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

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Emerging infectious diseases (EIDs) pose a risk to human welfare, both directly[1] and indirectly, by affecting managed livestock and wildlife that provide valuable resources and ecosystem services, such as the pollination of crops[2]. Honey bees (*Apis mellifera*), the prevailing managed insect crop pollinator, suffer from a range of emerging and exotic high impact pathogens[3,4] and population maintenance requires active management by beekeepers to control them. Wild pollinators such as bumble bees (*Bombus* spp.) are in global decline[5,6], one cause of which may be pathogen spillover from managed pollinators like honey bees[7,8] or commercial colonies of bumble bees[9]. In our study, a combination of infection experiments with landscape scale field data indicates that honey bee EIDs are indeed widespread infectious agents within the

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

Trading practices in domesticated animals allow infectious diseases to spread rapidly and to encounter novel hosts in newly sympatric wildlife[12]. This "spillover" of infectious disease from domesticated livestock to wildlife populations is one of the main sources of Emerging Infectious Disease (EIDs)[13]. Small or declining populations are particularly challenged, as the source host may act as a disease reservoir[14], giving rise to repeated spillover events and frequent disease outbreaks which, in the worst case, might drive already vulnerable or unmanaged populations to extinction[14]. Such severe impacts have been well documented over the past decades in vertebrates[10], but have largely been overlooked in invertebrates[15]. Recent years have seen elevated losses in multiple populations of one of the major crop pollinating insects, the honey bee (*Apis mellifera*)[16]. EIDs have been suggested as key drivers of decline, with deformed wing virus (DWV) (especially in combination with the exotic *Varroa* mite (*Varroa destructor*)) and *Nosema ceranae* (*N. ceranae*) being two likely causes for losses of *Apis*[17]. As generalist pollinators, honey bees are traded and now distributed almost worldwide for crop pollination and hive products. They share their diverse foraging sites with wild pollinators and thus facilitate interspecific transmission of pathogens, as has been suggested for intraspecific disease transmission from commercial to wild bumble bee populations[18]. Our focus is on inter-specific transmission, as EIDs in *Apis* are a potential threat to a range of wild

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

To examine the potential for *Apis* pathogens to cross host-genus boundaries, we tested the infectivity of the DWV complex (which includes the very closely related, co-occurring and recombinant *Varroa destructor* virus (VDV)[21,22]; we will refer to "DWV complex" as "DWV" throughout the text) and *N. ceranae*, in controlled inoculation experiments, to one of the most common *Bombus* species in Great Britain (*B. terrestris*). DWV is infective for *B. terrestris*; we found significantly more DWV infections 21 days after inoculating *B. terrestris* workers versus control (likelihood ratio test comparing the full model to one with only the intercept: $X^2 = 5.73$, df = 1, $p < 0.017$; Fig 1) and mean survival was reduced by 6 days. As for *Apis,* DWV causes deformed wings in *Bombus* when overtly infected[8], resulting in non-viable offspring and reduced longevity (Fig 1). *N. ceranae* is also infective for *B. terrestris*; infections increased in *Bombus* versus control ($X^2 = 17.76$, df = 1, $p < 0.001$; Fig 1), though overt symptoms were not seen (mean survival increased by 4 days).

