Title

Persistent effects of management history on honeybee colony virus abundances

Authors

Bartlett, Lewis J
Boots, Michael
Brosi, Berry J
et al.

Publication Date

2021-02-01

DOI

10.1016/j.jip.2020.107520

Peer reviewed

Persistent effects of management history on honeybee colony virus abundances.

Lewis J. Bartlett[1,4,5*], Michael Boots[1,2], Berry J. Brosi[3], Jacobus C. de Roode[4], Keith S. Delaplane[6], Catherine A Hernandez[2], Lena Wilfert[1,7]

1 Centre for Ecology and Conservation, University of Exeter, TR10 9FE, UK

2 Department of Integrative Biology, University of California, Berkeley, CA, 94720, USA

3 Department of Environmental Sciences, Emory University, Atlanta, GA, 30322, USA

4 Department of Biology, Emory University, Atlanta, GA, 30322, USA

5 Center for the Ecology of Infectious Diseases, University of Georgia, Athens, GA, 30602, USA

6 Department of Entomology, University of Georgia, Athens, GA, 30602, USA

7 Institute of Evolutionary Ecology and Conservation Genomics, University of Ulm, Ulm, D-89069, Germany

* l.bartlett@exeter.ac.uk

Author ORCiD Information

LJB: 0000-0002-4418-8071

BJB: 0000-0002-9233-1151

CAH: 0000-0002-1093-2747

KSD: 0000-0001-9323-441X

JCdR: NA

LW: 0000-0002-6075-458X

MB: 0000-0003-3763-6136

ASBTRACT

Infectious diseases are a major threat to both managed and wild pollinators. One key question is how the movement or transplantation of honeybee colonies under different management regimes affects honeybee disease epidemiology. We opportunistically examined any persistent effect of colony management history following relocation by characterising the virus abundances of honeybee colonies from three management histories, representing different management histories: feral, low-intensity management, and high-intensity “industrial” management. The colonies had been maintained for one year under the same approximate ‘common garden’ condition. Colonies in this observational study differed in their virus abundances according to management history, with the feral population management history showing qualitatively different viral abundance patterns compared to colonies from the two managed population management histories; for example, higher abundance of sacbrood virus but lower abundances of various paralysis viruses. Colonies from the high-intensity management history exhibited higher viral abundances for all viruses than colonies from the low-intensity management history. Our results provide evidence that management history has persistent impacts on honeybee disease epidemiology, suggesting that apicultural intensification could be majorly impacting on pollinator health, justifying much more substantial investigation.

KEYWORDS

Apis mellifera, industrial agriculture, honeybee, virus, management, pathogen.

INTRODUCTION

Loss of pollinators, both managed and wild, is of current and growing concern for both agriculture (Aizen and Harder, 2009; Brosi et al., 2008; Gallai et al., 2009) and conservation (Kleijn et al., 2015; Potts et al., 2016, 2010; Williams and Osborne, 2009). Bee pollinators are crucial for ecosystem function (Brosi and Briggs, 2013; Corbet et al., 1991) and agricultural fruit set (Garibaldi et al., 2013; Klein et al., 2007) and fruit quality (Knapp et al., 2017). They are also recognised for their cultural and recreational value (Bingham, 2006; Mace et al., 2012; Watson et al., 2011). One critical driver of bee declines is parasites and

infectious disease (Becher et al., 2013; Kent et al., 2018; Manley et al., 2015; Potts et al., 2010).

Managed honeybees, especially the western honeybee *Apis mellifera* L., have experienced emerging and re-emerging outbreaks of numerous parasites (Martin et al., 2012; McMahon et al., 2018, 2016; Mondet et al., 2014; Wilfert et al., 2016), and elevated losses to infectious disease for a variety of reasons (Genersch et al., 2010; Pettis and Delaplane, 2010; vanEngelsdorp et al., 2009; vanEngelsdorp and Meixner, 2010). Pollinator vulnerability to pathogens can be aggravated by invasive pests, poor forage, pesticide exposure, behavioural stress, and lack of bee genetic diversity (Aronstein et al., 2012; Bartlett et al., 2018; Conte et al., 2010; Dolezal et al., 2016; Forsgren and Fries, 2010; Goulson et al., 2015; Neumann and Carreck, 2010; Oldroyd, 2007; Pasquale et al., 2013; Rumkee et al., 2017; Sánchez-Bayo and Goka, 2014; van der Zee et al., 2012; Yang and Cox-Foster, 2005; Zee et al., 2014), all of which interact with intensification of management. Additionally, there is concern that intensifying pollinator management increases abundances of and selection for more virulent pathogens (Brosi et al., 2017; Graystock et al., 2016). As evidence mounts that managed pollinator pathogens can spill over into their wild counterpart populations (Cohen et al., 2017; Fürst et al., 2014; Graystock et al., 2016, 2015, 2013; Manley et al., 2019, 2015; McMahon et al., 2015), understanding the epidemiology of managed pollinators becomes increasingly important.

Pollination has intensified as a managed agricultural input in recent decades (Aebi et al., 2012; Aizen and Harder, 2009; Delaplane and Mayer, 2000; Graystock et al., 2016, 2013; Moritz and Erler, 2016; vanEngelsdorp and Meixner, 2010). Beekeeping in the USA has undergone a surge in industry-wide intensification (Brosi et al., 2017; Corbet et al., 1991) – reflecting changes in the wider agricultural environment experienced by beekeepers throughout the 20th century (Odoux et al., 2014; Otto et al., 2016). This intensification introduces profound changes in the population-level underpinnings of managed honeybee epidemiology. Critical aspects include much higher stocking densities (Seeley and Smith, 2015), cross-continental migratory beekeeping (Simone-Finstrom et al., 2016; vanEngelsdorp et al., 2013; Welch et al., 2009; Whynott, 1991), and pesticidal and antibiotic treatment for pests and pathogens (Delaplane, 2001; Dietemann et al., 2012). All of these are partially driven by moves away from honey production towards pollination services as a source of income (Bartlett et

al., 2018; Gallai et al., 2009; Hodges et al., 2001; Southwick and Southwick, 1992; USDA - NASS, 2012; Whynott, 1991).

There are now a number of theoretical studies that examine how aspects of intensified beekeeping could impact pathogen dynamics (Bartlett et al., 2019; Booton et al., 2017; Brosi et al., 2017; Giacobino et al., 2014; Lindström et al., 2008; Nolan and Delaplane, 2017; Simone-Finstrom et al., 2016; Wilfert et al., 2016). This includes predictions that feral *A. mellifera* populations will experience fewer pathogen outbreaks compared to their managed counterparts (Brosi et al., 2017; Seeley and Smith, 2015), on the basis that wild colonies are smaller and densities of wild colonies across a landscape much lower (Seeley, 2007), leading to lower transmission rates and disease burdens (Loftus et al., 2016), and that a lack of management leads to greater selection for social immunity behaviours or tolerance of parasites (Thaduri et al., 2019). Likewise, studies have hypothesised that traditional beekeeping – characterised by lower bee densities and lower rates of movement – may sustain lower pathogen burdens than modern high-intensity operations (Dynes et al., 2017; Mõtus et al., 2016; Nolan and Delaplane, 2017). There is some evidence of these adaptations amongst *Varroa* when comparing parasites taken from feral honeybees to those from managed populations (Dynes et al., 2020). However, recent modelling predicts that local (apiary-scale) apicultural intensification leads to only limited increases in pathogen prevalence, because even in small-scale beekeeping few individual bees can escape contracting a ubiquitous pathogen (Bartlett et al., 2019). Infection severity further depends on factors affecting honeybee health at a more primary level – including factors such as forage availability and quality, genetic diversity or predisposition towards emphasis on immune-behaviours, or pesticide exposure as detailed prior. Colony-level viral abundances have been used as indicators, or identified as drivers, of colony collapse (Dainat and Neumann, 2013; Highfield et al., 2009; McMenamin and Genersch, 2015); additionally, viruses are a current focus of research examining the spill-over of honeybee pathogens into other bee populations (Manley et al., 2019, 2019, 2015; McMahon et al., 2015; Wilfert et al., 2016). Understanding how honeybee management affects colony virus abundances is therefore a critical part of wider bee epidemiology, including the possibility that management regimes have selected for differential evolution of parasites experiencing different host populations of honeybees (Brosi et al., 2017).

Pertinent to understanding bee health is the movement of honeybee colonies across landscapes. This is carried out as part of industrial migratory (nomadic) beekeeping, a management practise already posited to influence honeybee viral epidemiology (Brosi et al., 2017; Welch et al., 2009; Whynott, 1991). A kind of nomadic beekeeping is simulated when queens, packages of bees, and small incipient “nucleus” colonies are produced in one region and shipped to another. It is estimated that the production of bees for export, domestic or international, constitutes approximately 20% of all beekeeping industry in the United States (Ferrier et al., 2018). As colonies move between locations, or indeed between operations under different management regimes, they are likely to both acquire and transmit pathogens, including viruses. Higher viral abundances not only impact colony health but may make this transmission more likely. Here we opportunistically examine if colony management history persistently affects viral abundances; this work has implications for the management and epidemiology of managed honeybees and for viral spill-over into non-*Apis* species.

To begin to examine this question, we opportunistically sampled a ‘common garden’ occurrence where honeybee colonies had been sourced from three different management histories: feral populations, a ‘low-intensity’ traditional operation, and a ‘high-intensity’ industrial operation; these are the same populations studied by Dynes et al. (2020) who differentiated the burden on colonies caused by *Varroa* from feral vs managed population of honeybees. In this observation study, pre-dating Dynes et al. (2020), colonies had been maintained for one year under the same management regime and in approximately the same environment. We characterised the virus abundances of these colonies to ask whether there was evidence that colony management history had a persistent (>1 year) legacy effect.

A persistent effect of colony management history would indicate that the ecological history of a colony has a meaningful and lasting effect on its viral dynamics, and consequently its potential role in spill-over into other colonies or bee populations. There are numerous possible causes of this, including both the health and genetics of the host, but also the evolutionary history and past selection of pathogen (and putative parasite vector) strains circulating in these different honeybee populations. The plausible, three-way GxGxG interactions are challenging to investigate and require justification from initial exploratory studies. Interrogating these possible causes requires large scale, intensive

experiments and sampling to differentiate apiary and transient source effects, to specifically focus on honeybee vitality, viral characteristics, or adaptive host-pathogen interactions, and overcome pragmatic problems with field experiments. This study does not tackle these large-scale experimental challenges, but does justify their pursuit through an observational documentation of circumstantial evidence that management style and management history underpin bee pollinator epidemiology.

