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Abstract: Several viruses found in the Western honey bee (*Apis mellifera*) have recently been detected in other bee species, raising the possibility of spill-over from managed to wild bee species. Alternatively, these viruses may be shared generalists across flower-visiting insects. Here we explore the former hypothesis, pointing out weaknesses in the current evidence, particularly in relation to deformed wing virus (DWV), and highlighting research areas that may help test it. Data so far suggest that DWV spills over from managed to wild bee species and has the potential to cause population decline. That DWV and other viruses of *A. mellifera* are found in other bee species needs to be considered for the sustainable management of bee populations.



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Prof. Dr. Robert J. Paxton

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June 10th, 2016

Dear Editors,

We submit our revised manuscript, entitled:

Impact of managed honey bee viruses on wild bees (COVIRO-D-16-00038)

for consideration for publication in Current Opinion in Virology, as one of the invited papers of the special issue on Environmental Virology. We have now revised the ms in response to reviewer comments and attach our point-by-point response to those comments as well as a version of the ms with tracked changes.

All coauthors (Anja Tehel, Mark Brown and myself) have contributed to the ms. The work described in our ms has not been published previously, it is not under consideration for publication elsewhere, its publication is approved by all authors and, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically. We declare no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, the manuscript.

Yours faithfully,

Robert Paxton

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as the Western honey bee is fine, but don't then switch back and forth, just use this designation. I corrected the first few, but please go through and correct the remainder.

Response:

We have gone through the entire ms and homogenized Apis mellifera to 'Western honey bee', given specific names to other honey bees (e.g. Eastern honey bee) and made consistent our naming of other bee species. Where we used 'wild bees', we now use 'wild bee species' to make it crystal clear that we do not mean 'Apis mellifera'. We thought confusion might also arise because we sometimes used 'domestic' and sometimes 'managed' to describe the Western honey bee. There is some discussion over whether Apis mellifera is 'domesticated' or merely a wild species now kept in boxes i.e. managed. We therefore replaced 'domesticated' with 'managed' throughout, including in the title, so as to avoid confusion and so as to be consistent throughout the ms.

Comment:

Please use * for highlighting references, rather than *

Response:

We copied the style of other articles in Current Opinion in Virology and used a bold ''. We suspect there may be formatting problems when sending text via the journal's website because we could not differentiate between the two stars used above.*

Comment:

We usually aim for around 50 references. Would it be possible to use a few reviews to replace some of the primary references, especially for areas that are indirectly related to the topic of the paper, like EIDs in other animals?

Response:

We have cut the number of references from 79 to 59 by removing many of the primary references, as you suggested, and we have reduced the number of highlighted references to 6.

Comment:

Please correct the table as noted on the manuscript.

Response:

We have given the table a short title and placed the explanation to it as a footnote.

Additional comments scored directly on the pdf by the editor.

Comment:

Graphical Abstract: can you please define these families? Most people will have no idea what they are.

Response:

We have added additional text to the graphical abstract to make clear the bee taxa and added names in common usage as well as genus and family names of the taxa.

Comment:

Please add something here to define BEEHAVE.

Response:

On lines 158-9 we added the following text to define BEEHAVE: The BEEHAVE model, a software that simulates the development of a honey bee colony and its foraging for nectar and pollen in a realistic landscape,

Comment:

Figure 1 caption: Please define the other species here, most readers won't know what they are.

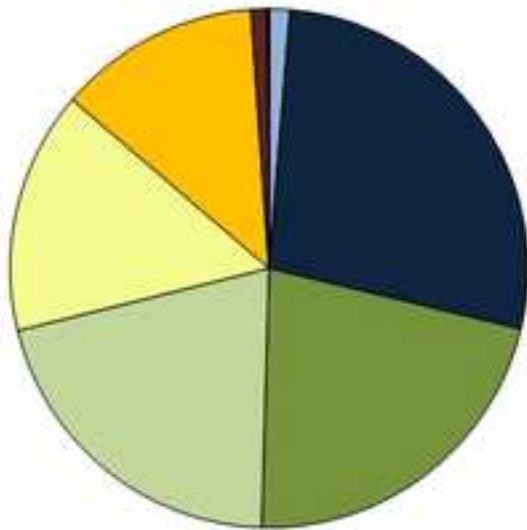
Response:

We updated the caption to Figure 1 to define the other species more thoroughly.