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

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

We estimated the GB-wide prevalence of the two pathogens in *Apis* and *Bombus* spp. based on our field survey data (Fig. 2). We found no evidence for spatial clustering of DWV presence in *Bombus* (Moran's I = 0.023, $p > 0.211$) or either of the pathogens in *Apis* (DWV presence: Moran's I = 0.03, $p > 0.186$; *Nosema*: Moran's I = -0.061, $p > 0.649$). There was, however, weak clustering of DWV infection in *Bombus* (Moran's I = 0.061, $p < 0.044$) and very strong clustering of *N. ceranae* in *Bombus* (Moran's I = 0.25, $p < 0.001$), indicating disease hotspots for DWV in *Bombus* in the south west and east of GB and for *N. ceranae* in *Bombus* in the south east of GB (Fig. 2). Because prevalence was lower in *Bombus* than *Apis*, we modelled pathogen prevalence in *Bombus* as dependent on pathogen prevalence in *Apis*, *Bombus* to *Apis* density, and *Apis* abundance, including biologically relevant interactions, whilst controlling for latitude, longitude, and sunlight hours, and adding collection site and species identity as random factors. Our full model for DWV presence was significantly better than the null model without any of the test predictors and their interactions included (likelihood ratio test: $X^2 = 19.03$, df = 5, $p < 0.002$). After removal of the non-significant interactions (GLMM: *Bombus* to *Apis* density X DWV presence in *Apis*: estimate ± SE = -0.105 ± 1.376, p = 0.939; *Apis* abundance X DWV presence in *Apis*: 0.425 ± 1.309, p=0.745), it is clear that prevalence of DWV in *Apis* has a strong positive effect on DWV prevalence in *Bombus* (GLMM: 2.718 ± 0.921, z = 2.951, $p < 0.004$)(Fig. 2, Extended Data Fig. 3), while none of the other predictors played a role (GLMM: *Bombus* to *Apis* density: 0.315 ± 0.387, z = 0.814, $p < 0.416$; *Apis* abundance : -0.085 ± 0.364, z = -0.232, $p < 0.816$). In the case of *N. ceranae*, our full model was significantly better than the null model ($X^2 = 15.8$, df = 5, $p < 0.008$). Specifically there was an effect of *Nosema* prevalence in *Apis* on *Nosema* prevalence 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 sampling[23]
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* populations[18]. 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.'s[25]

non-age-controlled design, indicating age dependent differential susceptibility in *B. terrestris,* as has been shown to be the case in honey bees[26].

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

Methods summary

Bombus inoculation experiment

Two day old workers of *Bombus terrestris audax* colonies (Biobest) were individually 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 analysis[29]. *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 lme4[35]. All analyses were run in R[36].
173

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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: $X2 = 11.93$, $df = 4.17$; $p < 0.021$,
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

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

Methods

Bombus inoculation experiment

Each of the 7 experimental *Bombus terrestris* colonies (Biobest) was tested for presence of the two treatment pathogens DWV and *N. ceranae.* Daily, callows (newly emerged workers) were removed from the colony, assigned sequentially to random treatment blocks and housed individually in small Perspex boxes on an *ad libitum* diet of 50% sucrose solution and artificial pollen (Nektapoll), as natural pollen has been shown to contain viable *N. ceranae* spores and DWV virions [19,37]. Two day old bumble bee workers were individually inoculated with a treatment dependent inoculum in 10 µl sucrose. Crude hindgut extracts of 5 *Apis* workers propagating *N. ceranae* were purified by the triangulation method[38] with slight adaptations.

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

DWV virus inoculum was prepared according to Bailey & Ball[39] with modifications. Honeybees with DWV symptoms (crippled wings and body deformities) were crushed in 0.5M potassium phosphate buffer (pH 8.0), filtered and clarified by slow speed centrifugation

(8000*g* for 10 minutes) before being diluted and injected (1µl) into white-eyed pupae for bulk propagation of virus. After 5 days, up to 100 pupae were harvested, and after a further screen by qRT-PCR, virus was purified as follows. Virus extraction buffer consisted of 0.5M potassium phosphate pH 8.0, 0.2% DEICA, 10% diethyl ether. Purification consisted of two slow speed clarifications (8000*g* for 10 minutes), one high speed clarification (75000*g* for 3 hours) followed by re-suspension in 0.5M potassium phosphate buffer (ph8.0) and a final slow speed clarification. Virus preparations were aliquoted and stored at -80°C until use in inoculation experiments.

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

A duplicate dilution series of external DNA standards covering 10^2 to 10^8 molecules (reaction efficiencies: 90-110%, r^2: 0.95-0.99) were included in qRT-PCR runs to quantify DWV genome equivalents present in the inoculum. For absolute quantification of virus dose, an external DNA standard was generated by amplifying a genomic fragment of 241bp using the primers F8668*std* (5'-GAT GGG TTT GAT TCG ATA TCT TGG-3') and B8757*std* (5'-GGC AAA CAA GTA TCT TTC AAA CAA TC-3') via RT-PCR that contained the 136bp fragment amplified by the DWV-specific qRT-PCR primers F8668/B8757[40].