METHODS

HONEYBEE COLONY SOURCING AND MAINTENANCE

We sampled 14 colonies from each of three different management histories sourced in 2013. Two management histories were managed backgrounds (beekeeping operations), which we refer to as ‘high-’ and ‘low-’ intensity management histories. The high-intensity management history colonies came from a commercial beekeeping operation in south Georgia fully fitting the industrial paradigm, in which colonies are maintained in extremely large, dense apiaries (potentially many hundreds of colonies), subject to frequent management interventions such as re-queening and chemical application, and trucked annually across the USA to pollinate crops and collect diverse honey floral types (Brosi et al., 2017; Welch et al., 2009). The low-intensity management history colonies came from a smaller operation representative of most beekeepers for whom beekeeping is a hobby or side-line business; in such low-intensity operations, colonies are typically maintained at reduced densities in smaller stationary apiaries, receive fewer severe management interventions, and any colony relocation is limited to much smaller distances at local or, at most, regional scales. It is important to note that these operations still practice active management, and they are not to be confused with “natural” or “organic” treatment-free beekeeping whose adherents often practice little or no invasive management. We cannot name the suppliers due to data protection and commercial interest concerns. The third management history sources were colonies trapped as reproductive swarms from populations of feral honeybees living in either the federally designated wilderness area constituting part of the Okefenokee Swamp in southeast Georgia USA or the Oconee National Forest in central Georgia USA. Such areas preclude any agricultural activity, and the size of these areas makes it likely that these feral swarms are not ‘recently feral’ but from sustained feral populations with potentially little immigration from managed

honeybee populations, in line with other such studied populations identified in the USA (Schiff et al., 1994; Seeley, 2007). Collections were undertaken with approval and in line with federal and state laws governing the use of designated wilderness areas for scientific research; in particular, we secured research permits from the Okefenokee National Wildlife Refuge. These three management history sources are the same as those from which *Varroa* were sourced for study by Dynes et al. (2020).

All colonies were then maintained in standard 10-frame Langstroth equipment hives in an approximate ‘common garden’ approach, using three separate apiaries surrounding one location (University of Georgia Horticultural Farm, Watkinsville, GA, USA). Colony maintenance was undertaken by a team of professional apicultural technicians. Colonies were separated by management history into three apiaries around this location, with each location at least 5km from any other known apiary to help prevent cross-inoculation (Dynes et al., 2017). Isolating each background in separate apiaries was a crucial part of this observational study, as this prevents any rapid displacement of ‘host -native’ pathogen strains by ‘alien’ strains, which rapidly spread within apiaries (Bartlett et al., 2019) and underpins one hypothesis of why management may influence honeybee epidemiology (Brosi et al., 2017); this isolation distance requirement is a current limiting factor on efforts to produce better ‘designed experiments’ interrogating the question this manuscript addresses. Colonies were maintained as though they were ordinary colonies under beekeeper care, following standard practise for the region, with the exception that no *Varroa* mite control treatments were applied. Any queen supersedure that occurred was a result of natural queen replacement by an open-mated daughter; no queens were intentionally replaced with outsourced genetic stock; it is thought that more frequent supersedure is adaptive in reducing pathogen burdens in feral populations (Brosi et al., 2017), and may therefore have a role in governing persistent honeybee viral dynamics. Colonies were managed from the summer of 2013 onwards, with samples for this study collected in May 2014, meaning approximately one year of common garden management for all colonies, varying by one or two months. All individuals in the colony, excepting in some instances the queen, were therefore replaced multiple times by subsequent generations between transplantation and sampling.

SAMPLE COLLECTION AND MOLECULAR PROCESSING

To compare the virus abundances of colonies, we randomly selected 30 adult honeybees from the brood frames of each colony. Samples from all colonies were gathered during foraging hours within a three day period to eliminate potential seasonal effects on viral dynamics (Sumpter and Martin, 2004; Tentcheva et al., 2004). For each sample, the 30 live honeybees were sealed in a 50ml centrifuge tube and immediately placed on dry ice before storage at -80 C^{o}.

Samples were processed for RNA extraction and conversion of RNA to cDNA on-site at the UGA Horticulture Farm; cDNA sequence targets were quantified at U.C. Berkeley using digital droplet PCR (ddPCR). An expanded protocol including all volumes, reagents, and extraction conditions is provided in the Appendix, with key points summarised here for brevity.

RNA was extracted from the thirty sampled honeybees in per-colony pooled batches, using similar protocols for RNA extraction by phase-separation techniques as seen elsewhere across RNA studies (Simms et al., 1993), including commonly for studies on bee viruses (Manley et al., 2019; Wilfert et al., 2016). RNA was converted to cDNA using a standard first-strand RT-PCR synthesis protocol with random hexamers (Promega, USA) and M-MLV enzyme (Amresco, USA), and measured with a NanoDrop (ThermoFisher; see Table S1). After RNA extraction but prior to cDNA synthesis we introduced ‘no-sample’ controls of molecular-grade water to check for potential contamination in downstream analysis. We quantified a number of viral targets by ddPCR: the ABPV/KBV/IAPV (here ‘AKIV’) ‘acute paralysis virus complex’ (de Miranda et al., 2010a), chronic bee paralysis virus (‘CBPV’), slow bee paralysis virus (‘SBPV’), sacbrood virus (‘SBV’), black queen cell virus (‘BQCV’), two deformed wing virus (‘DWV’) variants DWV-A and DWV-B (‘VDV-1’) (McMahon et al., 2016, 2015; Wilfert et al., 2016), and four strains of Lake Sinai virus (‘LSV1-4’) (Daughenbaugh et al., 2015; Ravoet et al., 2015). We also quantified a common housekeeping gene, *Apis mellifera* β-actin, which is expressed at a relatively constant level in honeybee tissues, therefore providing a reference level for viral titre (Lourenço et al., 2008). We used BioRad’s QX200TM Droplet Digital™ PCR system (ddPCR) to quantify sequence targets specific to the housekeeping gene and eight viral sequence targets – see Table 1 for targets and references. ddPCR uses emulsions of microscopic droplets to perform many thousands of small volume PCRs, ideally forming tight ‘clusters’ of fluorescence values (Miotke et al., 2014; Pinheiro et al., 2012). The proportion of droplets in each cluster can be used to estimate the

concentration of the target sequence in the original sample. All primer sequences have been previously tested and used in the honeybee virus literature for equivalent qPCR virus quantification studies (see Table 1).

Sequence targets were grouped such that DWV-A and DWV-B were quantified on the same plate simultaneously, as were ABPV/KBV/IAPV and SBPV (see Table 1). The five other sequence targets were subject to separate reactions owing to different reaction temperatures. Raw fluorescence data was then exported for further handling and statistical analysis.

VIRAL QUANTIFICATION

All experimental samples tested positive for all sequence targets, we therefore forwent positive controls for main quantification as they proved difficult to acquire for some targets. Our negative controls, introduced prior to the M-MLV step to generate cDNA, showed the expected tight bands of extremely low background fluorescence (Supp. Fig. S1) indicating an absence of sequence targets. Our experimental samples showed large variability in droplet fluorescence both between samples and within each sample, for both the housekeeping gene and viral sequence targets (Supp. Fig. S1). This was indicative of large differences in between-sample RNA/cDNA quality and inhibitor concentrations carried over from extraction. cDNA synthesis is especially sensitive to inhibitor activity when processing honeybee RNA (Forsgren et al., 2017). Large variability of positive droplet fluorescence amplitudes in ddPCR is a demonstrable effect of increased inhibitor concentrations (Dingle et al., 2013). Additionally, our target sequence concentrations were high enough that almost all droplets appeared positive (samples were ‘flooded’). Limitations in time and resources prevent us from repeating quantification using diluted samples.

To account for the suspected disruptive action of variable inhibitor concentrations and inter-sample variability in sequence quality, we compared fluorescence readings for each viral target to the fluorescence readings for the β-actin housekeeping gene. While work (unfortunately subsequent to this experiment) has documented the rapid loss of certain mRNA targets including β-actin following collection of live honeybees(Forsgren et al., 2017), we note our samples were placed immediately on dry ice and so were quickly euthanised before storage at -80° within 2 hours of collection, which should preserve β-actin as a suitable mRNA standard. Following this approach, within each sample and for each target sequence, each droplet will vary in amplitude based on 1)

inhibitor concentrations (Dingle et al., 2013) and 2) concentration of the target sequence in the droplet (Corbisier et al., 2015; Miotke et al., 2014; Pinheiro et al., 2012). Between-sample variation caused by differences in sample quality can be controlled for using the β-actin housekeeping gene, which will have been equally represented across all samples at the point of live *A. mellifera* collection (Lourenço et al., 2008). We therefore use the relative fluorescence of viral ddPCR in comparison to the sample's β-actin fluorescence as our measure of viral abundance in each sample, essentially a ratio of the concentration of β-actin sequence to viral target sequence in each sample.

DATA PROCESSING AND STATISTICAL ANALYSIS

We conducted all data handling and analysis in R (v 3.6.1. 'Action of the Toes') (R Core Team, 2019). We provide a full annotated R script of analysis for further detail and reproducibility (see Appendix and GitHub repository https://github.com/LBartlett/BackgroundViromes2020.git). We exported all raw fluorescence reads from the BioRad ddPCR system for downstream analysis. We excluded our negative control samples, and then calculated a mean fluorescence for each target sequence for each sample (9 targets x 42 samples). We tested for batch effects on sample quality using a one-way ANOVA to test whether sample (colony) management history had a significant effect on the mean fluorescence of the housekeeping gene target sequence, β-actin. For the eight viral sequence targets, we scaled each sample's mean fluorescence values against that sample's β-actin mean fluorescence to calculative a 'relative viral abundance' metric for analysis.

We undertook a community approach to test for grouping of viral community by management history using an adonis analysis. We also used a non-metric multidimensional scaling (NMDS) as a dimensionality reduction visualisation of the same viral community dissimilarity matrix and plotted the NMDS by colony management history. We used a Euclidean dissimilarity index, as our measure of relative viral abundance is an unusual metric for community ecology (it is a continuous measure that can be negative or positive, whereas typically discrete and positive counts of organisms are used in community similarity indices), and Euclidean distances are widely used across a wide variety of natural sciences and are therefore defensibly robust to many data types (Chao et al., 2006). We conducted both the adonis and NMDS using the 'vegan' package for R (Oksanen et al., 2019).