Many additional, minor comments were added to the text**Response:**

We incorporated all the suggested changes into the text.

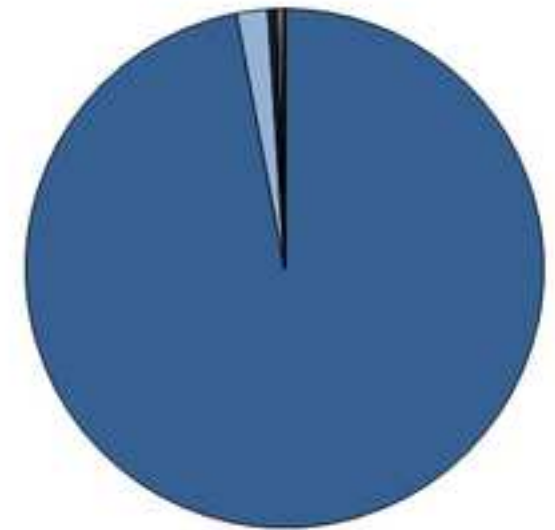
Number of bee species per taxon



Bee taxa (genus; family)

- honey bees (*Apis*; Apidae)
- bumble bees (*Bombus*; Apidae)
- other apid bees (Apidae)
- sweat bees (Halictidae)
- leafcutter and mason bees (Megachilidae)
- mining bees (Andrenidae)
- silk bees (Colletidae)
- oil bees (Melittidae)
- stenotritid bees (Stenotritidae)

Number of publications on viruses per bee taxon



Highlights

- Over 24 viruses have been associated with managed honey bees
- Many of these are also prevalent in wild bee species
- Evidence suggests spill-over of viruses from honey bees to wild bee species
- The impact of these viruses on wild bee species is poorly known

1 **Impact of managed honey bee viruses on wild bees**

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20 **Abstract**

21 Several viruses found in the Western honey bee (*Apis mellifera*) have recently been detected in
22 other bee species, raising the possibility of spill-over from managed to wild bee species.
23 Alternatively, these viruses may be shared generalists across flower-visiting insects. Here we explore
24 the former hypothesis, pointing out weaknesses in the current evidence, particularly in relation to
25 deformed wing virus (DWV), and highlighting research areas that may help test it. Data so far
26 suggest that DWV spills over from managed to wild bee species and has the potential to cause
27 population decline. That DWV and other viruses of *A. mellifera* are found in other bee species needs
28 to be considered for the sustainable management of bee populations.

30 **Keywords:**

31 Hymenoptera, *Apis mellifera*, bumble bee, spill-over, Deformed wing virus

33 **Introduction: EIDs and viral pathogens of the Western honey bee (*Apis mellifera*)**

34
35 We, our domestic animals, and wildlife are increasingly challenged by a range of emerging infectious
36 diseases (EIDs [1, 2]) that threaten respectively our health, our farming and the ecosystem services
37 we derive from the natural world. After cattle and pigs and before poultry, the Western honey bee
38 (*Apis mellifera*) has been considered the third most important domestic animal [3, 4]; pollination by
39 insects, including by the Western honey bee, is an important ecosystem service that contributes
40 over US \$ 200 billion to global agricultural output at today's prices and, directly and indirectly, to
41 one in every three mouthfuls of food [5]. Wild bee species also make a major contribution to crop
42 pollination [6]. Elevated losses of Western honey bees have been reported across northern
43 temperate regions of the world over the past decade [7], with EIDs considered a primary cause of
44 mortality [8], possibly because of increased viral virulence [9]. Wild bee species are also in decline in
45 northern temperate regions of the world concomitant with a decline in the wild flowers they visit
46 and pollinate [10], though causes of population decline, range contraction and wild bee species loss
47 are diverse [11, 12]. Given the importance of bees for pollination, these are serious concerns that
48 could affect food security and the functioning of terrestrial ecosystems [13].