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

Sampling scheme

The mainland sampling sites were chosen across Great Britain along a north-south transect (12 sampling points with fixed latitude, but free in longitude) and across two east-west transects (12 sampling points with fixed longitude, but free within a narrow latitudinal corridor). Each of the mainland sites were at least 30 km apart (mean ± SD of nearest neighbour = 69.21 ± 26.39). The island sites were chosen deliberately to gain background data for both *Apis* and *Bombus* disease prevalence in the absence of *Varroa*, the main transmission route for DWV in *Apis*. At each sampling site we collected approximately 30 workers for each of the following species: *Apis mellifera*, *Bombus terrestris* (verified by RFLP-analysis[29]), and the next most common bumble bee on site. We collected free flying bees from flowers rather than bees from colonies as this is the most likely point of contact in the field. By collecting from flowers we lowered the likelihood of collecting bumblebees from different colonies. While we ran the risk of collecting multiple honeybees from the same hive, this nevertheless represents the potential force of infection for both genera in the field.

Each collection took place along a continuous transect, where maximally ten bees per ten metre stretch were collected before moving on to the next ten metre stretch. At each site, the collection area covered at least 1000 m^2 (e.g., 10 x 100m, 20 x 50m). Each sampling point was within one of the following landcover types: urban areas (gardens and parks), farmland (hedgerows, border strips, crops, and wildflower meadows), coastal cliffs, sand dunes and heather moorland.

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

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

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

RNA work

RNA extraction followed the standard RNeasy plant mini kit (Qiagen) protocol with the final elutate (in RNase free ddH2O) of 30 µl being run over the column twice (for optimal RNA concentration). For reverse transcription of RNA to cDNA we followed the standard protocol of the Nanoscript Kit (Primerdesign). Our priming was target specific in separate reactions for *N. ceranae* (primer pair *N. ceranae*[41]), DWV (primer pair F15/B23[42]) and a housekeeping gene (primer pair ACTB[43]) as a positive control for RNA extraction efficiency. Bees were transferred to liquid N_2 prior to dissection. Each bee's abdomen was cut with a sterile scalpel dorsoventrally along the sagittal plane. One half was submerged in RLT buffer (Qiagen) for RNA extraction, and the second half was archived at -80C. Tissue disruption and homogenisation of individual half-abdomens was performed on a tissue lyser II (Qiagen) at 30Hz for 2 minutes followed by 20Hz for 2 minutes. RNA quality and quantity were checked on a Spectrometer (Nanodrop, Thermo Scientific). cDNA preparation was conducted at 65°C for 5 minutes for the initial priming immediately before the addition of the reverse transcriptase. For the extension, samples were incubated at 25°C for 5 minutes followed by 55 °C for 20 minutes and then heat inactivated for 15 minutes at 75°C. cDNA was used as template in a standard PCR with 57°C, 54°C, and 57°C annealing temperatures, respectively. Results were visualized on a 2% agarose gel with EtBr under UV light. Agarose gels were

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

Detection of negative strand DWV

Detection of pathogens in pollinators in the field does not provide proof of infection, as pathogens are likely being ingested on shared, contaminated food resources and therefore are inevitably present in the gut-lumen as passive contaminants without necessarily infecting the host. To minimize these cases, we tested all our DWV positive *Bombus* samples and a subset of DWV positive *Apis* samples for virus replication, a strong indicator for infection[44]. DWV is a positive strand virus whose negative strand is only present in a host once the virus is actively replicating[39]. Reverse transcription was conducted using a tagged primer tagB23[45] for the initial priming to target exclusively the negative strand. The resulting cDNA was used in a PCR with the tag sequence and F15 as primers[30,45]. We tested all *Bombus* samples that were positive for DWV presence and, where possible, 2 DWV-positive *Apis* samples from each site where we found DWV in *Bombus*.

