

1 **Disease associations between honeybees and bumblebees as a threat to wild pollinators**

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15 **Emerging infectious diseases (EIDs) pose a risk to human welfare, both directly¹ and**
16 **indirectly, by affecting managed livestock and wildlife that provide valuable resources**
17 **and ecosystem services, such as the pollination of crops². Honey bees (*Apis mellifera*),**
18 **the prevailing managed insect crop pollinator, suffer from a range of emerging and**
19 **exotic high impact pathogens^{3,4} and population maintenance requires active**
20 **management by beekeepers to control them. Wild pollinators such as bumble bees**
21 **(*Bombus* spp.) are in global decline^{5,6}, one cause of which may be pathogen spillover**
22 **from managed pollinators like honey bees^{7,8} or commercial colonies of bumble bees⁹. In**
23 **our study, a combination of infection experiments with landscape scale field data**
24 **indicates that honey bee EIDs are indeed widespread infectious agents within the**

25 **pollinator assemblage. The prevalence of deformed wing virus (DWV) and the exotic**
26 ***Nosema ceranae* is linked between honey bees and bumble bees, with honey bees having**
27 **higher DWV prevalence, and sympatric bumble bees and honey bees sharing DWV**
28 **strains; *Apis* is therefore the likely source of at least one major EID in wild pollinators.**
29 **Lessons learned from vertebrates^{10,11} highlight the need for increased pathogen control**
30 **in managed bee species to maintain wild pollinators, as declines in native pollinators**
31 **may be caused by interspecies pathogen transmission originating from managed**
32 **pollinators.**

33 Trading practices in domesticated animals allow infectious diseases to spread rapidly and to
34 encounter novel hosts in newly sympatric wildlife¹². This “spillover” of infectious disease
35 from domesticated livestock to wildlife populations is one of the main sources of Emerging
36 Infectious Disease (EIDs)¹³. Small or declining populations are particularly challenged, as the
37 source host may act as a disease reservoir¹⁴, giving rise to repeated spillover events and
38 frequent disease outbreaks which, in the worst case, might drive already vulnerable or
39 unmanaged populations to extinction¹⁴. Such severe impacts have been well documented over
40 the past decades in vertebrates¹⁰, but have largely been overlooked in invertebrates¹⁵. Recent
41 years have seen elevated losses in multiple populations of one of the major crop pollinating
42 insects, the honey bee (*Apis mellifera*)¹⁶. EIDs have been suggested as key drivers of decline,
43 with deformed wing virus (DWV) (especially in combination with the exotic *Varroa* mite
44 (*Varroa destructor*)) and *Nosema ceranae* (*N. ceranae*) being two likely causes for losses of
45 *Apis*¹⁷. As generalist pollinators, honey bees are traded and now distributed almost worldwide
46 for crop pollination and hive products. They share their diverse foraging sites with wild
47 pollinators and thus facilitate interspecific transmission of pathogens, as has been suggested
48 for intraspecific disease transmission from commercial to wild bumble bee populations¹⁸. Our
49 focus is on inter-specific transmission, as EIDs in *Apis* are a potential threat to a range of wild

50 pollinators worldwide. Whilst evidence from small scale studies suggests that wild pollinators
51 like *Bombus* spp. may already harbour some honey bee pathogens^{7,8,19,20}, the true infectivity
52 and landscape scale distribution of these highly virulent EIDs in wild pollinator populations
53 remains unknown

54 To examine the potential for *Apis* pathogens to cross host-genus boundaries, we tested the
55 infectivity of the DWV complex (which includes the very closely related, co-occurring and
56 recombinant *Varroa destructor* virus (VDV)^{21,22}; we will refer to “DWV complex” as
57 “DWV” throughout the text) and *N. ceranae*, in controlled inoculation experiments, to one of
58 the most common *Bombus* species in Great Britain (*B. terrestris*). DWV is infective for *B.*
59 *terrestris*; we found significantly more DWV infections 21 days after inoculating *B. terrestris*
60 workers versus control (likelihood ratio test comparing the full model to one with only the
61 intercept: $X^2 = 5.73$, $df = 1$, $p < 0.017$; Fig 1) and mean survival was reduced by 6 days. As
62 for *Apis*, DWV causes deformed wings in *Bombus* when overtly infected⁸, resulting in non-
63 viable offspring and reduced longevity (Fig 1). *N. ceranae* is also infective for *B. terrestris*;
64 infections increased in *Bombus* versus control ($X^2 = 17.76$, $df = 1$, $p < 0.001$; Fig 1), though
65 overt symptoms were not seen (mean survival increased by 4 days).

66 Having established both DWV and *N. ceranae* as infective for *B. terrestris*, we conducted a
67 structured survey across 26 sites in GB and the Isle of Man, collecting 10 *Apis* samples, and
68 20 *Bombus* samples per site to assess EID prevalence (for details on species identity across
69 sites, see Extended Data Fig. 1). We analysed a total of 745 bees from 26 sites for DWV
70 presence, DWV infection (replicating DWV) and *N. ceranae* presence. DWV was present in
71 20% (95% confidence interval (CI) 17-23%) of all samples; 36% (95% CI: 30-43%) of *Apis*
72 and 11% (95% CI: 9-15%) of *Bombus*. Of the *Apis* harbouring DWV, 88% (95% CI: 70-
73 98%) of the samples tested had actively replicating virus, whilst 38% (95% CI: 25-53%) of
74 *Bombus* harbouring DWV had replicating virus (see Extended Data Fig. 2 and Extended Data

75 Table 1). *N. ceranae* was less frequent, being detected in 7% (95% CI: 6-10%) of all samples;
76 9% (95% CI: 6-13%) of *Apis* samples and 7% (95% CI: 5-9%) of *Bombus* samples.