We further analysed these data to gain more detailed understanding of how different viral titres varied across the management backgrounds, using a linear mixed modelling approach, accounting for our mixed-design using the ‘afex’ package (Singmann et al., 2019) which relies on the ‘lme4’ linear mixed modelling engine (Bates et al., 2015, p. 4). The response variable was the relative amplitude; interacting fixed effects were virus (‘target’) and management history (‘treatment’); random effects were specified as virus (‘target’) nested under colony, to account for our repeated measures as part of our mixed design. We followed this with post-hoc testing using the ‘emmeans’ package (Lenth, 2019) to identify pairwise differences between management histories for each viral target, with *p*-values corrected for multiple comparisons using the Benjamini-Hochberg correction (Benjamini and Hochberg, 1995).

Table 1 - Primers used in this study to target specific cDNA sequences for amplification and quantification using ddPCR.

Target	**Forward Primer Sequence**	**Reverse Primer Sequence**	**Amplicon Length**	**T_R - Reaction Temperature (°C)**	**Reference**	**Primer Name (Forward)**	**Primer Name (Reverse)**
ABPV/KBV/ IAPV	GGCGAGCCACTATGTGCTAT	ATCTTCAGCCCACTT	401	50.0	(de Miranda et al., 2010a; Evans, 2001)	AKIF8140	AKIFR8507
CBPV	CAACCTGCCTCAACACAG	AATCTGGCAAGGTTGACTGG	276	53.0	(Ryabov et al., 2017)	CBPV1FqF1818	CBPV1FqB2077
SBPV	GCGCTTTAGTTCAATTGCC	ATTATAGGACGTGAAAATATAC	226	50.0	(de Miranda et al., 2010b)	SPV-F3177	SPV-B3363
SBV	TTGGAACTACGCATTCTCTG	GCTCTAACCTCGCATCAAC	335	54.0	(Locke et al., 2012)	SBV-F3164	SBV-B3461
BQCV	AGTGGCGGAGATGTATGC	GGAGGTGAAGTGGCTATATC	294	53.0	(Locke et al., 2012)	BQCV-F7893	BQCV-B8150
DWV-A	TGTCTTCATTAAAGCCACCTGGAA	TTTCCTCATTAACTGTGTCGTTGAT	140	57.3	(McMahon et al., 2015)	DWV-F2	DWV-R2a
DWV-B (VDV-1)	TATCTTCATTAAAACCGCCAGGCT	CTTCCTCATTAACTGAGTTGTTGTC	140	57.3	(McMahon et al., 2015)	VDV-F2	VDV-R2a
LSV 1-4	CGTGCGGACCTCATTTCTTCATGT	CTGCGAAGCACTAAAGCGTT	152	59.5	(Daughenbaugh et al., 2015)	LSV1-4-F-2157	LSV1-4-R-2309
Beta-Actin (*A. mellifera*)	CGTGCCGATAGTATTCTTG	CTTCGTCACCAACATAGG	271	52.0	(Locke et al., 2012; Lourenço et al., 2008)	Am-actin2-qF	Am-actin2-qB

RESULTS

We estimated the relative abundance of 8 viral sequence targets in 14 colonies from 3 apiaries (42 colonies total, 336 relative viral abundance values total). Each apiary represented a different colony management history (feral, low-intensity managed, or high-intensity managed) maintained under approximately equivalent field environments and the same management regime for one year.

Our adonis analysis of community composition found significant grouping of virus abundance by management history ($F_{2,39} = 2.72$, $p = 0.039$, $R^2 = 0.12$), i.e. honeybees of different management backgrounds harbour significantly different viral communities. This significant clustering was, we tentatively interpret, driven by the feral colonies and possibly the low-intensity colonies (barring one outlier) as shown visually in our two-dimensional NMDS plot (Fig. 1); stress value for the NMDS (k=2) was 0.052.

To further investigate and better understand the effect of colony management history, we used a linear mixed-effects modelling approach as described previously. We found that different viral species had significantly different relative abundances (main effect of viral species, $p < 0.0001$). We also found a significant interaction between viral species and colony management history ($p = 0.0007$), but no single effect of colony management history alone on relative viral abundance ($p = 0.16$). The corresponding data are shown in Fig. 2. We find evidence of a batch effect on sample quality; our one-way ANOVA found a significant effect of colony management history on the housekeeping gene (β-actin) mean fluorescence ($F_{2,39} = 8.23$, $p = 0.001$). However, the lack of any significant single effect of management history on our main result suggests our use of the β-actin housekeeping gene to adjust for variation in sample quality was successful.

We caution against comparisons being drawn based on relative abundance between viruses. The significant single effects of viral sequence target on relative abundance may be, at least in part, reflections of differences in efficiencies of the molecular reactions used to amplify and quantify the sequence targets, and so comparisons of relative abundance between viruses may not be biologically informative. Further, comparing copy number between different viruses with different pathologies is not informative for honeybee health. Rather, differences in copy number of the same virus between different colonies is of interest.

We undertook post-hoc testing to understand the significant interaction between colony management history and viral target. We examined the pairwise differences between colony management histories for each viral target, with p-values adjusted for multiple comparisons using the Benjamini-Hochberg correction (Benjamini and Hochberg (1995). The AKIV, LSV, and SBV sequence targets showed significant differences between management histories. Feral management history colonies had significantly lower relative abundances of AKIV compared to high-intensity management history colonies ($p = 0.0072$); however, they had significantly higher relative abundances of LSV and SBV compared to the low-intensity management history ($p = 0.0004$, $p = 0.0414$ respectively). High-intensity management history colonies appeared to have higher relative abundances of every viral target compared to the low-intensity management history colonies, and in the case of LSV this was significant ($p = 0.0399$). For BQCV, CBPV, DWV A & B, and SBPV, no significant pairwise differences were found; however, the high-intensity management history always showed a higher relative abundance compared to the low-intensity management history, even though the direction of the differences amongst these viruses varied for comparisons between the feral management history and high or low -intensity management histories (Fig. 2).

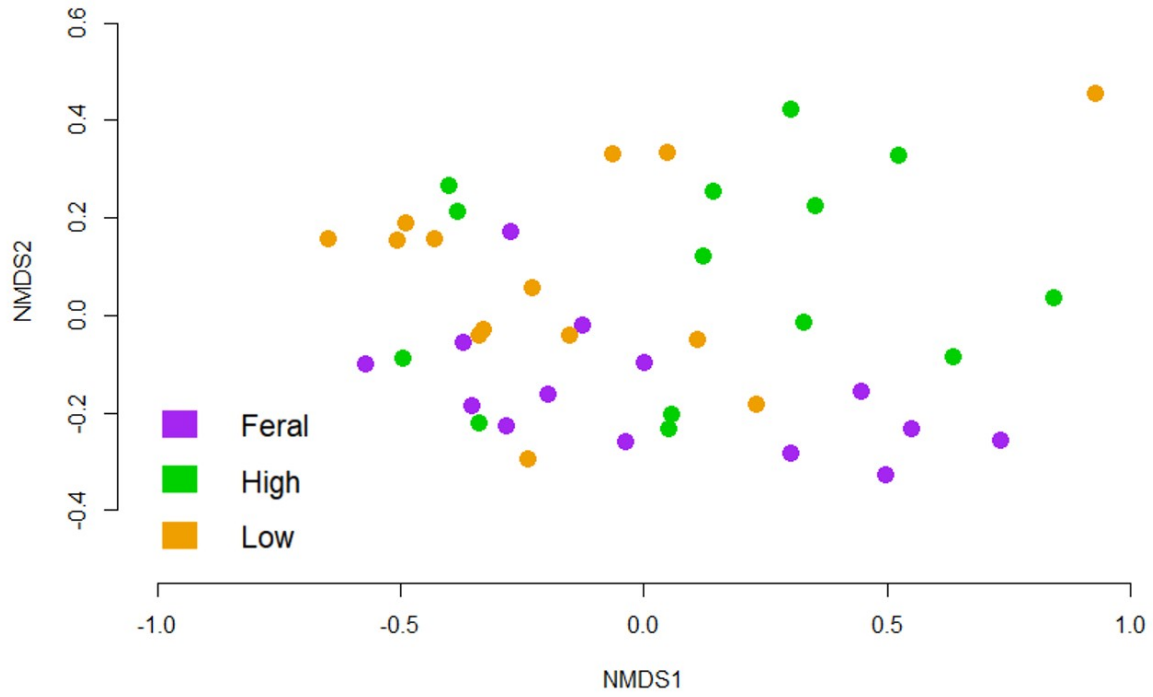
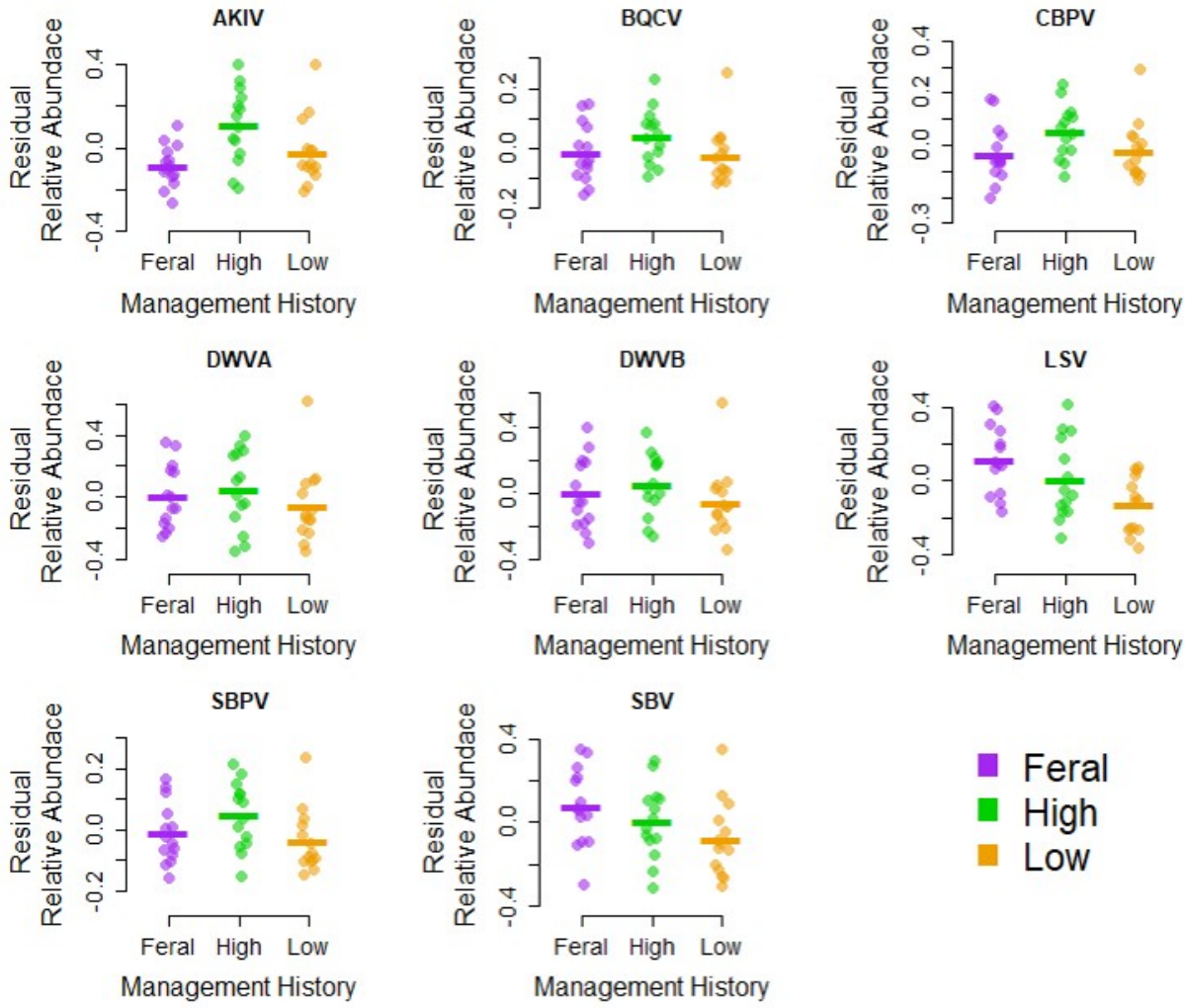