Sequencing

DWV sequence diversity was analysed by sequencing up to 5 independent clones per DWV negative-strand infected *Bombus* sample from 5 sites (H, L, Q, R, X; chosen for their high DWV infected prevalence in *Bombus*) and 5 clones of DWV infected *Apis* samples from the same sites (we checked extra *Apis* samples for DWV infection if necessary to match *Bombus* DWV infections). All *Bombus* samples were *B. lapidarius* with the exception of one sample from site L (clone05), which was *B. pascuorum* (this sample is not included in any of the 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 pair[42] 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 virus [24]. 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.3.0[46] maximum-likelihood (ML) bootstrapping (500 replicates) and MrBayes v3.1.2[47],
422 under a GTR model of sequence evolution and a gamma (Γ) model, using 4 categories to

accommodate rate variation across sites. Burn-in cutoffs were inspected manually for each parameter file in Tracer v1.4[48]. Inspection of the standard deviation of split frequencies confirmed that runs had converged (0.0093). To test alternative *a priori* hypotheses of virus diversification, for each virus (DWV and VDV) we constrained clades according to site (H, L, Q, R and X) or host genus (*Apis* and *Bombus*), and performed stepping stone sampling[23] as implemented in MrBayes v3.1.2 to accurately estimate marginal log likelihoods. MCMC sampling was conducted for 50 steps of 39000 generations each, with the first 9000 generations of every step discarded as burn-in. The model with the highest likelihood score was used as the null hypothesis. We compared Bayes Factors (BF) for both models and used a threshold of 2 ln (BF) > 10 as decisive support for the null against the alternative hypothesis[49] (Supplementary Table 2). We repeated stepping stone sampling to confirm run stability (data not shown).

Statistics

Mean survival of control treatments, free of the two test pathogens, was 14.2 ± 4.2 (mean ± sd) days, while DWV treated bees survived for 8.1 ± 5.8 (mean ± sd) days. To assess the effect of infection on survival we fitted a Cox mixed effects model with treatment as a fixed factor and colony origin as random factor and compared it to the null model [50](R library coxme, version 2.2-3, function coxme). The model was fitted with the penalized partial likelihood (PPL) and showed a significant negative impact of infection on longevity (X^2 = 11.93, df = 4.17; $p < 0.021$).

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

True prevalences with 95% confidence intervals were computed to correct for varying sample sizes (due to the different species of bumble bee at the sampling sites) and test sensitivity was set to a conservative 95% [51]. Confidence interval estimates are based on Blaker's (2000) method for exact two sided confidence intervals [52] for each sampling site and for each species sampled [31](R library epiR, version 0.9-45, function epi.prev).

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

To investigate spatial structure and disease hotspots we used spatial autocorrelation statistics of the true prevalence of each of the pathogens in the different host genera from the 26 collection sites. To identify whether or not the pathogens we found were spatially clustered, we computed the spatial autocorrelation coefficient Moran's I[54] with an inverse spatial distance weights matrix, as implemented in Gittleman and Kot [55] (R library ape, version 3.0-7, function Moran.I). Moran's I is a weighted measure describing the relationship of the prevalence values associated with spatial points. The coefficient ranges from -1 (perfect dispersion) through 0 (no spatial autocorrelation (random distribution)) to 1 (perfect clustering).

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

binomial error structure and logit link function using the function lmer of the R package lme4[35]. Latitude, longitude, sunlight hours (a proxy for favourable foraging weather that would enable disease transmission; calculated cumulatively from March until the month of collection [data were collected from the MET office webpage: http://www.metoffice.gov.uk/climate/uk/anomacts/, averaging over area sunlight hour ranges]) and landcover type were included in the model as fixed control effects (present in the full as well as the null model) while site and species were included in the model as random effects (present in the full as well as the null model). Before running the model we inspected all predictors for their distribution, as a consequence of which we log transformed "*Bombus* to *Apis* density" and "*Apis* abundance" to provide more symmetrical distributions. Thereafter we z-transformed all quantitative predictors to a mean of zero and a standard deviation of one to derive more comparable estimates and to aid interpretation of interactions[56]. Since changes in "*Bombus* to *Apis* density" and "*Apis* abundance" could lead to changes in pathogen prevalence in *Bombus* because of a change in pathogen prevalence in *Apis*, we included the interactions between "*Bombus* to *Apis* density" and pathogen prevalence in *Apis*, and "*Apis* abundance" and pathogen prevalence in *Apis*. To test the overall effect of our three test predictors, we compared the full model with a reduced model (null model) using a likelihood ratio test comprising latitude, longitude, sunlight hours and landcover type with the same random effects structure. Model stability was assessed by excluding data points one by one and comparing the estimates derived from these reduced models with estimates from the full model (revealing a stable model). Site G had to be excluded from this analysis as no *Apis* samples were found on site.