77 We estimated the GB-wide prevalence of the two pathogens in *Apis* and *Bombus* spp. based
78 on our field survey data (Fig. 2). We found no evidence for spatial clustering of DWV
79 presence in *Bombus* (Moran's I = 0.023, $p > 0.211$) or either of the pathogens in *Apis* (DWV
80 presence: Moran's I = 0.03, $p > 0.186$; *Nosema*: Moran's I = -0.061, $p > 0.649$). There was,
81 however, weak clustering of DWV infection in *Bombus* (Moran's I = 0.061, $p < 0.044$) and
82 very strong clustering of *N. ceranae* in *Bombus* (Moran's I = 0.25, $p < 0.001$), indicating
83 disease hotspots for DWV in *Bombus* in the south west and east of GB and for *N. ceranae* in
84 *Bombus* in the south east of GB (Fig. 2). Because prevalence was lower in *Bombus* than *Apis*,
85 we modelled pathogen prevalence in *Bombus* as dependent on pathogen prevalence in *Apis*,
86 *Bombus* to *Apis* density, and *Apis* abundance, including biologically relevant interactions,
87 whilst controlling for latitude, longitude, and sunlight hours, and adding collection site and
88 species identity as random factors. Our full model for DWV presence was significantly better
89 than the null model without any of the test predictors and their interactions included
90 (likelihood ratio test: $X^2 = 19.03$, $df = 5$, $p < 0.002$). After removal of the non-significant
91 interactions (GLMM: *Bombus* to *Apis* density X DWV presence in *Apis*: estimate \pm SE = -
92 0.105 ± 1.376 , $p = 0.939$; *Apis* abundance X DWV presence in *Apis*: 0.425 ± 1.309 , $p = 0.745$),
93 it is clear that prevalence of DWV in *Apis* has a strong positive effect on DWV prevalence in
94 *Bombus* (GLMM: 2.718 ± 0.921 , $z = 2.951$, $p < 0.004$) (Fig. 2, Extended Data Fig. 3), while
95 none of the other predictors played a role (GLMM: *Bombus* to *Apis* density: 0.315 ± 0.387 , z
96 $= 0.814$, $p < 0.416$; *Apis* abundance : -0.085 ± 0.364 , $z = -0.232$, $p < 0.816$). In the case of *N.*
97 *ceranae*, our full model was significantly better than the null model ($X^2 = 15.8$, $df = 5$, $p <$
98 0.008). Specifically there was an effect of *Nosema* prevalence in *Apis* on *Nosema* prevalence
99 in *Bombus* and this varied with *Apis* abundance (interaction between *Nosema* prevalence in

100 *Apis* and *Apis* abundance: $X^2 = 7.835$, $df = 2$, $p < 0.02$), while *Bombus* to *Apis* density did not
101 explain *Nosema* prevalence in *Bombus* (GLMM: 8.386 ± 6.793 , $z = 1.235$, $p = 0.217$)(Fig. 2,
102 Extended Data Fig. 3).

103 The prevalence data implied local transmission of DWV between *Apis* and *Bombus*. To test
104 this, we sequenced up to 5 isolates per DWV infected *Bombus* sample from 5 sites matched
105 by up to 5 isolates of sympatric DWV infected *Apis* samples. If a pathogen is transmitted
106 between these two hosts, we would expect *Apis* and *Bombus* to share the same DWV strain
107 variants within a site. Marginal log likelihoods estimated by stepping stone sampling²³
108 decisively support clades constrained by site as opposed to host, indicating pathogen
109 transmission within site (Fig. 3, Extended Data Table 2).

110 Our results provide evidence for an emerging pathogen problem in wild pollinators that may
111 be driven by *Apis*. Our data cannot demonstrate directionality in the interspecific
112 transmission of DWV. However, the high prevalence of DWV in honey bees, which is a
113 consequence of the exotic vector *Varroa destructor*²⁴, is consistent with their acting as the
114 major source of infection for the pollinator community. Similar results have been found for
115 intraspecific transmission of *Bombus*-specific pathogens from high prevalence commercial
116 *Bombus* colonies to low prevalence wild *Bombus* populations¹⁸. Our field estimates of
117 prevalence are conservative for DWV, as highly infected individuals have deformed wings,
118 are incapable of flight, and thus would not be captured by our sampling protocol.
119 Consequently, DWV prevalence and, as a result, impact are likely to be higher in managed
120 and wild populations than our data suggest. Interestingly, *N. ceranae* prevalence in *Bombus*
121 depends positively on *Apis* abundance, but only when *N. ceranae* prevalence in *Apis* is low,
122 suggesting a possible environmental saturation effect of *N. ceranae* spores. In contrast to the
123 low impact of *N. ceranae* on the survival of *B. terrestris* in our study, Graystock et al.²⁵ found
124 very high virulence. This might be explained by our use of young bees vs Graystock et al.'s²⁵

125 non-age-controlled design, indicating age dependent differential susceptibility in *B. terrestris*,
126 as has been shown to be the case in honey bees²⁶.