Figure 1 – Plot showing a non-metric multidimensional scaling (k = 2) of virus relative abundance data across colonies. Stress value after NMDS = 0.052. Each point corresponds to one colony and is colour coded by known management history. A restructured plot of the data used for these analyses (see Fig. 2) is presented in the Appendix (Fig. S2). Our corresponding adonis analysis found a significant grouping of colony virus abundances by management history (R^2 = 0.12, p = 0.039).



418 Figure 2 – Mean relative abundances of each virus for each colony, plotted
419 according to viral target (panel) and colony management history (x-axes and
420 colour). Y-axes scales differ between panels and are plotted as residuals to
421 dissuade from making comparisons between the relative abundance of different
422 viruses, as explained in the results. Our analysis shows that some viruses
423 significantly differed between backgrounds, but that background alone had no
424 significant single directional effect; differences between backgrounds changed
425 direction depending on the virus. AKIV – acute/Kashmir/Israeli paralysis virus
426 complex; BQCV – black queen cell virus; CBPV – chronic bee paralysis virus;
427 DWVA – deformed wing virus (A strain); DWVB – deformed wing virus (B strain, '
428 VDV-1'); LSV – Lake Sinai virus complex, Lake Sinai viruses 1 – 4; SBPV – slow
429 bee paralysis virus; SBV – sacbrood virus.

430

DISCUSSION

We present evidence that a honeybee colony's management history has a meaningful persistent effect on its future virus abundances, justifying much more involved experimental examination of this question. Despite a year in an approximate common garden, we show that there are substantial differences in virus abundances of colonies from our three sampled management histories (Fig. 2), with significant grouping of the virus abundances according to background based on our adonis analysis. Notably, when we look in detail we find that these differences are virus-specific, rather than generalisable across all viruses. It is not simply that colonies from one management history had elevated viral titres across all viruses, but rather that colonies from the feral management history showed qualitatively different viral abundance patterns to the two managed management histories. Amongst colonies from the two managed management histories, those sourced from the high-intensity management history exhibited higher viral abundances for all viruses compared to those from the low-intensity management history. Whether these effects were present at the point of acquiring the colonies (and subsequently persisted) or whether they developed following transplantation remains to be addressed in future studies with more study apiaries and better replication at the source-population level.

The finding of elevated viral titres in colonies from the 'high-intensity' background is consistent with the idea that the industrialisation of beekeeping is negatively impacting honeybee health. As industrial high-intensity practices become more common amongst, and more necessary for, beekeepers (Odoux et al., 2014; Whynott, 1991) this effect becomes increasingly relevant to the industry and elsewhere. We present evidence that a history of experiencing such high-intensity management, or the genetic stock used by high-intensity operations, leads to colonies either inheriting, or gaining, elevated viral titres; although we caution that we sampled colonies from only one single 'high-intensity' and one single 'low-intensity' management history, and that they were kept in close but separate apiaries. Nevertheless, the low-intensity management history honeybees in this observational study appeared to exhibit persistently lower viral burdens than their high-intensity counterparts. These findings call for a need to perform studies encompassing larger numbers of source management histories, as well as to keep colonies in isolation, in many small apiaries, and in

mixed apiaries to better control for site effects and investigate different explanatory hypotheses for this results.

The scale of this possible management effect, between low- and high- intensity, is interesting to compare to the effect of a feral management history. For half of our target viruses, the magnitude of difference between the two managed management histories was greater than the difference between either managed management history and the feral (Fig. 2). This is despite feral honeybees exhibiting population ecologies profoundly different from their managed counterparts, including colony spatial densities up to thousands of times lower, swarming more frequently, smaller colony sizes, and higher gentotypic variation (Brosi et al., 2017; Loftus et al., 2016; Loper et al., 2006; Schiff et al., 1994; Seeley, 2007). These differences appear to leave a lasting effect on colony virus abundances at a scale equivalent to comparing a low-intensity management regime to a high-intensity management regime. Speculation on the effects of management industrialisation has been made (Brosi et al., 2017; Nolan and Delaplane, 2017; Oldroyd, 2007; Seeley and Smith, 2015), however the size of these effects is difficult to quantify; our empirical evidence that the magnitude of these management-type impacts is comparable in size to when we compare managed bees with feral bees is notable.

Alongside these specific differences in viral abundances, our community analysis of the overall ‘colony virus abundances’ provided evidence of grouping by management history as well. Our adonis analysis showed a significant clustering of viral community according to management history, with visual interpretation of this in the plotted two-dimensional NMDS (Fig. 1) perhaps suggesting this is due to the viral characteristics of feral colonies, and potentially the lower abundances of the low-intensity colonies barring one outlier colony (easily identifiable in both Fig. 1 and Fig. 2).

An important caveat to interpretation of these significant effects of management history on colony viral characteristics is that we do not have access to these colonies’ initial virus abundances, and so it is not clear what degree of change occurred in their viral dynamics after being transplanted into the shared ‘common garden’ environment. Future work will be needed to establish the dynamics underpinning these differences, revealing why these effects manifest and persist. For example, differences at the point of management history, genetic differentiation of either honeybee or pathogen populations, differences in

queen quality, or lasting effects of stressors from management regimes, could all be drivers of the observed results. We consider this study a justification of pursuing the substantial experimental undertaking necessary to begin to differentiate the plausible drivers of the between-apiary differences presented here.

While our opportunistic sampling did not allow for holistic colony health appraisals, we can speculate on some of the dynamics plausibly at play by comparing the results here to those presented in Dynes et al. (2020), who subsequently took *Varroa* from the colonies in this study to assess the differential parasitic virulence of *Varroa* based on their population of origin, testing hypotheses laid out in evolutionary beekeeping literature (Brosi et al., 2017; Loftus et al., 2016; Seeley, 2007; Seeley and Smith, 2015). Interestingly, the *Varroa* assayed from these populations showed differentiation in their induced parasite burden when comparing feral to managed mites, whereby the feral mites showed significantly lower induced parasite burden whilst the two managed backgrounds were undifferentiated; this is in line with evolutionary predictions and findings elsewhere. However, Dynes et al. (2020) show parasite burdens qualitatively different to the viral abundances we found here comparing between the management histories, where the low-intensity managed colonies showed on average lower viral burdens than the high-intensity. This apparent contradiction between viral abundances and *Varroa* may be a consequence of numerous factors we have briefly mentioned here, including *Varroa* x honeybee x virus GxGxG interactions. Further, in spite of the feral-origin *Varroa* inducing the lowest parasite burden in Dynes et al. (2020), the feral mites were the only ones associated with a loss of colony health or productivity. This is in isolation a puzzling result, but may be linked to the viral abundance profiles we associate here with the feral colonies which show highest burdens for specific viruses including some lake sinai viruses and sacbrood virus, the latter of which is implicated with *Varroa* (McMahon et al., 2018). Taken as a whole, it becomes clear that the link between *Varroa*, viruses, and bee health is nuanced; it mandates detailed and thoughtful study, but is not necessarily contrary to evolutionary thinking even if certain results in isolation are unanticipated.

Migratory beekeeping has critical ramifications for continental-scale bee viral dynamics beyond just *Apis mellifera*, particularly if viral characteristics persist through many generations of honeybees. There are many speculated candidate

mechanisms for how such migration may foster elevated viral abundances (Goulson et al., 2015). Such colonies may be more likely to be nutritionally stressed due to experiencing principally monocultured crops (Becher et al., 2013; Odoux et al., 2014; Otto et al., 2016; Pasquale et al., 2013; Potts et al., 2010), exposed to more pesticides (Bartlett et al., 2018; Sánchez-Bayo et al., 2016; Sánchez-Bayo and Goka, 2014) and a wider variety of pathogens (Brosi et al., 2017; vanEngelsdorp and Meixner, 2010). It is also possible that industrial practices that reduce spatial structuring of the honeybee (host) populations have recently selected for more virulent viral variants (Boots et al., 2004; Boots and Mealor, 2007; Boots and Sasaki, 1999; Kamo and Boots, 2006; McMahon et al., 2016), leading to elevated viral titres.

If migratory beekeeping establishes elevated viral titres in colonies, those colonies may be moved to many locations over several months before they are returned to their home counties or states (Whynott, 1991). We have shown that it is possible these elevated viral titres persist (or subsequently develop) for extended periods even after moving from a specific management regime. There is now a large and growing body of literature documenting how honeybee viruses spill over into native bee populations (Choi et al., 2010; Forsgren et al., 2015; Forzan et al., 2017; Graystock et al., 2016, 2013; Guzman-Novoa et al., 2015; Li et al., 2011; Manley et al., 2019, 2015; Mazzei et al., 2014; Reynaldi et al., 2013; Santamaria et al., 2017; Singh et al., 2010; Zhang et al., 2012), a phenomenon which is conceivably more likely if higher viral abundances are present in migratory colonies. Our observation that high-intensity management history honeybees show the most elevated viral abundances establishes them as potential super-spreaders (Stein, 2011). They are more infectious and, through migratory beekeeping, are exposed to far more native pollinator populations, potentially infecting many more threatened populations. This double risk driver – to native bees and to non-migratory beekeeping operations – is significant for conservationists (Kleijn et al., 2015; Potts et al., 2016; Williams and Osborne, 2009), beekeepers (Brosi et al., 2008; Connell et al., 2012; Pettis and Delaplane, 2010), and policymakers in the US (FWS, 2016) and anywhere migratory beekeeping is becoming more common (Odoux et al., 2014).