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

We investigated the effect of pathogen treatment on disease status of an individual with a Generalized Linear Mixed Model (GLMM) [34]) with binomial error structure and logit link function using the function lmer of the R package lme4[35]. Colony of origin was entered into the model as a random effect. As described before, we checked model stability (the model with interaction terms included was unstable; however it stabilised once the non-significant interaction terms were removed), before testing the full model against the null model using a likelihood ratio test. All analyses were run in R[36].

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

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

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

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

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

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

Extended Data Table 1

species (N)	*Apis* (250)	*B.ter* (170)	*B.luc* (60)	*B.lap* (175)	*B.pas* (60)	*B.hor* (20)	*B.mon* (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]
N. ceranae	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]

Extended Data Table 2

Model		Marginal likelihood (ln)	Difference	BF	2 ln (BF)	Preferred model
Site (S)	Null	-1512.71	-94.92	$>10^{41}$	189.84	S
Host (H)		-1607.63				

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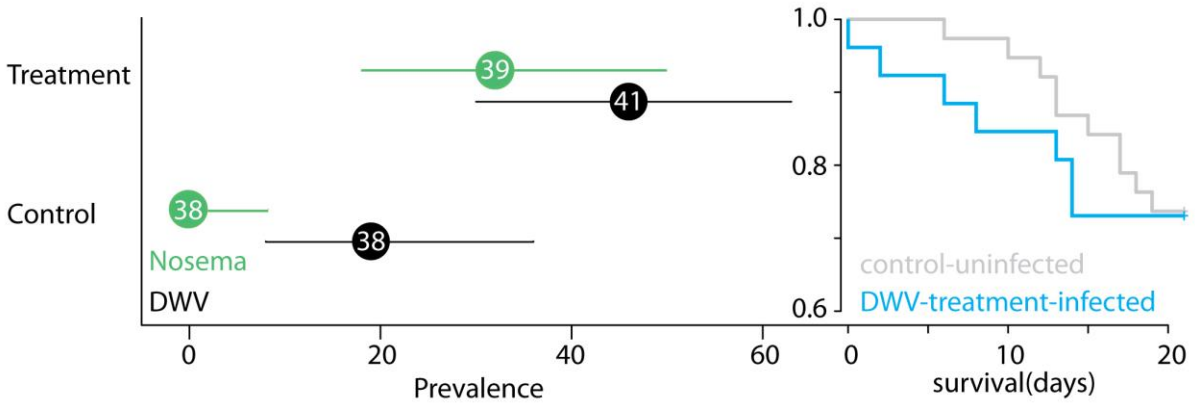
597 Figure 1.



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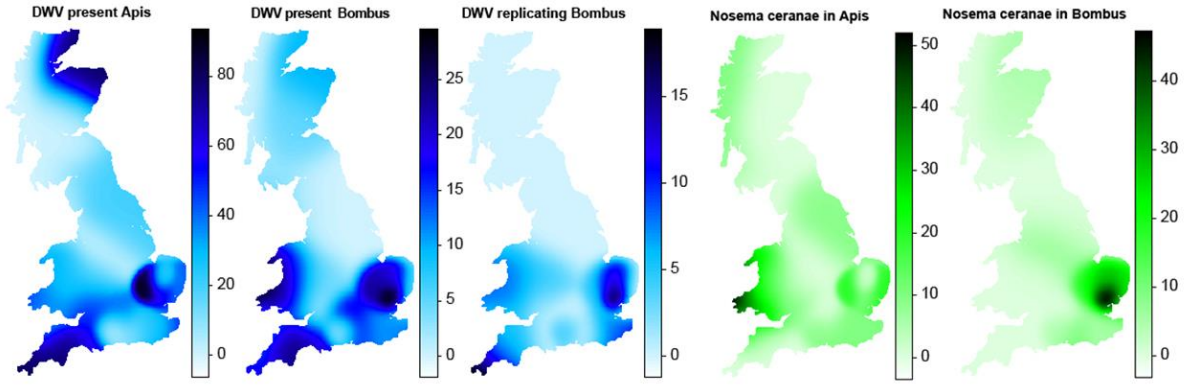
601 Figure 2