127 Ongoing spillover of EIDs could represent a major cause of mortality of wild pollinators
128 wherever managed bees are maintained. While our data are only drawn from GB, the
129 prerequisites for honey bees to be a source or reservoir for these EIDs – high colony densities
130 and high parasite loads – are present at a global scale. In addition, global trade in both honey
131 bees and commercial *Bombus* may exacerbate this impact^{6,27}. Reducing the pathogen burden
132 in managed honey bees so as to reduce the risk of transmission to wild pollinators is not
133 straightforward. Tighter control of importation and hygiene levels of transported colonies
134 could be imposed with regulation, but policies developed in this direction must learn from the
135 past; such regulation is difficult to implement and hard to evaluate^{9,28}. Clearly, it is essential
136 to ensure that those managing bees (including commercial producers, growers and
137 beekeepers) have access to the methods and skills to monitor, manage and control EIDs for
138 the benefit of their managed colonies, and the wider pollinator community. A consensus on
139 the threat of EIDs for wild pollinators can only be reached with greater knowledge of their
140 epidemiology, global extent and impact, and it will be crucial to involve key stakeholders
141 (e.g. the beekeeping community, *Bombus* exporters) in any decision process, as any progress
142 made will largely be driven by their actions.

143

144 **Methods summary**

145

146 ***Bombus* inoculation experiment**

147 Two day old workers of *Bombus terrestris audax* colonies (Biobest) were individually
148 inoculated with either 10^5 spores/bee purified *N. ceranae* or 10^9 genome equivalents/bee

149 purified DWV in 10 µl sucrose solution. Bees surviving for 21 days were freeze killed and
150 molecularly tested for pathogen presence.

151

152 **Sampling scheme**

153 Sampling took place at 24 mainland sites and two currently *Varroa destructor* (the main
154 vector for DWV in *Apis mellifera*) free islands: Colonsay and the Isle of Man (see Extended
155 Data Fig. 1 for site distribution). Cryptic *Bombus* species were identified by PCR-RFLP-
156 analysis²⁹. *Apis* and *Bombus* densities were estimated for each site by timing the collection
157 effort for 20 samples from each genus simultaneously. Samples collected were freeze-killed
158 at -20 °C and transferred to -80 °C as soon as possible thereafter. RNA and DNA preparation
159 followed standard protocols. Virus strand specific RT-PCR was carried out following Craggs,
160 et al.³⁰.

161

162 **Statistics**

163 True prevalences with 95% confidence intervals were computed based on Stevenson, et al.³¹
164 (R library epiR, version 0.9-45, function epi.prev).

165 Overall prevalence for each of our parasites was calculated using Gaussian kernel estimators
166 with an adaptive bandwidth of equal number of observations (set to 3x the maximum
167 observations per site)³² (R library prevR, version 2.1, function kde).

168 Moran's I was calculated as implemented in Paradis, et al.³³ (R library ape, version 3.0-7,
169 function Moran.I).

170 We ran Generalized Linear Mixed Models (GLMM)³⁴ to investigate both effects on disease
171 status of individuals 21 days after pathogen challenge and also pathogen prevalence in
172 *Bombus* using the function lmer of the R package lme4³⁵. All analyses were run in R³⁶.

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 251 Experiments were designed by M.A.F. and M.J.F.B.; M.A.F prepared the manuscript;
 252 M.J.F.B., D.P.M., R.J.P. and J.O. edited the manuscript. M.A.F. carried out the experimental
 253 work, molecular work and analyses apart from the phylogenetic analysis carried out by
 254 D.P.M..

255 **Author information** Viral RNA sequences have been deposited in GeneBank under accession
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257 Readers are welcome to comment on the online version of the paper. Correspondence and
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260

261 **Figures:**

262 **1 Infectivity:** Prevalence of infections in treated *Bombus terrestris* workers 21 days after
263 inoculation (in percent). Bars indicate 95% confidence intervals. Colours indicate treatment,
264 with *Nosema* treated samples in green and DWV treated samples in black. Sample sizes are
265 given inside the mean data point. The survival graph over the 21 day test period shows
266 uninfected control treatments in grey compared to infected DWV treatments in blue (Cox
267 mixed effects model fitted with penalized partial likelihood: $X^2 = 11.93$, $df = 4.17$; $p < 0.021$,
268 see Supplementary Information).

269 **2 Prevalence:** Estimated pathogen prevalence in *Apis* and *Bombus* across Great Britain and
270 the Isle of Man. Colour gradient (based on Gaussian kernel estimators with an adaptive
271 bandwidth of equal number of observations over 26 sites, see Methods) corresponds to
272 percent prevalence (note different scales). DWV prevalence is displayed in blue and *Nosema*
273 prevalence in green.

274 **3 Viral strain relations:** RNA-dependent RNA polymerase (RdRp) partial gene phylogeny
275 of pollinator viruses (see main text). Gene trees were estimated using PhyML v.3.0
276 maximum-likelihood (ML) bootstrapping (500 replicates) and MrBayes v3.1.2 (see
277 Methods). Coloured boxes correspond to sites H, L, Q, R and X (as shown on the map) while

278 text colours correspond to host (Red: *Bombus*; Black: *Apis*). Symbols represent node support
279 values: posterior probability (left), bootstrap support (right). Filled circle: >90%, Target
280 symbol: >70%, Empty circle: >50%. Branches (//) one third of true length.

281 **Methods**

282 ***Bombus* inoculation experiment**

283 Each of the 7 experimental *Bombus terrestris* colonies (Biobest) was tested for presence of
284 the two treatment pathogens DWV and *N. ceranae*. Daily, callows (newly emerged workers)
285 were removed from the colony, assigned sequentially to random treatment blocks and housed
286 individually in small Perspex boxes on an *ad libitum* diet of 50% sucrose solution and
287 artificial pollen (Nektapoll), as natural pollen has been shown to contain viable *N. ceranae*
288 spores and DWV virions^{19,37}. Two day old bumble bee workers were individually inoculated
289 with a treatment dependent inoculum in 10 µl sucrose. Crude hindgut extracts of 5 *Apis*
290 workers propagating *N. ceranae* were purified by the triangulation method³⁸ with slight
291 adaptations.