The role of feral honeybees in the bee virus landscape is also worth considering. Honeybees are not native to the Americas. However, feral honeybees are hypothesised to foster far lower viral abundances, and possibly less virulent

strains, compared to managed honeybees (Brosi et al., 2017; Loftus et al., 2016), however see recent evidence on the evolution of viral tolerance in feral honeybees (Thaduri et al., 2019) and documentation of higher DWV loads in feral colonies (Thompson et al., 2014). Our evidence, though limited, points to feral colonies indeed sustaining higher titres of certain viruses, and may align with tolerance-based mechanisms of honeybee persistence, including mediated through differential control or tolerance of *Varroa* mites amongst colonies from different backgrounds, or differences in *Varroa* populations themselves. Whilst our observation of this common-garden cannot give direct insight into viral dynamics of feral populations, our results suggest it is possible that feral populations of honeybees sustain circulation of the well-characterised viruses examined here, and in some cases (such as sacbrood virus and the Lake Sinai viruses) possibly at higher per-colony abundances than in managed populations; this has been documented elsewhere with DWV (Thompson et al., 2014), although we note we do not find that to be the case here. Sacbrood virus has been implicated in *Varroa* mite mediated losses (Nielsen et al., 2008), whilst Lake Sinai viruses are fairly understudied (Daughenbaugh et al., 2015; McMahon et al., 2018). It is possible that even in protected areas, honeybees may be sustaining viral circulation with the capacity to spill-over into native bee populations. From an apicultural perspective, pursuing eradication of various honeybee parasites will also prove difficult if feral populations act as reservoirs for *Apis* parasites and pathogens.

Overall, our results putatively support hypotheses that colony management history, and likely management history, have persistent effects on colony epidemiology with respect to honeybee viruses. Notably, comparing two populations from very different management regimes revealed that the ‘industrial’ population exhibited greater viral abundances. Our findings are relevant to ongoing efforts to control managed pollinator diseases and to understand how industrial and migratory beekeeping practices are influencing the epidemiology of embattled bee populations. Additionally, our evidence runs counter to hypotheses predicting universally lower pathogen burden in feral colonies, which here showed the highest abundances of certain viruses. This unintuitive result invites further thought on and investigation into our understanding of the evolutionary dynamics of insect viruses across landscapes. Overall, this observational study justifies the substantial and intensive

undertakings required to address this question with well-designed experimental studies.

DATA ACCESSIBILITY

All raw molecular read data will be made available at a suitable repository (e.g. Dryad, Mendeley Data) upon acceptance for publication. We provide an annotated R script for reproducibility of analyses undertaken in this work, which can be accessed from GitHub (https://github.com/LBartlett/BackgroundViromes2020.git).

ACKNOWLEDGEMENTS

We would like to thank Norma Morella for advice and guidance with ddPCR, Jennifer Berry for insightful discussion on commercialised industrial beekeeping, and Jack Garrison and Nicholas Weaver for assistance with colony management and sample collection. LJB acknowledges funding from a Natural Environment Research Council training grant (NE/L002434/1). MB acknowledges funding from a Biotechnology and Biological Sciences Research Council grant (BB/L010879/1). BJB, JCdR and KSD acknowledge funding from National Institutes of Health (R01-109501); the content of this study is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

AUTHOR CONTRIBUTIONS

L.J.B. and M.B. conceptualised the study, with input from K.S.D., B.J.B., J.C.d.R., and L.W. Colony sourcing and management was undertaken by K.S.D., with assistance from B.J.B. and J.C.d.R. Molecular work was undertaken by L.J.B. and C.A.H. with guidance from L.W. L.J.B. analysed all data with guidance from B.J.B. and L.W.; L.J.B. drafted the manuscript, with contributions from all authors.

COMPETING INTERESTS

The authors declare no competing interests.

BIBLIOGRAPHY

Aebi, A., Vaissière, B.E., vanEngelsdorp, D., Delaplane, K.S., Roubik, D.W., Neumann, P., 2012. Back to the future: *Apis* versus non-*Apis* pollination—a response to Ollerton et al. Trends Ecol. Evol. 27, 142–143. https://doi.org/10.1016/j.tree.2011.11.017

637 Aizen, M.A., Harder, L.D., 2009. The Global Stock of Domesticated Honey Bees Is
638 Growing Slower Than Agricultural Demand for Pollination. Curr. Biol. 19,
639 915–918. https://doi.org/10.1016/j.cub.2009.03.071
640 Aronstein, K.A., Saldivar, E., Vega, R., Westmiller, S., Douglas, A.E., 2012. How
641 *Varroa* Parasitism Affects the Immunological and Nutritional Status of the
642 Honey Bee, *Apis mellifera*. Insects 3, 601–615.
643 https://doi.org/10.3390/insects3030601
644 Bartlett, L.J., Carlson, C.J., Boots, M., 2018. Identifying regions of risk to honey
645 bees from Zika vector control in the USA. J. Apic. Res. 57, 709–719. https://
646 doi.org/10.1080/00218839.2018.1494914
647 Bartlett, L.J., Rozins, C., Brosi, B.J., Delaplane, K.S., Roode, J.C. de, White, A.,
648 Wilfert, L., Boots, M., 2019. Industrial bees: The impact of apicultural
649 intensification on local disease prevalence. J. Appl. Ecol. 56, 2195–2205.
650 https://doi.org/10.1111/1365-2664.13461
651 Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects
652 Models using lme4. J. Stat. Softw. 67, 1–48.
653 https://doi.org/10.18637/jss.v067.i01.
654 Becher, M.A., Osborne, J.L., Thorbek, P., Kennedy, P.J., Grimm, V., 2013. REVIEW:
655 Towards a systems approach for understanding honeybee decline: a
656 stocktaking and synthesis of existing models. J. Appl. Ecol. 50, 868–880.
657 https://doi.org/10.1111/1365-2664.12112
658 Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A
659 Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B
660 Methodol. 57, 289–300.
661 Bingham, N., 2006. Bees, Butterflies, and Bacteria: Biotechnology and the Politics
662 of Nonhuman Friendship. Environ. Plan. Econ. Space 38, 483–498.
663 https://doi.org/10.1068/a38436
664 Booton, R.D., Iwasa, Y., Marshall, J.A.R., Childs, D.Z., 2017. Stress-mediated Allee
665 effects can cause the sudden collapse of honey bee colonies. J. Theor. Biol.
666 420, 213–219. https://doi.org/10.1016/j.jtbi.2017.03.009
667 Boots, M., Hudson, P.J., Sasaki, A., 2004. Large Shifts in Pathogen Virulence
668 Relate to Host Population Structure. Science 303, 842–844. https://doi.org/
669 10.1126/science.1088542
670 Boots, M., Mealor, M., 2007. Local Interactions Select for Lower Pathogen
671 Infectivity. Science 315, 1284–1286.
672 https://doi.org/10.1126/science.1137126
673 Boots, M., Sasaki, A., 1999. ‘Small worlds’ and the evolution of virulence:
674 infection occurs locally and at a distance. Proc. R. Soc. Lond. B Biol. Sci.
675 266, 1933–1938.
676 Brosi, B.J., Armsworth, P.R., Daily, G.C., 2008. Optimal design of agricultural
677 landscapes for pollination services. Conserv. Lett. 1, 27–36. https://doi.org/
678 10.1111/j.1755-263X.2008.00004.x
679 Brosi, B.J., Briggs, H.M., 2013. Single pollinator species losses reduce floral
680 fidelity and plant reproductive function. Proc. Natl. Acad. Sci. 110, 13044–
681 13048. https://doi.org/10.1073/pnas.1307438110
682 Brosi, B.J., Delaplane, K.S., Boots, M., de Roode, J.C., 2017. Ecological and
683 evolutionary approaches to managing honeybee disease. Nat. Ecol. Evol.
684 1, 1250. https://doi.org/10.1038/s41559-017-0246-z
685 Chao, A., Chazdon, R.L., Colwell, R.K., Shen, T.-J., 2006. Abundance-Based
686 Similarity Indices and Their Estimation When There Are Unseen Species in
687 Samples. Biometrics 62, 361–371.
688 Choi, Y.S., Lee, M.Y., Hong, I.P., Kim, N.S., Kim, H.K., Byeon, K.H., Yoon, H.J.,
689 2010. Detection of Honeybee Virus from Bumblebee (*Bombus terrestris* L.
690 and *Bombus ignitus*). J. Apic.

Cohen, H., Quistberg, R.D., Philpott, S.M., DeGrandi-Hoffman, G., 2017. Vegetation Management and Host Density Influence Bee–Parasite Interactions in Urban Gardens. Environ. Entomol. https://doi.org/10.1093/ee/nvx155

Connell, J., Delaplane, K.S., Donohue, S., Esaias, W., Gross, B., Hayes Jr, J., Lengerich, E.J., Pettis, J., Rennich, K., Underwood, R., others, 2012. The Bee Informed Partnership: using beekeeper's real-world experience to solve beekeepers' real-world problems. Am. Entomol. 58.

