson and May, 1982; Ewald, 1983).

As a possible example, in the recent West African Ebola epidemic, a single mutation originating in an ancestral node of the Ebola lineage (A82V in the glycoprotein) could have been the major driver of the vast majority of human infections during the 3-year (2013–16) epidemic. The mutation is associated with increased virus load (and virulence) in humans but not rodents or canines (Diehl et al., 2016). Furthermore, the A82V mutation—combined with subsequent mutations conferring primate-specific infectivity—carries the cost of reduced infectivity in cells from bats (Urbanowicz et al., 2016),

which are the presumed original hosts of the 2013–16 human outbreak. Although it is not clear whether between- or within-host selection was the dominant driver of Ebola emergence, the fact that the predominant mutation emerged very early on in the epidemic shortly after the putative host-switch event implicates adaptation toward the novel human immune system as the primary cause of enhanced Ebola virulence (Bedford and Malik, 2016). Similar patterns of molecular adaptation have also been observed in the early epidemic dynamics of influenza (Chen et al., 2006).

A related component of this is whether host phylogenetic relatedness, and the assumed similarity between immune systems, could be a useful predictor of pathogen spread or virulence (Faria et al., 2013; Streicker et al., 2010). A comparative analysis of pathogens that have jumped species and even kingdom barriers shows that pathogenicity toward novel hosts may be based on traits that evolved to ensure survival in the original host, and/or are associated with the exploitation of highly conserved host defense molecules such as antimicrobial peptides (van Baarlen et al., 2007). Although studies in this area are lacking in bees, there is some evidence in *Drosophila* that while original-to-new host relatedness per se may not be correlated with virulence, host phylogeny does play a role in explaining variation in virulence (Longdon et al., 2015).

It is worth noting that novel host–pathogen interactions can be highly maladaptive, both for the host and pathogen, with hosts mounting immune responses that inflict major self-damage (Graham et al., 2005), or viruses undergoing short-sighted within host adaptation that inadvertently kills the host and prevents transmission (Levin and Bull, 1994). Clearly, outcomes are likely to be highly host–pathogen specific, reflecting complex interactions between molecular and evolutionary ecology (Schmid-Hempel and Ebert, 2003). Resolving molecular mechanisms for a wide range of host–virus systems and alternative transmission routes will help to generate predictions about how novel hosts and viruses play out in nature.

4.3 Human-Driven Ecological Disturbances as Drivers of Disease Emergence

Changes in the dynamics and ecology of host–parasite systems play important roles in the emergence of parasites, pathogens, and disease. While host–parasite systems are always changing in response to natural variation in weather, population dynamics, distribution, and other such factors, human impacts have led to much more rapid state changes in the epidemiology of disease agents. Major anthropogenic drivers of emergence include the global

movement of crops and domesticated animals, invasive species, changes in landscape structure, the global use of agrochemicals (Mitchell et al., 2017), and climate change (Lafferty, 2009), which will lead to dramatic shifts in the distributional ranges of hosts and their parasites (Estrada-Peña et al., 2014). One of the earliest examples of such shifts was the inadvertent introduction of rinderpest virus to Africa from Asia, caused by the transfer of infected Asian cattle to Ethiopia. Rinderpest rapidly spread through direct transmission across sub-Saharan Africa, devastating African cattle populations as well as wild populations of buffalo, giraffe, and wildebeest (Normile, 2008). In bees, the transport of nonnative bee, including non-*Apis* bees, has led to the spread and emergence of disease. Examples include the use of nonnative commercially produced bumble bees in South America (Maharramov et al., 2013; Schmid-Hempel et al., 2014), Japan (Goka et al., 2006), and New Zealand (Dafni et al., 2010).

Such anthropogenically driven changes in the dynamics of directly transmitted viruses are only part of the picture. In fact, the dynamics of vector-transmitted viruses are both more complex and less easy to manage and predict. For example, environmentally driven shifts in the ranges of vectors such as the Asian tiger mosquito may enable the emergence of viruses in naïve host populations (Taber et al., 2017), while the rapid spread of the invasive mite *V. destructor* through its novel host *A. mellifera* has had dramatic impacts on the viral landscape within this host (Martin et al., 2012; Mondet et al., 2014; Wilfert et al., 2016). Vectors that share multiple host species can also be dramatically affected by anthropogenically driven changes, leading to host-switching opportunities in the viruses they transmit. Changes in host assemblages driven by invasive host species and habitat fragmentation can enhance encounter rates between vectors and primary hosts, leading to higher prevalence and transmission of the vectored pathogen (Allan et al., 2003; Hoyer et al., 2017). All of these changes in dynamics may have direct impacts on viral evolution because patterns of transmission and host encounter act as agents of natural selection on viral functional traits.

While our understanding of these selective pressures and their effects is in its infancy, we can make some broad predictions based on fundamental evolutionary principles. Shifts in host or vector range that disrupt current host–viral systems should select for more generalism in viruses with respect to host infection and exploitation strategies. In contrast, where landscape changes strengthen current vector–host interactions, higher viral specificity may result. The higher viral prevalence that may arise from both of these drivers is likely to produce higher genetic variability in the viral population,

providing the substance on which further selection can act. However, how such trends might affect traits such as viral virulence is unclear and may depend upon the idiosyncratic details of particular host–vector–virus systems. It is for this reason that we believe major research effort should be focused on exploring the direct and indirect impacts of *V. destructor,* as discussed throughout this review. In the near term, we look forward to rapid advances in this area, which will enhance not only our understanding of bee–virus systems but also promise to shed light on viral evolutionary and ecological dynamics in general.

5. OPEN CHALLENGES AND CONCLUDING REMARKS

Two features currently hamper the study of the bee–virus system. First, the field lacks a bee cell culture in which to replicate bee viruses. Though infective clones (e.g., of DWV; see Lamp et al., 2016) may go some way to resolve the shortfall, the field would benefit from a system of in vitro viral cultivation. Second, though bees can be readily collected from flowers in the field and their individual movements even tracked in real time (Wolf et al., 2016), we have little idea of their long-distance natal dispersal (either before or after hibernation), making it difficult to evaluate the potential movement of them and the viruses they harbor over two or more host generations (Wilfert et al., 2016), although the genetic tools to measure bumblebee family lineages across landscapes over multiple seasons are now well established (Carvell et al., 2017). Combined with the advances in landscape genomics (Lozier and Zayed, 2017) and phylodynamics (Grenfell et al., 2004), these developments may help by allowing finer inference of virus dispersal and host-switching events. Given the increase in attention now being paid to bees and their pathogens, we are confident that many of these issues can be resolved. One prediction we can already make: research into the bee–virus system will surge in the coming years, and will bring with it a much richer and deeper understand of virus ecology and evolution, with relevance not only for the health and wellbeing of bees but of other host–virus systems, including *Homo sapiens*.

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