292 We used small cages with 30 *N. ceranae* infected honey bees to propagate *N. ceranae* spores
293 for the inoculum. Every second day we collected 5 honey bees from these cages, and
294 removed and ground the hindguts. The resulting extract was filtered through cotton and
295 washed with 0.9% insect ringer (Sigma Aldrich). We triangulated extracts using Eppendorf
296 tubes and spin speeds of 0.5g for 3 minutes, purifying *N. ceranae* spores over 7 tubes. Spore
297 numbers were quantified in a Neubauer counting chamber. In parallel, we extracted and
298 purified *N. ceranae* free bees to use for control inoculations.

299 DWV virus inoculum was prepared according to Bailey & Ball³⁹ with modifications.
300 Honeybees with DWV symptoms (crippled wings and body deformities) were crushed in
301 0.5M potassium phosphate buffer (pH 8.0), filtered and clarified by slow speed centrifugation

302 (8000g for 10 minutes) before being diluted and injected (1µl) into white-eyed pupae for bulk
303 propagation of virus. After 5 days, up to 100 pupae were harvested, and after a further screen
304 by qRT-PCR, virus was purified as follows. Virus extraction buffer consisted of 0.5M
305 potassium phosphate pH 8.0, 0.2% DEICA, 10% diethyl ether. Purification consisted of two
306 slow speed clarifications (8000g for 10 minutes), one high speed clarification (75000g for 3
307 hours) followed by re-suspension in 0.5M potassium phosphate buffer (pH8.0) and a final
308 slow speed clarification. Virus preparations were aliquoted and stored at -80°C until use in
309 inoculation experiments.

310 The purified virus was checked by quantitative Reverse Transcription (qRT) PCR for the
311 presence of DWV and the absence of other common honey bee RNA viruses: BQCV, IAPV,
312 SBV, CBPV, ABPV, and SBPV by PCR.

313 A duplicate dilution series of external DNA standards covering 10^2 to 10^8 molecules (reaction
314 efficiencies: 90-110%, r^2 : 0.95-0.99) were included in qRT-PCR runs to quantify DWV
315 genome equivalents present in the inoculum. For absolute quantification of virus dose, an
316 external DNA standard was generated by amplifying a genomic fragment of 241bp using the
317 primers F8668*std* (5'-GAT GGG TTT GAT TCG ATA TCT TGG-3') and B8757*std* (5'-
318 GGC AAA CAA GTA TCT TTC AAA CAA TC-3') via RT-PCR that contained the 136bp
319 fragment amplified by the DWV-specific qRT-PCR primers F8668/B8757⁴⁰.

320 Shortly before administering, inocula were prepared to a total concentration of 10^5 spores/bee
321 in 10 µl (10^4 spores/µl sucrose solution). Inocula were administered individually in a small
322 Petri dish after 30-60 minutes starvation. Only workers ingesting the full 10 µl within 1h
323 were used in the experiment.

324

325 **Sampling scheme**

326 The mainland sampling sites were chosen across Great Britain along a north-south transect
327 (12 sampling points with fixed latitude, but free in longitude) and across two east-west
328 transects (12 sampling points with fixed longitude, but free within a narrow latitudinal
329 corridor). Each of the mainland sites were at least 30 km apart (mean \pm SD of nearest
330 neighbour = 69.21 ± 26.39). The island sites were chosen deliberately to gain background
331 data for both *Apis* and *Bombus* disease prevalence in the absence of *Varroa*, the main
332 transmission route for DWV in *Apis*. At each sampling site we collected approximately 30
333 workers for each of the following species: *Apis mellifera*, *Bombus terrestris* (verified by
334 RFLP-analysis²⁹), and the next most common bumble bee on site. We collected free flying
335 bees from flowers rather than bees from colonies as this is the most likely point of contact in
336 the field. By collecting from flowers we lowered the likelihood of collecting bumblebees
337 from different colonies. While we ran the risk of collecting multiple honeybees from the
338 same hive, this nevertheless represents the potential force of infection for both genera in the
339 field.

340 Each collection took place along a continuous transect, where maximally ten bees per ten
341 metre stretch were collected before moving on to the next ten metre stretch. At each site, the
342 collection area covered at least 1000 m² (e.g., 10 x 100m, 20 x 50m). Each sampling point
343 was within one of the following landcover types: urban areas (gardens and parks), farmland
344 (hedgerows, border strips, crops, and wildflower meadows), coastal cliffs, sand dunes and
345 heather moorland.

346 If possible, we collected all bees within a single day. In the case of adverse weather, we
347 returned as soon as possible to finish the collection at the exact same site. To estimate *Apis*
348 and *Bombus* densities at each site we timed the collection effort simultaneously. Time taken

349 to collect 20 *Bombus* workers (of any *Bombus* species) and 20 *Apis* workers was recorded,
350 respectively. Timed collecting efforts took place on a single day only.

351 Samples collected were put in sampling tubes, transferred straight onto ice, then freeze-killed
352 at -20°C and transferred to -80°C as soon as possible thereafter to ensure optimal RNA
353 (DWV) preservation.