Conte, Y.L., Ellis, M., Ritter, W., 2010. *Varroa* mites and honey bee health: can *Varroa* explain part of the colony losses? Apidologie 41, 353–363. https://doi.org/10.1051/apido/2010017

Corbet, S.A., Williams, I.H., Osborne, J.L., 1991. Bees and the Pollination of Crops and Wild Flowers in the European Community. Bee World 72, 47–59. https://doi.org/10.1080/0005772X.1991.11099079

Corbisier, P., Pinheiro, L., Mazoua, S., Kortekaas, A.-M., Chung, P.Y.J., Gerganova, T., Roebben, G., Emons, H., Emslie, K., 2015. DNA copy number concentration measured by digital and droplet digital quantitative PCR using certified reference materials. Anal. Bioanal. Chem. 407, 1831–1840. https://doi.org/10.1007/s00216-015-8458-z

Dainat, B., Neumann, P., 2013. Clinical signs of deformed wing virus infection are predictive markers for honey bee colony losses. J. Invertebr. Pathol. 112, 278–280. https://doi.org/10.1016/j.jip.2012.12.009

Daughenbaugh, K., Martin, M., Brutscher, L., Cavigli, I., Garcia, E., Lavin, M., Flenniken, M., Daughenbaugh, K.F., Martin, M., Brutscher, L.M., Cavigli, I., Garcia, E., Lavin, M., Flenniken, M.L., 2015. Honey Bee Infecting Lake Sinai Viruses. Viruses 7, 3285–3309. https://doi.org/10.3390/v7062772

de Miranda, J.R., Cordoni, G., Budge, G., 2010a. The Acute bee paralysis virus–Kashmir bee virus–Israeli acute paralysis virus complex. J. Invertebr. Pathol. 103, Supplement, S30–S47. https://doi.org/10.1016/j.jip.2009.06.014

de Miranda, J.R., Dainat, B., Locke, B., Cordoni, G., Berthoud, H., Gauthier, L., Neumann, P., Budge, G.E., Ball, B.V., Stoltz, D.B., 2010b. Genetic characterization of slow bee paralysis virus of the honeybee (*Apis mellifera* L.). J. Gen. Virol. 91, 2524–2530. https://doi.org/10.1099/vir.0.022434-0

Delaplane, K.S., 2001. *Varroa destructor*: revolution in the making. Bee World 82, 157–159. https://doi.org/10.1080/0005772X.2001.11099522

Delaplane, K.S., Mayer, D.F., 2000. Crop pollination by bees. Cabi, Cambridge.

Dietemann, V., Pflugfelder, J., Anderson, D., Charrière, J.-D., Chejanovsky, N., Dainat, B., Miranda, J. de, Delaplane, K., Dillier, F.-X., Fuch, S., Gallmann, P., Gauthier, L., Imdorf, A., Koeniger, N., Kralj, J., Meikle, W., Pettis, J., Rosenkranz, P., Sammataro, D., Smith, D., Yañez, O., Neumann, P., 2012. *Varroa destructor*: research avenues towards sustainable control. J. Apic. Res. 51, 125–132. https://doi.org/10.3896/IBRA.1.51.1.15

Dingle, T.C., Sedlak, R.H., Cook, L., Jerome, K.R., 2013. Tolerance of Droplet-Digital PCR vs Real-Time Quantitative PCR to Inhibitory Substances. Clin. Chem. 59, 1670–1672. https://doi.org/10.1373/clinchem.2013.211045

Dolezal, A.G., Carrillo-Tripp, J., Miller, W.A., Bonning, B.C., Toth, A.L., 2016. Pollen Contaminated With Field-Relevant Levels of Cyhalothrin Affects Honey Bee Survival, Nutritional Physiology, and Pollen Consumption Behavior. J. Econ. Entomol. 109, 41–48. https://doi.org/10.1093/jee/tov301

Dynes, T.L., Berry, J.A., Delaplane, K.S., de Roode, J.C., Brosi, B.J., 2020. Assessing virulence of *Varroa destructor* mites from different honey bee management regimes. Apidologie 51, 276–289. https://doi.org/10.1007/s13592-019-00716-6

Dynes, T.L., Roode, J.C.D., Lyons, J.I., Berry, J.A., Delaplane, K.S., Brosi, B.J., 2017. Fine scale population genetic structure of *Varroa destructor*, an ectoparasitic mite of the honey bee (*Apis mellifera*). Apidologie 48, 93–101. https://doi.org/10.1007/s13592-016-0453-7

Evans, J.D., 2001. Genetic Evidence for Coinfection of Honey Bees by Acute Bee Paralysis and Kashmir Bee Viruses. J. Invertebr. Pathol. 78, 189–193. https://doi.org/10.1006/jipa.2001.5066

Ferrier, P.M., Rucker, R.R., Thurman, W.N., Burgett, M., 2018. Economic effects and responses to changes in honey bee health (No. 246), Economic Research Service Economic Research Report. United States Department of Agriculture.

Forsgren, E., Fries, I., 2010. Comparative virulence of *Nosema ceranae* and *Nosema apis* in individual European honey bees. Vet. Parasitol. 170, 212–217. https://doi.org/10.1016/j.vetpar.2010.02.010

Forsgren, E., Locke, B., Semberg, E., Laugen, A.T., Miranda, J.R. de, 2017. Sample preservation, transport and processing strategies for honeybee RNA extraction: Influence on RNA yield, quality, target quantification and data normalization. J. Virol. Methods 246, 81–89. https://doi.org/10.1016/j.jviromet.2017.04.010

Forsgren, E., Wei, S., Guiling, D., Zhiguang, L., Tran, T.V., Tang, P.T., Truong, T.A., Dinh, T.Q., Fries, I., 2015. Preliminary observations on possible pathogen spill-over from *Apis mellifera* to *Apis cerana*. Apidologie 46, 265–275. https://doi.org/10.1007/s13592-014-0320-3

Forzan, M., Felicioli, A., Sagona, S., Bandecchi, P., Mazzei, M., 2017. Complete Genome Sequence of Deformed Wing Virus Isolated from *Vespa crabro* in Italy. Genome Announc. 5, e00961-17. https://doi.org/10.1128/genomeA.00961-17

Fürst, M.A., McMahon, D.P., Osborne, J.L., Paxton, R.J., Brown, M.J.F., 2014. Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature 506, 364. https://doi.org/10.1038/nature12977

FWS, 2016. Endangered and Threatened Wildlife and Plants; Endangered Status for 49 Species From the Hawaiian Islands [WWW Document]. Fed. Regist. URL https://www.federalregister.gov/documents/2016/09/30/2016-23112/endangered-and-threatened-wildlife-and-plants-endangered-status-for-49-species-from-the-hawaiian (accessed 11.7.16).

Gallai, N., Salles, J.-M., Settele, J., Vaissière, B.E., 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol. Econ. 68, 810–821. https://doi.org/10.1016/j.ecolecon.2008.06.014

Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A., Kremen, C., Carvalheiro, L.G., Harder, L.D., Afik, O., Bartomeus, I., Benjamin, F., Boreux, V., Cariveau, D., Chacoff, N.P., Dudenhöffer, J.H., Freitas, B.M., Ghazoul, J., Greenleaf, S., Hipólito, J., Holzschuh, A., Howlett, B., Isaacs, R., Javorek, S.K., Kennedy, C.M., Krewenka, K.M., Krishnan, S., Mandelik, Y., Mayfield, M.M., Motzke, I., Munyuli, T., Nault, B.A., Otieno, M., Petersen, J., Pisanty, G., Potts, S.G., Rader, R., Ricketts, T.H., Rundlöf, M., Seymour, C.L., Schüepp, C., Szentgyörgyi, H., Taki, H., Tscharntke, T., Vergara, C.H., Viana, B.F., Wanger, T.C., Westphal, C., Williams, N., Klein, A.M., 2013. Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance. Science 339, 1608–1611. https://doi.org/10.1126/science.1230200

Genersch, E., Ohe, W. von der, Kaatz, H., Schroeder, A., Otten, C., Büchler, R., Berg, S., Ritter, W., Mühlen, W., Gisder, S., Meixner, M., Liebig, G., Rosenkranz, P., 2010. The German bee monitoring project: a long term

study to understand periodically high winter losses of honey bee colonies. Apidologie 41, 332–352. https://doi.org/10.1051/apido/2010014
Giacobino, A., Cagnolo, N.B., Merke, J., Orellano, E., Bertozzi, E., Masciangelo, G., Pietronave, H., Salto, C., Signorini, M., 2014. Risk factors associated with the presence of *Varroa destructor* in honey bee colonies from east-central Argentina. Prev. Vet. Med. 115, 280–287. https://doi.org/10.1016/j.prevetmed.2014.04.002
Goulson, D., Nicholls, E., Botías, C., Rotheray, E.L., 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347, 1255957. https://doi.org/10.1126/science.1255957
Graystock, P., Blane, E.J., McFrederick, Q.S., Goulson, D., Hughes, W.O.H., 2016. Do managed bees drive parasite spread and emergence in wild bees? Int. J. Parasitol. Parasites Wildl., Including articles from the 25th WAAVP Conference, Liverpool, August 2015 5, 64–75. https://doi.org/10.1016/j.ijppaw.2015.10.001
Graystock, P., Goulson, D., Hughes, W.O.H., 2015. Parasites in bloom: flowers aid dispersal and transmission of pollinator parasites within and between bee species. Proc R Soc B 282, 20151371. https://doi.org/10.1098/rspb.2015.1371
Graystock, P., Yates, K., Evison, S.E.F., Darvill, B., Goulson, D., Hughes, W.O.H., 2013. The Trojan hives: pollinator pathogens, imported and distributed in bumblebee colonies. J. Appl. Ecol. 50, 1207–1215. https://doi.org/10.1111/1365-2664.12134
Guzman-Novoa, E., Hamiduzzaman, M.M., Anguiano-Baez, R., Correa-Benítez, A., Castañeda-Cervantes, E., Arnold, N.I., 2015. First detection of honey bee viruses in stingless bees in North America. J. Apic. Res. 54, 93–95. https://doi.org/10.1080/00218839.2015.1100154
Highfield, A.C., Nagar, A.E., Mackinder, L.C.M., Noël, L.M.-L.J., Hall, M.J., Martin, S.J., Schroeder, D.C., 2009. Deformed Wing Virus Implicated in Overwintering Honeybee Colony Losses. Appl. Environ. Microbiol. 75, 7212–7220. https://doi.org/10.1128/AEM.02227-09
Hodges, A., Mulkey, D., Philippakos, E., Sanford, M., 2001. Economic impact of the Florida apiculture industry. Am. Bee J. 141, 361–363.
Kamo, M., Boots, M., 2006. The evolution of parasite dispersal, transmission, and virulence in spatial host populations. Evol. Ecol. Res. 8, 1333–1347.
Kent, C.F., Dey, A., Patel, H., Tsvetkov, N., Tiwari, T., MacPhail, V.J., Gobeil, Y., Harpur, B.A., Gurtowski, J., Schatz, M.C., Colla, S.R., Zayed, A., 2018. Conservation Genomics of the Declining North American Bumblebee Bombus terricola Reveals Inbreeding and Selection on Immune Genes. Front. Genet. 9. https://doi.org/10.3389/fgene.2018.00316
Kleijn, D., Winfree, R., Bartomeus, I., Carvalheiro, L.G., Henry, M., Isaacs, R., Klein, A.-M., Kremen, C., M'Gonigle, L.K., Rader, R., Ricketts, T.H., Williams, N.M., Lee Adamson, N., Ascher, J.S., Báldi, A., Batáry, P., Benjamin, F., Biesmeijer, J.C., Blitzer, E.J., Bommarco, R., Brand, M.R., Bretagnolle, V., Button, L., Cariveau, D.P., Chifflet, R., Colville, J.F., Danforth, B.N., Elle, E., Garratt, M.P.D., Herzog, F., Holzschuh, A., Howlett, B.G., Jauker, F., Jha, S., Knop, E., Krewenka, K.M., Le Féon, V., Mandelik, Y., May, E.A., Park, M.G., Pisanty, G., Reemer, M., Riedinger, V., Rollin, O., Rundlöf, M., Sardiñas, H.S., Scheper, J., Sciligo, A.R., Smith, H.G., Steffan-Dewenter, I., Thorp, R., Tscharntke, T., Verhulst, J., Viana, B.F., Vaissière, B.E., Veldtman, R., Ward, K.L., Westphal, C., Potts, S.G., 2015. Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. Nat. Commun. 6, 7414. https://doi.org/10.1038/ncomms8414

Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. Lond. B Biol. Sci. 274, 303–313. https://doi.org/10.1098/rspb.2006.3721

Knapp, J.L., Bartlett, L.J., Osborne, J.L., 2017. Re-evaluating strategies for pollinator-dependent crops: How useful is parthenocarpy? J. Appl. Ecol. 54, 1171–1179. https://doi.org/10.1111/1365-2664.12813

Lenth, R., 2019. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.3.5.1.