49
50 To date, 24 viruses have been associated with the Western honey bee [14], many of which are
51 positive sense single strand (+) RNA viruses. One of these in particular, Deformed wing virus (DWV),
52 has risen to prominence because of its association with the honey bee exotic ectoparasitic mite
53 *Varroa destructor* [15*], a possibly synergistic association for virus and mite [16]. *Varroa destructor*
54 (henceforth varroa mite) was originally an ectoparasite of the Asiatic Eastern honey bee
55 (*Apis cerana*), but jumped to the Western honey bee and is nowadays widespread across most of the
56 world with its new host, the Western honey bee, on which it vectors DWV and many other viruses
57 [8, 14]. Introduction of varroa mites to a naïve population of Western honey bees leads to a

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tremendous rise in DWV prevalence and titer per individual host [17, 18], and DWV is the leading contender as the causal agent of colony decline [14].

Prominent among EIDs of a diverse range of animals are RNA viruses that exhibit high mutation (and substitution) rates [19] and cross species barriers to emerge (*i.e.* become more prevalent) in novel hosts, including humans [20] *e.g.* Ebola and Zika. DWV and other (+) RNA viruses of the Western honey bee have now been detected in wild bee species [21**], though knowledge of their impact on wild bee hosts remains limited (Fig. 1; Table 1). That a virus is found across two or more host species, be they managed or wild bees, is important because it dictates the epidemiology and evolutionary trajectory of that pathogen [22, 23*]. Here we bring together recent literature to explore the hypothesis that viral spill-over from managed Western honey bees negatively impacts wild bee species.

DWV prevalence and impact

Before the introduction of the varroa mite to the Western honey bee in Europe *ca.* half a century ago, serological (antibody-based) detection of DWV suggested it was at extremely low prevalence [24]. Varroa mites are now found in most, if not all, Western honey bee colonies across all of Europe (except isolated offshore islands and in extreme northern latitudes) and, using PCR-based screening, DWV is the most commonly detected virus, often found at high titer [25]. Careful screening of Western honey bee colonies on the archipelagos of Hawaii and New Zealand has clearly demonstrated that DWV rose to 100% prevalence of colonies after the introduction of varroa mites [17, 18]. It is arguably present in all Western honey bee colonies that harbor varroa mites, and phylogenetic analysis of viral gene sequences suggest it has likely followed the introduction of varroa mites to North America and elsewhere to become an EID of Western honey bees worldwide [15*].

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83 Australia remains the only large land mass that, to date, has not reported varroa mites in its
84 managed Western honey bee colonies.
85
86 Concomitant with its rise in prevalence across Western honey bee colonies and in titer in individual
87 honey bees, DWV is the most commonly reported virus detected by PCR in a range of other bee
88 species (Fig. 2). It has been found in three Asiatic honey bee (*Apis*) species in Asia [25, 26]. Across
89 Europe and North America, DWV has also been detected in bumble bees (*Bombus* spp.) [27**, 28,
90 29, 30, 31**, 32, 33, 34], other wild bee species [27**, 29, 35, 36, 37] and even taxonomically more
91 distant hosts such as ants, wasps and cockroaches [28, 29, 38]. These represent an eclectic mix of
92 host species, and the field would benefit from more systematic surveying of the prevalence of DWV,
93 both in terms of geographic and taxonomic (host species) coverage.

94
95 Whether these increases in host range of DWV beyond Western honey bees are a consequence of
96 pathogen spill-over of this EID of the Western honey bee are equivocal because most studies merely
97 report PCR-based detection of DWV. For the Asiatic honey bees that also host varroa mites,
98 phylogenetic analysis of DWV isolates from these different host species supports the suggestion of
99 EID spill-over from introduced, managed Western honey bees harboring varroa mites and DWV [25].
100 Given the mechanistic link between varroa mites and DWV transmission, EID spill-over into Asiatic
101 honey bee species is a plausible interpretation of the phylogenetic data.

102
103 As only honey bees (*Apis* spp.) host varroa mites that can vector DWV, it is assumed that bumble
104 bees and other wild bee species acquire DWV through robbing of DWV-infected colonies of Western
105 honey bees for honey or, more likely, by collection and ingestion of DWV with pollen or nectar from
106 flowers that are also visited by DWV-infected Western honey bee workers [27**, 39, 40]. Given this
107 assumption, we highlight two issues that confound the interpretation of data on DWV prevalence in
108 wild bee species in terms of impact on hosts.