354

355 **RNA work**

356 RNA extraction followed the standard RNeasy plant mini kit (Qiagen) protocol with the final
357 elutate (in RNase free ddH₂O) of 30 µl being run over the column twice (for optimal RNA
358 concentration). For reverse transcription of RNA to cDNA we followed the standard protocol
359 of the Nanoscript Kit (Primerdesign). Our priming was target specific in separate reactions
360 for *N. ceranae* (primer pair *N. ceranae*⁴¹), DWV (primer pair F15/B23⁴²) and a housekeeping
361 gene (primer pair ACTB⁴³) as a positive control for RNA extraction efficiency. Bees were
362 transferred to liquid N₂ prior to dissection. Each bee's abdomen was cut with a sterile scalpel
363 dorsoventrally along the sagittal plane. One half was submerged in RLT buffer (Qiagen) for
364 RNA extraction, and the second half was archived at -80C. Tissue disruption and
365 homogenisation of individual half-abdomens was performed on a tissue lyser II (Qiagen) at
366 30Hz for 2 minutes followed by 20Hz for 2 minutes. RNA quality and quantity were checked
367 on a Spectrometer (Nanodrop, Thermo Scientific). cDNA preparation was conducted at 65°C
368 for 5 minutes for the initial priming immediately before the addition of the reverse
369 transcriptase. For the extension, samples were incubated at 25°C for 5 minutes followed by
370 55 °C for 20 minutes and then heat inactivated for 15 minutes at 75°C. cDNA was used as
371 template in a standard PCR with 57°C, 54°C, and 57°C annealing temperatures, respectively.
372 Results were visualized on a 2% agarose gel with EtBr under UV light. Agarose gels were

373 scored without knowledge of sample ID. To verify the specificity of the amplicon, one
374 purified PCR product taken from *Apis* and one taken from *B. lapidarius* were sequenced
375 (Macrogen Inc.).

376

377 **Detection of negative strand DWV**

378 Detection of pathogens in pollinators in the field does not provide proof of infection, as
379 pathogens are likely being ingested on shared, contaminated food resources and therefore are
380 inevitably present in the gut-lumen as passive contaminants without necessarily infecting the
381 host. To minimize these cases, we tested all our DWV positive *Bombus* samples and a subset
382 of DWV positive *Apis* samples for virus replication, a strong indicator for infection⁴⁴. DWV
383 is a positive strand virus whose negative strand is only present in a host once the virus is
384 actively replicating³⁹. Reverse transcription was conducted using a tagged primer tagB23⁴⁵
385 for the initial priming to target exclusively the negative strand. The resulting cDNA was used
386 in a PCR with the tag sequence and F15 as primers^{30,45}. We tested all *Bombus* samples that
387 were positive for DWV presence and, where possible, 2 DWV-positive *Apis* samples from
388 each site where we found DWV in *Bombus*.

389

390 **Sequencing**

391 DWV sequence diversity was analysed by sequencing up to 5 independent clones per DWV
392 negative-strand infected *Bombus* sample from 5 sites (H, L, Q, R, X; chosen for their high
393 DWV infected prevalence in *Bombus*) and 5 clones of DWV infected *Apis* samples from the
394 same sites (we checked extra *Apis* samples for DWV infection if necessary to match *Bombus*
395 DWV infections). All *Bombus* samples were *B. lapidarius* with the exception of one sample
396 from site L (clone05), which was *B. pascuorum* (this sample is not included in any of the
397 other analyses, but revealed a DWV infection in an initial screening and was hence included

398 in the virus variant analysis). We sequenced a region of the DWV genome: the RNA-
399 dependent RNA polymerase (RdRp) gene (F15/B23 primer pair⁴² used throughout the study).
400 RdRp is thought to be a conserved region of the virus genome where non-synonymous
401 substitutions may have significant implications for the epidemiology of the virus²⁴. RT-PCRs
402 and PCR were run as described before. DWV PCR products were verified by gel
403 electrophoresis as described above; if a clear, clean single band was visible, we proceeded
404 directly to the cloning protocol. If not, we purified products from the agarose gel following a
405 standard protocol (Qiaquick Gel Extraction Kit, Qiagen) and used the purified fragment in an
406 additional PCR. PCR products were cloned using the Invitrogen TA cloning kit (Invitrogen),
407 according to the manufacturer's instructions. Plasmid DNA was isolated using the Spin
408 Miniprep kit (Qiagen) and the successful insertion of target sequence was tested by restriction
409 analysis (digested with EcoR I). Up to 5 clones per sample were sequenced in forward and
410 reverse orientation (Source BioSciences, Cambridge).

411

412 **Analysis of DWV sequences**

413 The 75 *Apis* and *Bombus* clones from sites H, L, Q, R and X were supplemented with DWV
414 and VDV reference RdRp sequences (accession nos. NC004830 and NC006494
415 respectively), resulting in a final alignment of 420bp from 77 sequences. Forward and reverse
416 sequences of each clone were assembled and the consensus sequence was used for further
417 analysis. Sequences were aligned using Geneious (R 6.1.6) with standard settings. Ends were
418 trimmed by hand. For the tree building we conducted two independent (MC)³ algorithms
419 running for 2 million generations, each with four chains (3 hot, 1 cold), sampling one tree in
420 1000, under the GTR+ Γ (nst = 6) substitution model. Gene trees were estimated using PhyML
421 v.3.0⁴⁶ maximum-likelihood (ML) bootstrapping (500 replicates) and MrBayes v3.1.2⁴⁷,
422 under a GTR model of sequence evolution and a gamma (Γ) model, using 4 categories to