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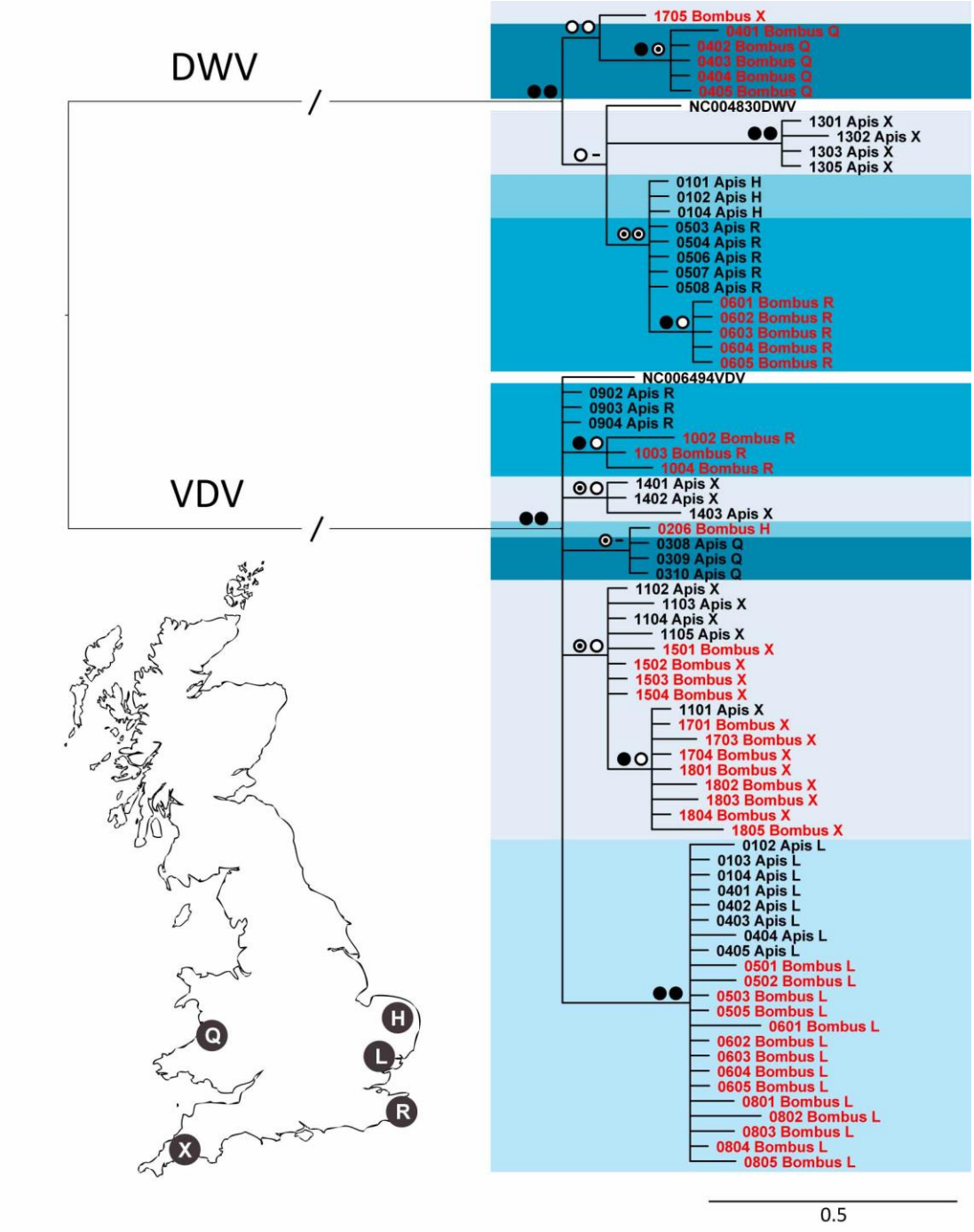
605 Figure 3



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