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110 Limitations to interpretation of data on DWV impact

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112 The first issue is that many studies of wild bee species merely report the detection (or not) of DWV,
113 often from a single site [36], making inference of DWV spill-over from Western honey bees difficult
114 at best. We know little of the parasite community of wild bee species [41], a research area that
115 deserves greater attention. Therefore, DWV as a natural pathogen of any given wild bee species
116 cannot in principle be ruled out; it is a plausible hypothesis that DWV is a generalist insect pathogen,
117 or one originally associated with a species other than the Western honey bee.

118
119 To infer spill-over, studies need to examine the prevalence of DWV in managed Western honey bees
120 and wild bee species across multiple sites, and couple this with proof that the pathogen in managed
121 Western honey bees and wild bee species is the same, *e.g.*, by analysis of genetic variants of DWV
122 [27**, 29]. The only study of which we are aware that currently fulfills both criteria has
123 demonstrated (i) a clear correlation between DWV prevalence in Western honey bees and in wild
124 bumble bees across multiple sites and (ii) sharing of the identical viral variant by Western honey
125 bees and wild bees (*Bombus* spp.) within a site [31**].

126
127 These data speak strongly for pathogen sharing between Western honey bees and wild bee species
128 but still leave open the question of directionality of pathogen transmission. DWV prevalence in
129 Western honey bees collected from flowers was approximately double that of DWV prevalence in
130 bumble bees, strongly suggesting spill-over from Western honey bees to wild bee species [31**]. Yet
131 interpretation of pathogen prevalence data is potentially conflated with virulence of that pathogen
132 in a host; for insect populations, mathematical models suggest that highly virulent pathogens exhibit
133 low prevalence and vice versa [42]. There is a clear need for additional research focused on
134 understanding the directionality of transmission of DWV (and other pathogens) among host species,

135 especially research integrating phylogenetic methods that infer transmission over long time scales
136 [15*] with experimental approaches that can test for on-going transmission [27**].

137

138 The second issue concerning PCR-based detection of DWV in wild bee species and other insects is
139 that it may merely reflect contamination of the exterior of a wild bee by DWV or, in the case of
140 wasps, ants and cockroaches, consumption of DWV-infected Western honey bees or hive products.
141 Though such contamination might also facilitate the dispersal of DWV [28, 43], it does not
142 necessarily represent a threat to the host, either the individual host insect, or the host colony (for
143 social species such as bumble bees) or the host population. Amplification of the negative (-) viral
144 strand of (+) RNA viruses like DWV can be used as an indicator of active infection [31**], though we
145 note that in principle it demonstrates viral replication and not host pathology. Similarly, the
146 detection of DWV-infected carpenter bees (*Xylocopa augusti*) with deformed wings suggests that
147 DWV can be infective and pathogenic for wild bee species [35], although other parasites can cause
148 similar pathologies [44]. The most direct means to determine virulence is through experimental
149 inoculation of a host species. Though laboratory experimentation is an abstraction of the real world,
150 it controls for confounding factors when testing for virulence. In experimental inoculation assays, DWV
151 has been shown to be a pathogen of the widespread European bumble bee *Bombus terrestris* [31**,
152 33].

153

154 We have little idea of how virulent DWV is in the other wild bee species in which it has been
155 detected. This is clearly an important area for future research if we are to understand the impact of
156 DWV spill-over from managed Western honey bees to wild bee species. For social wild bee species,
157 there is also a need to scale up from virulence at the level of the individual insect to virulence at the
158 level of the colony [45] and the population. The BEEHAVE model, a software that simulates the
159 development of a honey bee colony and its foraging for nectar and pollen in a realistic landscape,

160 can fulfill this propose for the Western honey bee [46], but there is a need for mathematical models
161 incorporating other forms of social organization, such as that of the bumble bee [47].

162

163 **Other viruses, their prevalence and impact**

164

165 Ten other viruses originally isolated from the Western honey bee, mostly (+) RNA viruses, have been
166 detected in wild bee species (Table 1). After DWV, the next most commonly reported virus in wild
167 bee species is Black queen cell virus (BQCV), followed by Israeli acute paralysis virus (IAPV) (Fig. 2),
168 both (+) RNA viruses.