423 accommodate rate variation across sites. Burn-in cutoffs were inspected manually for each
424 parameter file in Tracer v1.4⁴⁸. Inspection of the standard deviation of split frequencies
425 confirmed that runs had converged (0.0093). To test alternative *a priori* hypotheses of virus
426 diversification, for each virus (DWV and VDV) we constrained clades according to site (H,
427 L, Q, R and X) or host genus (*Apis* and *Bombus*), and performed stepping stone sampling²³ as
428 implemented in MrBayes v3.1.2 to accurately estimate marginal log likelihoods. MCMC
429 sampling was conducted for 50 steps of 39000 generations each, with the first 9000
430 generations of every step discarded as burn-in. The model with the highest likelihood score
431 was used as the null hypothesis. We compared Bayes Factors (BF) for both models and used
432 a threshold of $2 \ln(\text{BF}) > 10$ as decisive support for the null against the alternative
433 hypothesis⁴⁹ (Supplementary Table 2). We repeated stepping stone sampling to confirm run
434 stability (data not shown).

435

436 **Statistics**

437 Mean survival of control treatments, free of the two test pathogens, was 14.2 ± 4.2 (mean \pm
438 sd) days, while DWV treated bees survived for 8.1 ± 5.8 (mean \pm sd) days. To assess the
439 effect of infection on survival we fitted a Cox mixed effects model with treatment as a fixed
440 factor and colony origin as random factor and compared it to the null model⁵⁰ (R library
441 *coxme*, version 2.2-3, function *coxme*). The model was fitted with the penalized partial
442 likelihood (PPL) and showed a significant negative impact of infection on longevity ($X^2 =$
443 11.93 , $df = 4.17$; $p < 0.021$).

444 *N. ceranae* treated bees survived for 18 ± 1 (mean \pm sd) days. A model with treatment as
445 fixed factor and colony origin as random factor showed no improvement over the null model
446 (PPL: $X^2 = 0.12$, $df = 1$; $p > 0.735$).

447 True prevalences with 95% confidence intervals were computed to correct for varying sample
448 sizes (due to the different species of bumble bee at the sampling sites) and test sensitivity was
449 set to a conservative 95%⁵¹. Confidence interval estimates are based on Blaker's (2000)
450 method for exact two sided confidence intervals⁵² for each sampling site and for each species
451 sampled³¹(R library epiR, version 0.9-45, function epi.prev).

452 To investigate our spatially distributed dataset we undertook an exploratory data analysis
453 (EDA)⁵³ in which we calculated a prevalence surface for each of our parasites using Gaussian
454 kernel estimators with an adaptive bandwidth of equal number of observations. This is a
455 variant of the nearest neighbour technique, with bandwidth size being determined by a
456 minimum number of observations in the neighbourhood (set to 3 times the maximum
457 observations per site)³² (R library prevR, version 2.1, function kde). Estimated surfaces were
458 used for visual inspection only (Fig. 2); all the remaining analyses are based on the raw data
459 only.

460 To investigate spatial structure and disease hotspots we used spatial autocorrelation statistics
461 of the true prevalence of each of the pathogens in the different host genera from the 26
462 collection sites. To identify whether or not the pathogens we found were spatially clustered,
463 we computed the spatial autocorrelation coefficient Moran's I ⁵⁴ with an inverse spatial
464 distance weights matrix, as implemented in Gittleman and Kot⁵⁵ (R library ape, version 3.0-
465 7, function Moran.I). Moran's I is a weighted measure describing the relationship of the
466 prevalence values associated with spatial points. The coefficient ranges from -1 (perfect
467 dispersion) through 0 (no spatial autocorrelation (random distribution)) to 1 (perfect
468 clustering).

469 To investigate whether pathogen prevalence (*Nosema* and DWV were tested in separate
470 models) in *Apis*, *Bombus* to *Apis* relative density, or *Apis* absolute abundance had an effect on
471 pathogen prevalence in *Bombus*, we ran a Generalized Linear Mixed Model (GLMM)³⁴ with

472 binomial error structure and logit link function using the function lmer of the R package
473 lme4³⁵. Latitude, longitude, sunlight hours (a proxy for favourable foraging weather that
474 would enable disease transmission; calculated cumulatively from March until the month of
475 collection [data were collected from the MET office webpage:
476 <http://www.metoffice.gov.uk/climate/uk/anomacts/>, averaging over area sunlight hour
477 ranges]) and landcover type were included in the model as fixed control effects (present in
478 the full as well as the null model) while site and species were included in the model as
479 random effects (present in the full as well as the null model). Before running the model we
480 inspected all predictors for their distribution, as a consequence of which we log transformed
481 “*Bombus to Apis* density” and “*Apis* abundance” to provide more symmetrical distributions.
482 Thereafter we z-transformed all quantitative predictors to a mean of zero and a standard
483 deviation of one to derive more comparable estimates and to aid interpretation of
484 interactions⁵⁶. Since changes in “*Bombus to Apis* density” and “*Apis* abundance” could lead
485 to changes in pathogen prevalence in *Bombus* because of a change in pathogen prevalence in
486 *Apis*, we included the interactions between “*Bombus to Apis* density” and pathogen
487 prevalence in *Apis*, and “*Apis* abundance” and pathogen prevalence in *Apis*. To test the
488 overall effect of our three test predictors, we compared the full model with a reduced model
489 (null model) using a likelihood ratio test comprising latitude, longitude, sunlight hours and
490 landcover type with the same random effects structure. Model stability was assessed by
491 excluding data points one by one and comparing the estimates derived from these reduced
492 models with estimates from the full model (revealing a stable model). Site G had to be
493 excluded from this analysis as no *Apis* samples were found on site.