Li, J., Peng, W., Wu, J., Strange, J.P., Boncristiani, H., Chen, Y., 2011. Cross-Species Infection of Deformed Wing Virus Poses a New Threat to Pollinator Conservation. J. Econ. Entomol. 104, 732–739. https://doi.org/10.1603/EC10355

Lindström, A., Korpela, S., Fries, I., 2008. Horizontal transmission of Paenibacillus larvae spores between honey bee (*Apis mellifera*) colonies through robbing. Apidologie 39, 515–522. https://doi.org/10.1051/apido:2008032

Locke, B., Forsgren, E., Fries, I., Miranda, J.R. de, 2012. Acaricide Treatment Affects Viral Dynamics in *Varroa destructor*-Infested Honey Bee Colonies via both Host Physiology and Mite Control. Appl Env. Microbiol 78, 227–235. https://doi.org/10.1128/AEM.06094-11

Loftus, J.C., Smith, M.L., Seeley, T.D., 2016. How Honey Bee Colonies Survive in the Wild: Testing the Importance of Small Nests and Frequent Swarming. PLOS ONE 11, e0150362. https://doi.org/10.1371/journal.pone.0150362

Loper, G.M., Sammataro, D., Finley, J., Cole, J., 2006. Feral honey bees in southern Arizona 10 years after varroa infestation. Am. Bee J.

Lourenço, A.P., Mackert, A., Cristino, A. dos S., Simões, Z.L.P., 2008. Validation of reference genes for gene expression studies in the honey bee, Apis mellifera, by quantitative real-time RT-PCR. Apidologie 39, 372–385. https://doi.org/10.1051/apido:2008015

Mace, G.M., Norris, K., Fitter, A.H., 2012. Biodiversity and ecosystem services: a multilayered relationship. Trends Ecol. Evol. 27, 19–26. https://doi.org/10.1016/j.tree.2011.08.006

Manley, R., Boots, M., Wilfert, L., 2015. REVIEW: Emerging viral disease risk to pollinating insects: ecological, evolutionary and anthropogenic factors. J. Appl. Ecol. 52, 331–340. https://doi.org/10.1111/1365-2664.12385

Manley, R., Temperton, B., Doyle, T., Gates, D., Hedges, S., Boots, M., Wilfert, L., 2019. Knock-on community impacts of a novel vector: spillover of emerging DWV-B from *Varroa*-infested honeybees to wild bumblebees. Ecol. Lett. 22, 1306–1315. https://doi.org/10.1111/ele.13323

Martin, S.J., Highfield, A.C., Brettell, L., Villalobos, E.M., Budge, G.E., Powell, M., Nikaido, S., Schroeder, D.C., 2012. Global Honey Bee Viral Landscape Altered by a Parasitic Mite. Science 336, 1304–1306. https://doi.org/10.1126/science.1220941

Mazzei, M., Carrozza, M.L., Luisi, E., Forzan, M., Giusti, M., Sagona, S., Tolari, F., Felicioli, A., 2014. Infectivity of DWV Associated to Flower Pollen: Experimental Evidence of a Horizontal Transmission Route. PLOS ONE 9, e113448. https://doi.org/10.1371/journal.pone.0113448

McMahon, D.P., Fürst, M.A., Caspar, J., Theodorou, P., Brown, M.J.F., Paxton, R.J., 2015. A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees. J. Anim. Ecol. 84, 615–624. https://doi.org/10.1111/1365-2656.12345

McMahon, D.P., Natsopoulou, M.E., Doublet, V., Fürst, M., Weging, S., Brown, M.J.F., Gogol-Döring, A., Paxton, R.J., 2016. Elevated virulence of an

emerging viral genotype as a driver of honeybee loss. Proc R Soc B 283, 20160811. https://doi.org/10.1098/rspb.2016.0811
McMahon, D.P., Wilfert, L., Paxton, R.J., Brown, M.J.F., 2018. Emerging Viruses in Bees: From Molecules to Ecology. Adv. Virus Res. https://doi.org/10.1016/bs.aivir.2018.02.008
McMenamin, A.J., Genersch, E., 2015. Honey bee colony losses and associated viruses. Curr. Opin. Insect Sci., Ecology * Parasites/Parasitoids/Biological control 8, 121–129. https://doi.org/10.1016/j.cois.2015.01.015
Miotke, L., Lau, B.T., Rumma, R.T., Ji, H.P., 2014. High Sensitivity Detection and Quantitation of DNA Copy Number and Single Nucleotide Variants with Single Color Droplet Digital PCR. Anal. Chem. 86, 2618–2624. https://doi.org/10.1021/ac403843j
Mondet, F., Miranda, J.R. de, Kretzschmar, A., Conte, Y.L., Mercer, A.R., 2014. On the Front Line: Quantitative Virus Dynamics in Honeybee (Apis mellifera L.) Colonies along a New Expansion Front of the Parasite *Varroa destructor*. PLOS Pathog. 10, e1004323. https://doi.org/10.1371/journal.ppat.1004323
Moritz, R.F.A., Erler, S., 2016. Lost colonies found in a data mine: Global honey trade but not pests or pesticides as a major cause of regional honeybee colony declines. Agric. Ecosyst. Environ. 216, 44–50. https://doi.org/10.1016/j.agee.2015.09.027
Mõtus, K., Raie, A., Orro, T., Chauzat, M.-P., Viltrop, A., 2016. Epidemiology, risk factors and varroa mite control in the Estonian honey bee population. J. Apic. Res. 55, 396–412. https://doi.org/10.1080/00218839.2016.1251081
Neumann, P., Carreck, N.L., 2010. Honey bee colony losses. J. Apic. Res. 49, 1–6. https://doi.org/10.3896/IBRA.1.49.1.01
Nielsen, S.L., Nicolaisen, M., Kryger, P., 2008. Incidence of acute bee paralysis virus, black queen cell virus, chronic bee paralysis virus, deformed wing virus, Kashmir bee virus and sacbrood virus in honey bees (*Apis mellifera*) in Denmark. Apidologie 39, 310–314. https://doi.org/10.1051/apido:2008007
Nolan, M.P., Delaplane, K.S., 2017. Distance between honey bee *Apis mellifera* colonies regulates populations of *Varroa destructor* at a landscape scale. Apidologie 48, 8–16. https://doi.org/10.1007/s13592-016-0443-9
Odoux, J.-F., Aupinel, P., Gateff, S., Requier, F., Henry, M., Bretagnolle, V., 2014. ECOBEE: a tool for long-term honey bee colony monitoring at the landscape scale in West European intensive agroecosystems. J. Apic. Res. 53, 57–66. https://doi.org/10.3896/IBRA.1.53.1.05
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O'hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2019. Vegan: community ecology package.
Oldroyd, B.P., 2007. What's Killing American Honey Bees? PLOS Biol. 5, e168. https://doi.org/10.1371/journal.pbio.0050168
Otto, C.R.V., Roth, C.L., Carlson, B.L., Smart, M.D., 2016. Land-use change reduces habitat suitability for supporting managed honey bee colonies in the Northern Great Plains. Proc. Natl. Acad. Sci. 113, 10430–10435. https://doi.org/10.1073/pnas.1603481113
Pasquale, G.D., Salignon, M., Conte, Y.L., Belzunces, L.P., Decourtye, A., Kretzschmar, A., Suchail, S., Brunet, J.-L., Alaux, C., 2013. Influence of Pollen Nutrition on Honey Bee Health: Do Pollen Quality and Diversity Matter? PLOS ONE 8, e72016. https://doi.org/10.1371/journal.pone.0072016
Pettis, J.S., Delaplane, K.S., 2010. Coordinated responses to honey bee decline in the USA. Apidologie 41, 256–263. https://doi.org/10.1051/apido/2010013

Pinheiro, L.B., Coleman, V.A., Hindson, C.M., Herrmann, J., Hindson, B.J., Bhat, S., Emslie, K.R., 2012. Evaluation of a Droplet Digital Polymerase Chain Reaction Format for DNA Copy Number Quantification. Anal. Chem. 84, 1003–1011. https://doi.org/10.1021/ac202578x

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25, 345–353. https://doi.org/10.1016/j.tree.2010.01.007

Potts, S.G., Imperatriz-Fonseca, V., Ngo, H.T., Aizen, M.A., Biesmeijer, J.C., Breeze, T.D., Dicks, L.V., Garibaldi, L.A., Hill, R., Settele, J., Vanbergen, A.J., 2016. Safeguarding pollinators and their values to human well-being. Nature 540, 220–229. https://doi.org/10.1038/nature20588

R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ravoet, J., De Smet, L., Wenseleers, T., de Graaf, D.C., 2015. Genome sequence heterogeneity of Lake Sinai Virus found in honey bees and Orf1/RdRP-based polymorphisms in a single host. Virus Res. 201, 67–72. https://doi.org/10.1016/j.virusres.2015.02.019