169

170 BQCV is prevalent and widespread across managed Western honey bee populations in Europe [9, 48]
171 and North America [49]. A structured survey of 26 sites in Great Britain showed BQCV to be more
172 common in the Western honey bee than in bumble bee species, and prevalence of all viruses in
173 Western honey bees was a significant predictor of viral prevalence in bumble bees [21**]. These
174 data again suggest spill-over from domestic Western honey bees to wild bee species (but see
175 caveats above). Though BQCV is pathogenic for Western honey bee larvae following experimental
176 inoculation [50], its impact on wild bee species is unknown, but warrants investigation.

177

178 IAPV has been rarely reported from Europe, but in North America it is prevalent in managed
179 Western honey bees and was initially associated with so-called colony collapse disorder [51]. Like
180 the closely related Kashmir bee virus (KBV) and Acute bee paralysis virus (ABPV), IAPV is highly
181 pathogenic for Western honey bees [52]. It has been detected in wild bee species near IAPV-infected
182 managed Western honey bee colonies, but not in the same wild bee species near a non-IAPV-
183 infected apiary [27**]. Furthermore, greenhouse experiments [27**] have shown successful
184 transmission of IAPV between Western honey bees and wild bee species. Spill-over of IAPV from
185 Western honey bees to wild bee species in the field through shared use of flowers is therefore likely.

186 We note, though, that the related ABPV, also highly pathogenic for Western honey bees [53], has a
187 higher prevalence in wild bee species than Western honey bees [21**], suggesting spill-over from
188 wild bee species to domestic Western honey bees (but see caveats above).

189
190 Though studies on BQCV and IAPV in wild bee species are few (Fig. 2), they highlight the potentially
191 wide distribution of viruses across multiple bee species, likely promoted by shared use of flowers.

192 Experimental demonstration of IAPV cross-species transmission [27**] is exemplary. Similar
193 experiments with other viruses would help our understanding of the threat posed to wild bee
194 species by viruses of managed Western honey bees.

195

196 **Future directions**

197

198 Evidence is mounting that viruses originally detected in the managed Western honey bee are widely
199 distributed across wild bee species. For DWV, data support the idea that virus spills over from
200 Western honey bees to wild bee species, where it potentially reduces individual wild bee host fitness
201 [31**]. Yet viral prevalence varies considerably across wild bee species [21**], and some viruses
202 have higher prevalence in wild bees, suggesting spill-over to managed Western honey bees. A key
203 future aim is to determine the epidemiology and transmission dynamics of these viruses in the field.
204 Do they show source-sink dynamics, or active back-and-forth transmission? Understanding this will
205 give us tools to manipulate transmission in the field, for the benefit of both wild bee species and
206 managed Western honey bees. From the direct perspective of impact, an interesting question is
207 whether some wild bee species, particularly those that are rare or declining, are less tolerant of viral
208 pathogens than others; analysis of their anti-viral innate immune defence mechanisms [54], whose
209 complements vary among Dipteran species [55*], may be one productive means of investigation.
210 Similarly, genetic analyses of population size and bottlenecks in coupled wild bee-virus systems
211 might elucidate past impacts. Whether viral emergence in managed Western honey bees leads to

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212 host switching through the mass action principle [56, 57], *i.e.* through increasing density of infective
213 virions in the environment, versus viral adaptation is another question that would benefit from
214 greater attention, not only in understanding the threat posed to wild bee species by managed
215 Western honey bee viruses but also as a model of EID impacts on wildlife. The impact of viruses may
216 also vary with co-infection by two or more viruses [58] and through interactions with other so-called
217 ‘stressors’ of wild bee species such as pesticide exposure [8], as has been demonstrated at the
218 molecular level for the Western honey bee [59]. With such information to hand, we may be able to
219 understand how pathogen spill-over from Western honey bees impacts communities of wild bee
220 species, their populations, and the ecosystem service of pollination, from which terrestrial life and
221 human well-being benefit.

222

223 **Acknowledgements**

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225 thanks BB/N000668/1, and RJP thanks the DFG’s Priority Programme 1596 (Ecology and species
226 barriers in emerging viral diseases), project Pa 632/11.