494 We fitted linear models to assess the relationships of parasite prevalence among *Apis* and
495 *Bombus*.

496 We investigated the effect of pathogen treatment on disease status of an individual with a
 497 Generalized Linear Mixed Model (GLMM)³⁴) with binomial error structure and logit link
 498 function using the function lmer of the R package lme4³⁵. Colony of origin was entered into
 499 the model as a random effect. As described before, we checked model stability (the model
 500 with interaction terms included was unstable; however it stabilised once the non-significant
 501 interaction terms were removed), before testing the full model against the null model using a
 502 likelihood ratio test. All analyses were run in R³⁶.

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571

572 **Extended Data Table 1 Pathogen prevalence per species:** Pathogen prevalence is given in
 573 percent with 95% confidence intervals (% prevalence [95% CI]). Sample numbers (N) are
 574 shown in brackets.

575 Footnote to Extended Data Table 1: * out of the 31 DWV present *Apis* samples tested

576

577 **Extended Data Table 2** Alternative hypotheses for the diversification of DWV and VDV
 578 viruses in UK pollinators

579 **Extended Data Figure 1 Species and site distribution:** Distribution of sampling sites
 580 across Great Britain and the Isle of Man. The most common *Bombus* species on site is
 581 represented by coloured letters while the 2nd most common *Bombus* species is represented by
 582 differently coloured dots. Total sample sizes for each site are given in the table.

583 **Extended Data Figure 2 Prevalence per site and species:** Pathogen prevalence in *Bombus*
 584 spp. in percent per site (a. for DWV; b. for *N. ceranae*) and per species (c. for DWV; d. for *N.*
 585 *ceranae*). Bars indicate 95% confidence intervals. Note different scales

586 **Extended Data Figure 3 Prevalence raw data:** the linear models shown only illustrate the
 587 relationships but do not drive the conclusions in the main text. a) DWV presence in *Apis* and
 588 *Bombus* (adj $R^2 = 0.34$, $p < 0.001$); b) DWV replicating in *Bombus* and DWV presence in
 589 *Bombus* (adj $R^2 = 0.46$, $p < 0.001$); c) *N. ceranae* presence in *Apis* and *Bombus* (adj $R^2 = -$
 590 0.04 , $p > 0.728$). The line shows the best fit, and the dark grey region shows 95%CI of fit.

591

592 Extended Data Table 1

| species (N) | <i>Apis</i> (250) | <i>B.ter</i> (170) | <i>B.luc</i> (60) | <i>B.lap</i> (175) | <i>B.pas</i> (60) | <i>B.hor</i> (20) | <i>B.mon</i> (10) |
|-------------------|-------------------|--------------------|-------------------|--------------------|-------------------|-------------------|-------------------|
| DWV present | 36 [30, 43] | 9 [5, 14] | 18 [9, 29] | 16 [11, 23] | 4 [1, 12] | 0 [0, 17] | 11 [1, 47] |
| DWV replicating | 88 [70, 98]* | 1 [0, 3] | 4 [1, 12] | 10 [6, 15] | 0 [0, 6] | 0 [0, 17] | 11 [1, 47] |
| <i>N. ceranae</i> | 9 [6, 13] | 2 [1, 6] | 0 [0, 6] | 16 [11, 23] | 0 [0, 6] | 5 [0, 25] | 0 [0, 29] |
| single infection | 18 [14, 23] | 3 [1, 7] | 3 [1, 12] | 20 [14, 27] | 0 [0, 6] | 5 [0, 25] | 11 [1, 47] |
| co-infection | 1 [0, 3] | 0 [0, 2] | 0 [0, 6] | 3 [1, 7] | 0 [0, 6] | 0 [0, 17] | 0 [0, 29] |

593

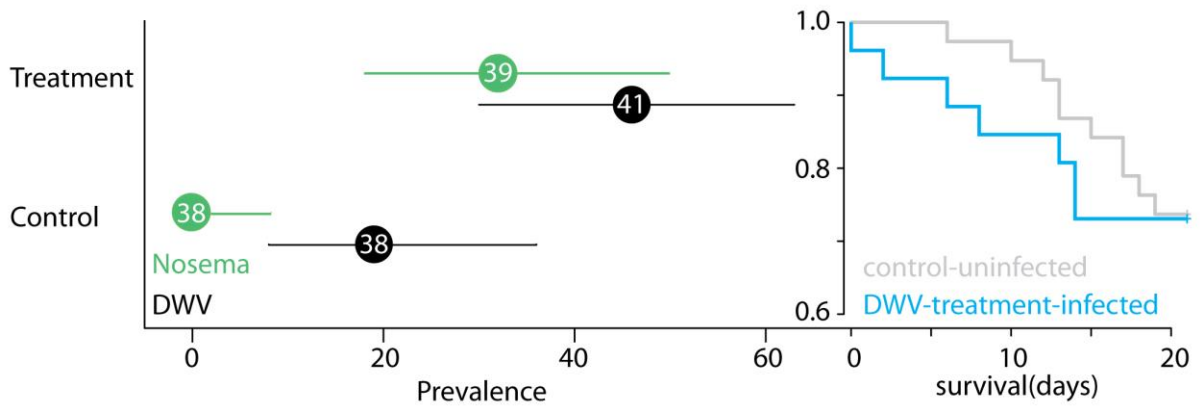
594 Extended Data Table 2

| Model | Marginal likelihood (ln) | Difference | BF | 2 ln (BF) | Preferred model |
|----------|--------------------------|------------|-------------------|-----------|-----------------|
| Site (S) | Null | -1512.71 | | | |
| Host (H) | | -1607.63 | >10 ⁴¹ | 189.84 | S |