Reynaldi, F.J., Sguazza, G.H., Albicoro, F.J., Pecoraro, M.R., Galosi, C.M., Reynaldi, F.J., Sguazza, G.H., Albicoro, F.J., Pecoraro, M.R., Galosi, C.M., 2013. First molecular detection of co-infection of honey bee viruses in asymptomatic Bombus atratus in South America. Braz. J. Biol. 73, 797–800. https://doi.org/10.1590/S1519-69842013000400016

Rumkee, J.C.O., Becher, M.A., Thorbek, P., Osborne, J.L., 2017. Modeling Effects of Honeybee Behaviors on the Distribution of Pesticide in Nectar within a Hive and Resultant in-Hive Exposure. Environ. Sci. Technol. 51, 6908–6917. https://doi.org/10.1021/acs.est.6b04206

Ryabov, E.V., Childers, A.K., Chen, Y., Madella, S., Nessa, A., vanEngelsdorp, D., Evans, J.D., 2017. Recent spread of Varroa destructor virus-1, a honey bee pathogen, in the United States. Sci. Rep. 7, 17447. https://doi.org/10.1038/s41598-017-17802-3

Sánchez-Bayo, F., Goka, K., 2014. Pesticide Residues and Bees – A Risk Assessment. PLOS ONE 9, e94482. https://doi.org/10.1371/journal.pone.0094482

Sánchez-Bayo, F., Goulson, D., Pennacchio, F., Nazzi, F., Goka, K., Desneux, N., 2016. Are bee diseases linked to pesticides? — A brief review. Environ. Int. 89–90, 7–11. https://doi.org/10.1016/j.envint.2016.01.009

Santamaria, J., Villalobos, E.M., Brettell, L.E., Nikaido, S., Graham, J.R., Martin, S., 2017. Evidence of *Varroa*-mediated deformed wing virus spillover in Hawaii. J. Invertebr. Pathol. https://doi.org/10.1016/j.jip.2017.11.008

Schiff, N.M., Sheppard, W.S., Loper, G.M., Shimanuki, H., 1994. Genetic Diversity of Feral Honey Bee (Hymenoptera: Apidae) Populations in the Southern United States. Ann. Entomol. Soc. Am. 87, 842–848. https://doi.org/10.1093/aesa/87.6.842

Seeley, T.D., 2007. Honey bees of the Arnot Forest: a population of feral colonies persisting with *Varroa destructor* in the northeastern United States. Apidologie 38, 19–29. https://doi.org/10.1051/apido:2006055

Seeley, T.D., Smith, M.L., 2015. Crowding honeybee colonies in apiaries can increase their vulnerability to the deadly ectoparasite *Varroa destructor*. Apidologie 46, 716–727. https://doi.org/10.1007/s13592-015-0361-2

Simms, D., Cizdziel, P.E., Chomczynski, P., 1993. TRIzol: A new reagent for optimal single-step isolation of RNA. Focus 15, 532–535.

Simone-Finstrom, M., Li-Byarlay, H., Huang, M.H., Strand, M.K., Rueppell, O., Tarpy, D.R., 2016. Migratory management and environmental conditions affect lifespan and oxidative stress in honey bees. Sci. Rep. 6.

Singh, R., Levitt, A.L., Rajotte, E.G., Holmes, E.C., Ostiguy, N., vanEngelsdorp, D., Lipkin, W.I., dePamphilis, C.W., Toth, A.L., Cox-Foster, D.L., 2010. RNA Viruses in Hymenopteran Pollinators: Evidence of Inter-Taxa Virus Transmission via Pollen and Potential Impact on Non-Apis Hymenopteran Species. PLOS ONE 5, e14357. https://doi.org/10.1371/journal.pone.0014357

Singmann, H., Bolker, B., Westfall, J., Aust, F., Ben-Shachar, M.S., 2019. afex: Analysis of Factorial Experiments. R Package.

Southwick, E.E., Southwick, L., 1992. Estimating the Economic Value of Honey Bees (Hymenoptera: Apidae) as Agricultural Pollinators in the United States. J. Econ. Entomol. 85, 621–633. https://doi.org/10.1093/jee/85.3.621

Stein, R.A., 2011. Super-spreaders in infectious diseases. Int. J. Infect. Dis. 15, e510–e513. https://doi.org/10.1016/j.ijid.2010.06.020

Sumpter, D.J.T., Martin, S.J., 2004. The dynamics of virus epidemics in *Varroa*-infested honey bee colonies. J. Anim. Ecol. 73, 51–63. https://doi.org/10.1111/j.1365-2656.2004.00776.x

Tentcheva, D., Gauthier, L., Zappulla, N., Dainat, B., Cousserans, F., Colin, M.E., Bergoin, M., 2004. Prevalence and Seasonal Variations of Six Bee Viruses in *Apis mellifera* L. and *Varroa destructor* Mite Populations in France. Appl Env. Microbiol 70, 7185–7191. https://doi.org/10.1128/AEM.70.12.7185-7191.2004

Thaduri, S., Stephan, J.G., Miranda, J.R. de, Locke, B., 2019. Disentangling host-parasite-pathogen interactions in a varroa-resistant honeybee population reveals virus tolerance as an independent, naturally adapted survival mechanism. Sci. Rep. 9, 1–10. https://doi.org/10.1038/s41598-019-42741-6

Thompson, C.E., Biesmeijer, J.C., Allnutt, T.R., Pietravalle, S., Budge, G.E., 2014. Parasite Pressures on Feral Honey Bees *(Apis mellifera* sp.). PLOS ONE 9, e105164. https://doi.org/10.1371/journal.pone.0105164

USDA - NASS, C. of A., 2012. USDA - NASS, Census of Agriculture - 2012 Census Volume 1, Chapter 1: U.S. National Level Data [WWW Document]. URL https://www.agcensus.usda.gov/Publications/2012/Full_Report/Volume_1,_Chapter_1_US/ (accessed 11.8.16).

van der Zee, R., Pisa, L., Andonov, S., Brodschneider, R., Charrière, J.-D., Chlebo, R., Coffey, M.F., Crailsheim, K., Dahle, B., Gajda, A., Gray, A., Drazic, M.M., Higes, M., Kauko, L., Kence, A., Kence, M., Kezic, N., Kiprijanovska, H., Kralj, J., Kristiansen, P., Martin Hernandez, R., Mutinelli, F., Nguyen, B.K., Otten, C., Özkırım, A., Pernal, S.F., Peterson, M., Ramsay, G., Santrac, V., Soroker, V., Topolska, G., Uzunov, A., Vejsnæs, F., Wei, S., Wilkins, S., 2012. Managed honey bee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008-9 and 2009-10. J. Apic. Res. 51, 100–114. https://doi.org/10.3896/IBRA.1.51.1.12

vanEngelsdorp, D., Evans, J.D., Saegerman, C., Mullin, C., Haubruge, E., Nguyen, B.K., Frazier, M., Frazier, J., Cox-Foster, D., Chen, Y., Underwood, R., Tarpy, D.R., Pettis, J.S., 2009. Colony Collapse Disorder: A Descriptive Study. PLoS ONE 4, e6481. https://doi.org/10.1371/journal.pone.0006481

vanEngelsdorp, D., Meixner, M.D., 2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. J. Invertebr. Pathol. 103, S80–S95. https://doi.org/10.1016/j.jip.2009.06.011

vanEngelsdorp, D., Tarpy, D.R., Lengerich, E.J., Pettis, J.S., 2013. Idiopathic brood disease syndrome and queen events as precursors of colony mortality in

migratory beekeeping operations in the eastern United States. Prev. Vet. Med. 108, 225–233. https://doi.org/10.1016/j.prevetmed.2012.08.004

Watson, R., Albon, S., Aspinall, R., Austen, M., Bardgett, B., Bateman, I., Berry, P., Bird, W., Bradbury, R., Brown, C., Bullock, J., Burgess, J., Church, A., Christie, C., Crute, I., Davies, L., Edwards-Jones, G., Emmett, B., Firbank, L., Fitter, A., Gibson, A., Hails, R., Haines-Young, R., L., H.A., Heathwaite, L., Hopkins, J., Jenkins, M., Jones, L., Mace, G., Malcolm, S., Maltby, E., Maskell, L., Norris, K., Ormerod, S., Osborne, J., Pretty, J., Quine, C., Russell, S., Simpson, L., Smith, P., Tierney, M., K., T., Van der Wal, R., Vira, B., Walpole, M., Watkinson, A., Weighell, A., Winn, J., Winter, M., 2011. UK National Ecosystem Assessment : understanding nature’s value to society. Synthesis of key findings (Report). Information Press.

Welch, A., Drummond, F., Tewari, S., Averill, A., Burand, J.P., 2009. Presence and Prevalence of Viruses in Local and Migratory Honeybees (*Apis mellifera*) in Massachusetts. Appl. Environ. Microbiol. 75, 7862–7865. https://doi.org/10.1128/AEM.01319-09

Whynott, D., 1991. Following the bloom: across America with the migratory beekeepers. Stackpole Books.

Wilfert, L., Long, G., Leggett, H.C., Schmid-Hempel, P., Butlin, R., Martin, S.J.M., Boots, M., 2016. Deformed wing virus is a recent global epidemic in honeybees driven by *Varroa* mites. Science 351, 594–597. https://doi.org/10.1126/science.aac9976

Williams, P.H., Osborne, J.L., 2009. Bumblebee vulnerability and conservation world-wide. Apidologie 40, 367–387. https://doi.org/10.1051/apido/2009025

Yang, X., Cox-Foster, D.L., 2005. Impact of an ectoparasite on the immunity and pathology of an invertebrate: Evidence for host immunosuppression and viral amplification. Proc. Natl. Acad. Sci. 102, 7470–7475. https://doi.org/10.1073/pnas.0501860102

Zee, R. van der, Brodschneider, R., Brusbardis, V., Charrière, J.-D., Chlebo, R., Coffey, M.F., Dahle, B., Drazic, M.M., Kauko, L., Kretavicius, J., Kristiansen, P., Mutinelli, F., Otten, C., Peterson, M., Raudmets, A., Santrac, V., Seppälä, A., Soroker, V., Topolska, G., Vejsnæs, F., Gray, A., 2014. Results of international standardised beekeeper surveys of colony losses for winter 2012–2013: analysis of winter loss rates and mixed effects modelling of risk factors for winter loss. J. Apic. Res. 53, 19–34. https://doi.org/10.3896/IBRA.1.53.1.02

Zhang, X., He, S.Y., Evans, J.D., Pettis, J.S., Yin, G.F., Chen, Y.P., 2012. New evidence that deformed wing virus and black queen cell virus are multi-host pathogens. J. Invertebr. Pathol. 109, 156–159. https://doi.org/10.1016/j.jip.2011.09.010