227

228 **References and recommended reading**

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

230 * of special interest **of outstanding interest

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31
 32 264 **This study applies Bayesian statistical methods to the analysis of three gene sequences of DWV**
 33 265 **from isolates collected across the world. There is strong support for the emergence of DWV in**
 34 266 **managed Western honey bees (*Apis mellifera*) as a consequence of varroa mite (*Varroa***
 35 267 ***destructor*) parasitism of honey bees, and its likely spread from Europe to North America and**
 36 268 **elsewhere.**

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322 **bumble bees (*Bombus* spp.). Sequencing of DWV variants gave unequivocal support for the on-**
323 **going sharing of DWV between bee taxa while lab experiments demonstrated the pathogenicity of**
324 **DWV for wild bumble bees when ingested with pollen.**

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398 **Table 1:** Reported taxonomic distribution in wild bee species of viruses initially detected in managed Western honey bees (*Apis mellifera*)

	DWV	BQCV	IAPV	SBPV	ABPV	SBV	KBV	LSV	CBPV	AmFV	VdMLV
<i>Andrena vaga</i> ^a		17	20					17		17	
<i>Andrena ventralis</i> ^a								17		17	
<i>Augochlora pura</i> ^b	20										
<i>Bombus agrorum</i> ^c					1						
<i>Bombus atratus</i> ^c	4, 18	4, 18		4	4	4, 18					
<i>Bombus hortorum</i> ^c		12		12	1, 12						
<i>Bombus huntii</i> ^c	9	15									
<i>Bombus impatiens</i> ^c	9, 19, 20	8, 20	8, 19	8	19		19		19		
<i>Bombus lapidarius</i> ^c	3	12		12, 14	12			14			14
<i>Bombus lucorum</i> ^c	3	12		12	1, 12						
<i>Bombus monticola</i> ^c	3										
<i>Bombus pascuorum</i> ^c	2, 5	12		12	12			14			14
<i>Bombus pratorum</i> ^c								14			
<i>Bombus ruderarius</i> ^c					1						
<i>Bombus ternarius</i> ^c	20	20	20								
<i>Bombus terrestris</i> ^c	2, 3, 5, 6	12	13, 16	12	1, 12	12, 20	13				
<i>Bombus vagans</i> ^c	8, 20	8, 20	20	8		20					
<i>Ceratina dupla</i> ^d	20										
<i>Heriades truncorum</i> ^e		17									
<i>Osmia bicornis</i> ^e	17							17		17	17
<i>Osmia cornuta</i> ^e	11	17						17		17	17
<i>Scaptotrigona mexicana</i> ^f	7	7									
<i>Xylocopa latreille</i> ^d	10										
<i>Xylocopa virginica</i> ^d	20	8, 20		8							

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400 For numbered references, see supplementary material Appendix A; cell shading in yellow: detection of a virus in a host species; in orange: detection of (-)
401 strand of virus in a host species, indicative of RNA replication; in red: demonstration of pathogenicity in a host species; DWV (Deformed wing virus), BQCV
402 (Black queen cell virus), IAPV (Israeli acute paralysis virus), SBPV (Slow bee paralysis virus), ABPV (Acute bee paralysis virus), SBV (Sacbrood virus), KBV
403 (Kashmir bee virus), LSV (Lake Sinai virus), CBPV (Chronic bee paralysis virus), AmFV (*Apis mellifera* filamentous virus), VdMLV (*Varroa destructor* macula-
404 like virus); ^a mining bees (family Andrenidae); ^b sweat bees (family Halictidae); ^c bumble bees (genus *Bombus*, family Apidae); ^d carpenter bees (members of
405 the family Apidae); ^e leafcutter and mason bees (family Megachilidae); ^f stingless bees (members of the family Apidae)