595

596

597 Figure 1.

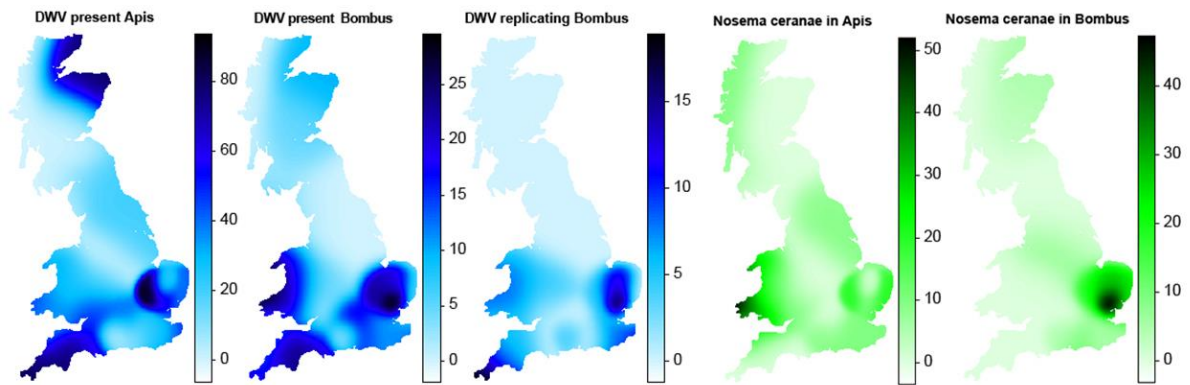


598

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601 Figure 2

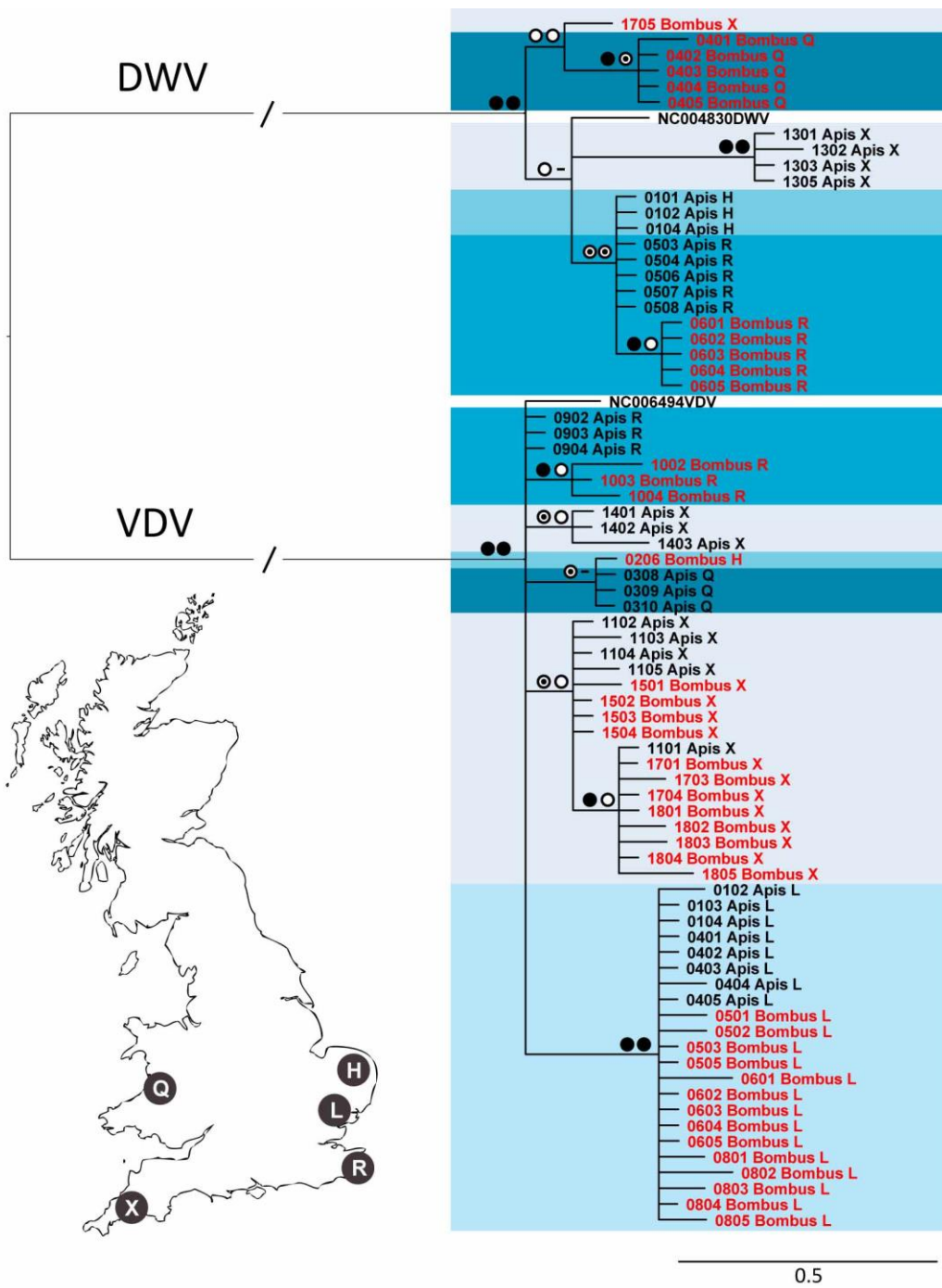


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605 Figure 3



606