440 **Figure captions:**

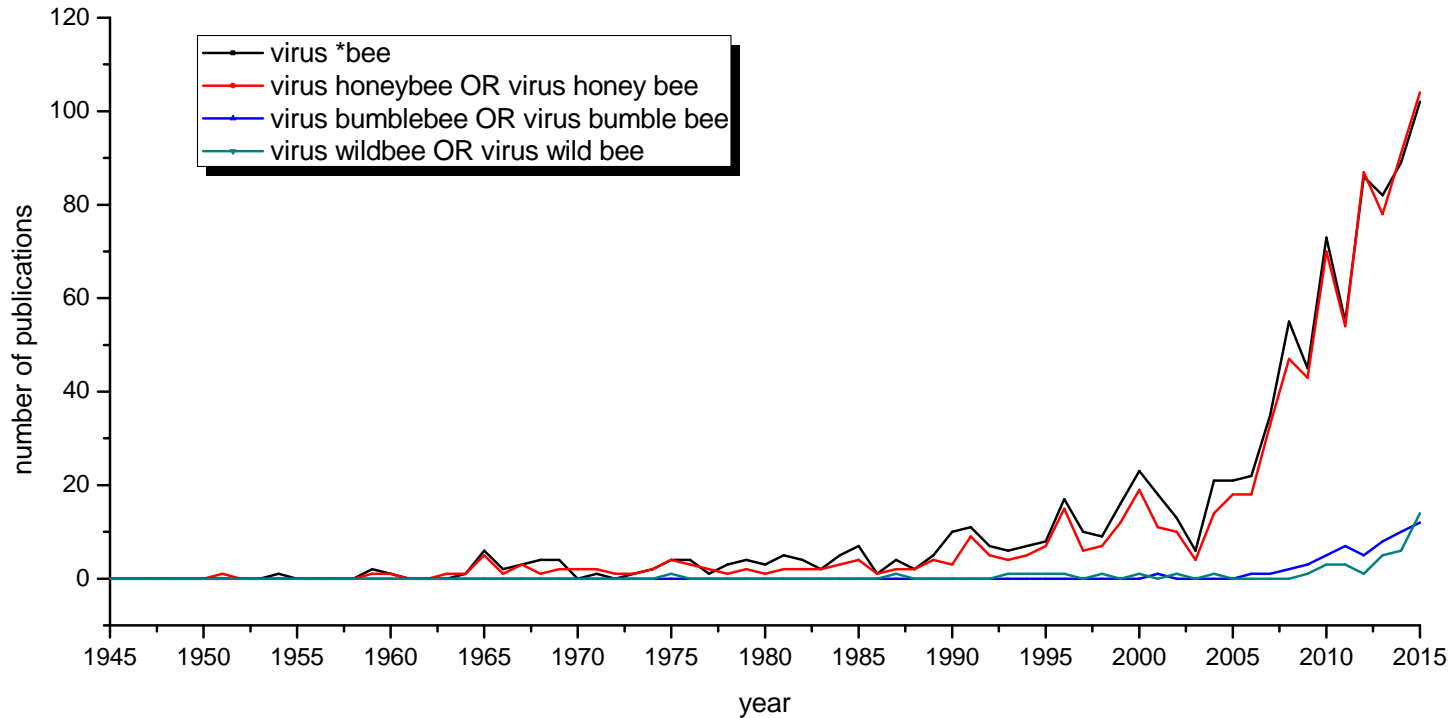
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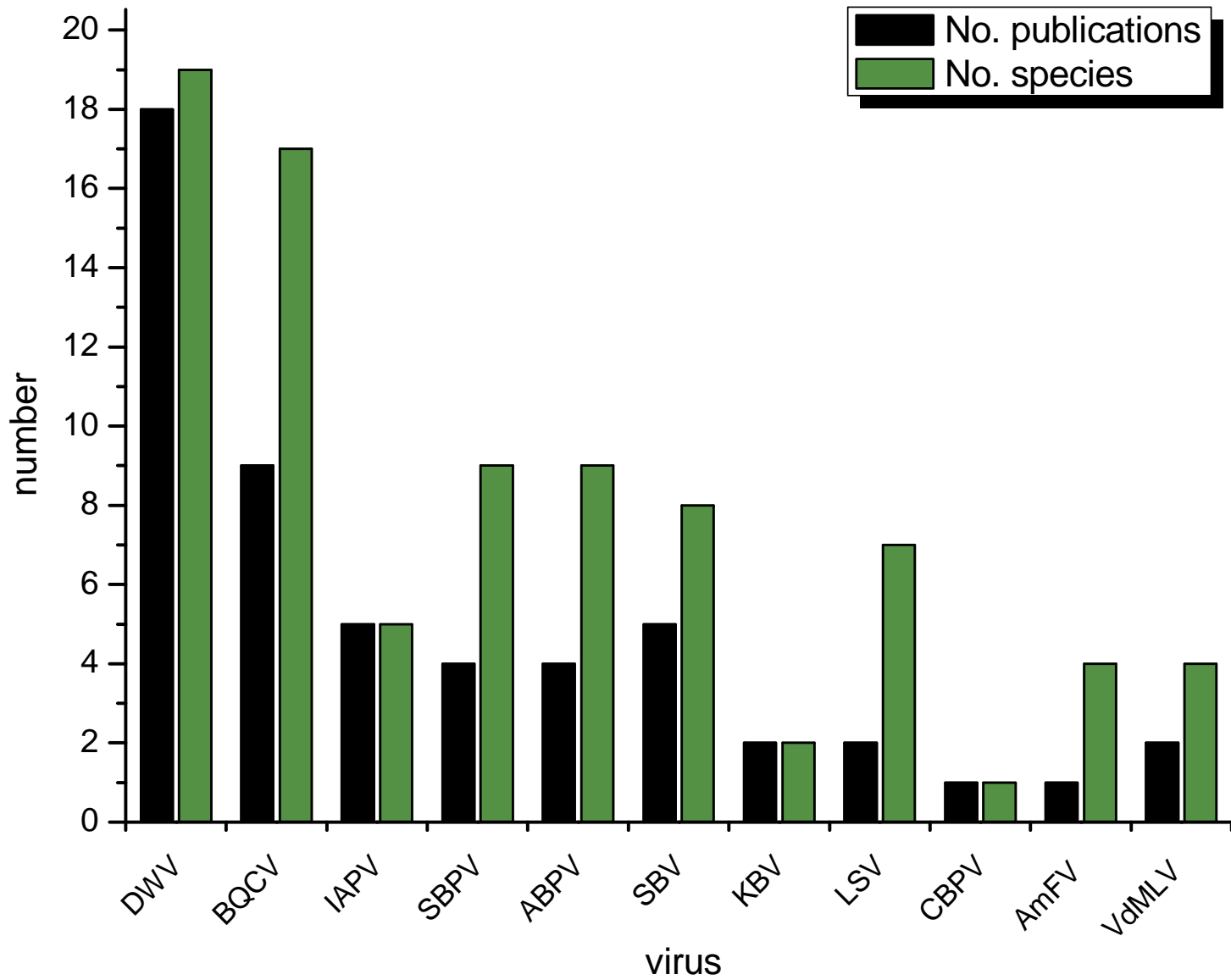
442 **Figure 1:** Increase in the number of publications on viruses across bee species over the past 70
443 years, showing that most relate to managed honey bees (the Western honey bee, *Apis mellifera*,
444 or other Asiatic honey bee species that all belong to the genus *Apis*), and relatively few to
445 bumble bees (genus *Bombus*) or other wild bees (species in the family Apidae other than honey
446 bees and bumble bees, plus all species in the families: Halictidae, Megachilidae, Andrenidae,
447 Colletidae, Melittidae and Stenotritidae); Web of Science search (accessed on March 18th, 2016)
448 using terms in the box

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450 **Figure 2:** The frequency with which putative honey bee (the Western honey bee, *Apis mellifera*,
451 or other Asiatic honey bee species that all belong to the genus *Apis*) viruses have been reported
452 in other bee species, both in terms of the total number of papers per virus and the number of
453 host species (excluding *Apis* spp.) per virus; DWV (Deformed wing virus), BQCV (Black queen cell
454 virus), IAPV (Israeli acute paralysis virus), SBPV (Slow bee paralysis virus), ABPV (Acute bee
455 paralysis virus), SBV (Sacbrood virus), KBV (Kashmir bee virus), LSV (Lake Sinai virus), CBPV
456 (Chronic bee paralysis virus), AmFV (*Apis mellifera* filamentous virus), VdMLV (*Varroa destructor*
457 macula-like virus)

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Supplementary Material

Appendix A: numbered references embedded within Table 1

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